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Award Number: DAMD17-02-P-0706

TITLE: The First Joint Symposium of the National and International Neurotrauma Societies 2002

PRINCIPAL INVESTIGATOR: W. Dalton Dietrich, Ph.D.

CONTRACTING ORGANIZATION: University of Miami
Miami, Florida 33136

REPORT DATE: December 2002

TYPE OF REPORT: Final Proceedings

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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20030317 105

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	December 2002	Final Proceedings (3 Jun 02 - 31 Dec 02)

4. TITLE AND SUBTITLE
The First Joint Symposium of the National and International Neurotrauma Societies 2002

5. FUNDING NUMBERS
DAMD17-02-P-0706

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9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
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Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates: All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

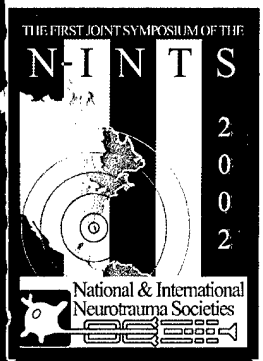
12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

The First Joint Symposium of the National and International Neurotrauma Societies was held October 27 through November 1, 2002, at the Saddlebrook Resort, Tampa, FL USA. Over 750 scientists/physicians participated in this five-day meeting, which represented a unique partnership between the International and National Neurotrauma Communities. Various research topics targeting CNS injury and repair were discussed by some of the leading authorities in the world. The success of the meeting was greatly dependent upon support from various funding agencies, including the Department of the Army. These moneys were used to support conference/symposium activities. A copy of the program, including the scientific committee, programs, and abstract/poster presentation is included with this report.

14. SUBJECT TERMS:	15. NUMBER OF PAGES
Neurotrauma	222
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited



THE FIRST **JOINT** SYMPOSIUM OF THE

NATIONAL AND INTERNATIONAL

NEUROTRAUMA SOCIETIES

The Twentieth Annual National Neurotrauma Society Symposium
& The Sixth International Neurotrauma Symposium

October 27 - November 1, 2002
Saddlebrook Resort
Tampa, FL USA

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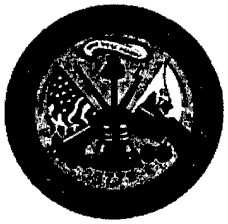

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THE FIRST JOINT SYMPOSIUM OF THE NATIONAL AND INTERNATIONAL NEUROTRAUMA SOCIETIES

N-I N T S 2002

National & International Neurotrauma Societies

www.neurotrauma2002.org

October 27-November 2, 2002
Saddlebrook Resort
Tampa, Florida

THE 20TH NATIONAL NEUROTRAUMA SOCIETY SYMPOSIUM
&
THE 6TH INTERNATIONAL NEUROTRAUMA SYMPOSIUM

PROGRAM CO-CHAIRPERSONS:

DOUGLAS K. ANDERSON, PH.D.
Malcolm Randall VA Medical Center/ University of Florida

W. DALTON DIETRICH, PH.D.
University of Miami

LINDA J. NOBLE, PH.D.
University of California at San Francisco

Conference Organizers:
David Price & Associates, Inc.
7487 SW 50th Terrace
Miami, FL 33155 USA
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Email: nints@dprice.com

October, 2002

Dear NINTS 2002 Attendees:

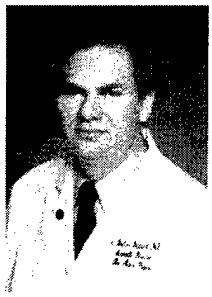
Welcome to the first joint meeting of the National and International Neurotrauma Societies (N-INTS) at the Saddlebrook Resort.

This five day meeting represents a unique partnership between both the international and national neurotrauma communities, both of whom have been historically committed to excellence in both research and the medical management of the brain and spinal cord injured patient. It is our hope that the joining of these two historically successful societies will provide a unique intellectual environment in which to promote the exchange of contemporary scientific communication on the basic mechanisms and treatments of acute and chronic CNS injury.

We express our sincere gratitude to the outstanding group of basic and clinical scientists who helped make this meeting possible by serving on the program committee and participating as faculty in the years' symposium. It is through their efforts that we can offer you such an extraordinary program.

Thank you for your participation in this year's symposium.
We hope that you will find it both challenging and enjoyable.

Warmest regards,



Douglas K. Anderson

W. Dalton Dietrich

Linda J. Noble

THE FIRST JOINT SYMPOSIUM OF THE NATIONAL AND INTERNATIONAL NEUROTRAUMA SOCIETIES

October, 2002

Dear N-INTS Attendee:

On behalf of the International Neurotrauma Society (INTS), it is my pleasure to welcome all participants and guests to the Sixth International Neurotrauma Symposium and the first to be held in conjunction with another neurotrauma organization, the National Neurotrauma Society.

The purpose of the INTS is to foster the worldwide dissemination of neurotrauma research and to supervise international neurotrauma symposia throughout the world. The intention continues to be to alternate the venue of the symposium meetings between Australasia, Europe and The Western Hemisphere every two or three years. To do so, the INTS authorizes a local host for each meeting and assists the local host's organizing committee thorough the International Scientific Advisory Board of the INTS.

Professors Douglas Anderson and Dalton Dietrich were chosen from scientists at several distinguished North American universities to organize and host this Sixth INTS meeting. They have had a most difficult task to achieve scientific and social comparability to that of the Fifth International Neurotrauma Symposium in Germany and have been given the additionally difficult task of integrating the INTS meeting with the meeting of the National Neurotrauma Society. This represents the first attempt of either society to present a combined meeting.

The INTS has been exceptionally impressed by the organizational capabilities of Professors Anderson and Dietrich and their teams from the University of Miami and the University of Florida. The cooperation of Professor Linda Noble and her colleagues in the National Neurotrauma Society has been superb and, thus, we are confident that we are about to experience a very special scientific and social program that will advance the science of neurotraumatology and will draw scientists from all nations much closer together. In this regard, I wish you a most productive symposium and pleasant camaraderie.

Thomas A. Genarelli, MD
President, International Neurotrauma Society

www.neurotrauma2002.org

October 27-November 2, 2002
Saddlebrook Resort
Tampa, Florida

THE 20TH NATIONAL NEUROTRAUMA SOCIETY SYMPOSIUM
&
THE 6TH INTERNATIONAL NEUROTRAUMA SYMPOSIUM

PROGRAM CO-CHAIRPERSONS:

DOUGLAS K. ANDERSON, PH.D.
Malcolm Randall VA Medical Center/ University of Florida

W. DALTON DIETRICH, PH.D.
University of Miami

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FACULTY DISCLOSURES

In compliance with the Accreditation council for Continuing Medical Education (ACCME) Standards for Commercial Support of CME, the Office of Continuing Medical Education discloses all current relationships that program faculty report with companies whose products they may discuss during their presentations.

The following invited speakers have disclosed relationships with commercial supporters:

- Doug Anderson, Ron Hayes and Paul Reier are faculty members at the McKnight Brain Institute.
- Arlene Chiu and Mary Ellen Michel are employed by the National Institute of Neurological Disorders and Stroke.
- W. Dalton Dietrich and Martin Oudega are faculty members at the Miami Project to Cure Paralysis.
- Ron Hart is a faculty member at the W.M. Keck Center for Collaborative Neuroscience.
- David Hovda is the Director of the UCLA Brain Injury Research Center.
- Claire Hulsebosch has received funding in the past from the Kent Waldrep National Paralysis Foundation, Pfizer and the Paralyzed Veterans of America.
- Robin Roof is employed by Pfizer.
- Marion Murray receives funding from EPVA.
- Andrew Maas is the Chairman of the Pharmos steering committee for International Multicenter Study.
- Graham Teasdale has received financial support from Pharmos through Glasgow University.

We have been unable to obtain information regarding relationships with commercial supporters from the following speakers: Ross Bullock, Susan Harkema, Jun Chen, Paul Reier.

The following session chairs have disclosed relationships with commercial supporters:

- Mary Bunge is a faculty member at the Miami Project to Cure Paralysis. She is also a member of the Ameritec board which determines the recipient of the Ameritec Prize for Paralysis Research and is on the Scientific Advisory Board for Acorda Therapeutics.
- Mary Eaton is a faculty member at the Miami Project to Cure Paralysis and has been a past recipient of grants from the Paralyzed Veterans of America.
- Edward Hall is a former employee of Pharmacia and is currently employed by Pfizer.
- Dena Howland is a faculty member at the McKnight Brain Institute.
- Bruce Lyeth and Paul Vespa receive funding from a research grant from the UCLA Brain Injury Research Center.

The following oral abstract presenters have disclosed relationships with commercial supporters:

- Paul Vespa (P333), Grace Griesbach (P115) and Cheri Osteen / Christopher Giza (P545) are all members of and/or receive support from the UCLA Brain Injury Research Center.
- Stephen Larner (P105) and Eric Johnson are both members of the McKnight Brain Institute.

All other oral abstract presenters report having no relationships with the commercial supporters of CME.

ACKNOWLEDGEMENTS OF COMMERCIAL SUPPORT

(Supporters listed alphabetically)

The National and International Neurotrauma Societies and the School of Medicine at Virginia Commonwealth University gratefully acknowledge the following companies for their generous support of N-INTS 2002:

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Eastern Paralyzed Veterans Association

Evelyn F. & William L. McKnight Brain Institute at the University of Florida

Geron Corporation

Kent Waldrep National Paralysis Foundation

Miami Project to Cure Paralysis

The National Institutes of Health

The National Institute for Child Health & Human Development

The National Institute of Neurological Disorders and Stroke

Paralyzed Veterans of America

Pfizer

Pharmacia

Pharmos Corporation

United States Department of the Army

United States Department of the Navy

United States Department of Veterans Affairs

UCLA Brain Injury Research Center

University of Miami Department of Veterans Administration

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Oxford Optronix

United States Department of Veterans Affairs

University of California at Irvine

W.M. Keck Center for Collaborative Neuroscience

Women in Neurotrauma (WINTR)

GENERAL INFORMATION

NINTS CREDENTIALS DESK AND BASIC INFORMATION

INFO DESK PHONE: EXTENSION 4322

Located in the Royal Palm Foyer, the Credentials Desk will be open daily from at 7am until 6pm. Conference staff will be on hand to assist participants in every possible way, so as to ensure your NINTS 2002 experience is both enjoyable and rewarding.

WWW.NEUROTRAUMA2002.ORG

The NINTS Conference website will be updated with Conference highlights, awards and winners of the Poster Competition following the completion of the conference.

FUTURE CONFERENCES

The Organizing Committee of the 7th International Neurotrauma Symposium invites all members of the international neurotrauma community to join their colleagues on September 12-16, 2004 in Adelaide, South Australia. For details, please contact events@plevin.com.au.

The 21st Annual Meeting of the National Neurotrauma Society will be held on Nov. 6-7, 2003. The meeting will be a satellite to the annual Neuroscience meeting in New Orleans, Louisiana. Student travel awards, student poster competition and an excellent scientific program are planned. Details will be available soon at www.neurotrauma.org.

OFFICIAL NINTS 2002 T-SHIRTS

Don't leave NINTS without an Official Conference T-Shirt! Available in various sizes for only $15.00. Don't forget to pick up a few extra for your colleagues back in the lab...The T-Shirt sales desk is located next to the NINTS Central Information Desk in the Royal Palm Foyer. Supplies are limited!

NAME BADGES

Name badges are required for access to all sessions, meals and evening events included with registration. Please wear your name badge at all times. In addition, please be sure to bring your tickets with you for admittance to ticketed events.

RESPONSIBILITY

By registering and participating in NINTS 2002, the attendee shall hold harmless the National and International Neurotrauma Societies, Society Administration and elected officials, Conference Co-Chairs and Conference Organizers in the case of any damage or personal injury claims. In addition non of the party's shall be liable for non-performance including but not limited to, strikes or labor unrest, delay in transportation, delay in delivery by suppliers, fire, wars, acts of governments, unavailability of power or other utilities, or acts of nature.

CME ACCREDITATION

This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME) by the School of Medicine, Virginia Commonwealth University, Medical College of Virginia Campus (VCU) and the National Neurotrauma Society. VCU is accredited by the ACCME to provide continuing medical education for physicians.

Physicians may claim up to 33.5 hours in Type 1 or Type 2 CME on the Virginia Board of Medicine Continued Competency and Assessment Form required for renewal of an active medical license.

VCU designates this educational activity for a maximum of 33.5 hours in category 1 credits toward the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the activity.

This continuing education activity meets the criteria of Virginia Commonwealth University and the Southern Association of Colleges and Schools. 3.0 CEUs will be awarded and recorded with the University.

SOCIAL EVENTS INCLUDED WITH REGISTRATION

Continental Breakfast will be held in the Royal Palm Ballroom each morning for all registered delegates and registered accompanying guests.

Sunday, October 27th

Welcome Reception
6:30 pm - 8:00 pm

Attendance is included for all registered delegates and registered accompanying guests.

Monday, October 28th

Delegate Luncheon (*Provided by Pharmacia)*
11:45 am - 1:00 pm, Pegasus Ballroom

Attendance is included for all registered delegates.

Thursday, October 31st

Masquerade Gala Dinner
7:30 pm - 10:30 pm, Grand Pavilion

Join us for a traditional American Halloween celebration at Saddlebrook Resort!

We invite you to attend and, if you like,
Dress in costume or wear a mask
for our festive celebration on All Hollow's Eve.

Attendance is included for all registered delegates and registered accompanying guests.

OPTIONAL TICKETED SOCIAL EVENTS

To purchase tickets for these events, please visit the Credentials Desk unless otherwise noted.

Monday, October 28th

"An Evening in the Tropics" Dinner
7:00 pm, $58.00 pp

All participants are cordially invited to attend an evening celebrating the local flavors and rich cultures of tropical Florida.

Tuesday, October 29th

Women in Neurotrauma Luncheon Ticket
11:45 am-1:00 pm, $14.00 pp

Women in Neurotrauma Reception
5:30 pm-6:30 pm, $12.00pp

Wednesday, October 30th

National Neurotrauma Society Business Meeting
11:45 pm-1:00 pm, $22.00 pp

All National Neurotrauma Society members are invited to attend. Lunch tickets may be purchased in advance.

Thursday, October 31st

Lunch at Saddlebrook
11:45 am-1:00 pm, $22.00 pp

Avoid the rush at the restaurants and network with your colleagues during a casual buffet lunch.

GENERAL INFORMATION

INSTRUCTIONS FOR AUTHORS

PUBLICATION

All accepted abstracts will be published in the Journal of Neurotrauma which is included with the attendees' credential kits.

POSTERS

All posters will be displayed in the Royal Palm Ballroom. For your convenience, push pins will be provided for your use in displaying your poster. Be sure to set up and remove your posters during the times indicated below. Posters left remaining after their session will be discarded. Please be sure you are stationed at your poster at the scheduled session time to present your abstract:

Abstract Numbers	Poster Session	Date	Set up	Session Time	Removal
*101-132	Top Poster Abstracts Final Judging	Monday, Oct 28 to Friday, Nov 1	7:00-8:00 am on Monday	Refer to Sessions 1, 2 & 3 for session times	12:15-12:30 pm on Friday
133-208	1	Monday, Oct 28	7:00-8:00 am	10:15-11:45 am	11:45-12:00 pm
209-284	2	Monday, Oct 28	12:00-12:30 pm	3:00-4:30 pm	After 4:30 pm
285-360	3	Tuesday, Oct 29	7:00-8:00 am	10:15-11:45 am	11:45-12:00 pm
361-436	4	Tuesday, Oct 29	12:00-12:30 pm	3:00-4:30 pm	After 4:30 pm
437-512	5	Wednesday, Oct 30	7:30-10:15 am	11:45-1:15 pm	1:15-1:30 pm
513-588	6	Thursday, Oct 31	7:00-8:00 m	10:15-11:45 am	11:45-12:00 pm

*Posters 101-132 will be reviewed by the Abstract Judging Committee on Monday and Tuesday during Poster Sessions 1-3. Please be sure you are present at all three judging sessions to answer any questions the judges may have. All posters being reviewed by the committee will be displayed for the entire week.

ORAL PRESENTATIONS

Each abstract selected for oral presentation will be allotted 10 minutes for their presentation, followed by 5 minutes for discussion. Please be sure to arrive at Royal Palm Ballroom East (1/2/3) at least 15 minutes prior to your presentation with your presentation on a PC formatted disk in order to coordinate with the A/V technician. Please refer to the schedule below for the date & time of your presentation:

Free Communications Session	Date	Session Time	Abstract Number (in order of presentation)
1	Monday, Oct 28	4:30-6:00 pm	107, 108, 113, 123, 128, 318
2	Tuesday, Oct 29	4:30-6:00 pm	105, 115, 116, 120, 126, 313
3	Wednesday, Oct 30	10:15-11:45 am	104, 110, 114, 122, 127, 519
4	Thursday, Oct 31	3:00-4:30 pm	106, 125, 131, 333, 375, 545

TRAVEL GRANTS

Thanks to generous support in the form of a grant from NIH, NINTS is able to offer $10,000 in travel grants encouraging students to attend the conference. The travel grants are awarded based on financial need and merit. We are pleased to announce that this year's awardees are: *Maria Briones-Galang, Oleg Butovsky, Szu-Fu Chen, Jyoti Chuckowree, Pauline Dergham, Julie Friedland, Maria Jimenez Harmann, Caitlin Hill, Nicole Klapka, Iris Kulbatski, Paul Lea, Jie Liu, Yan Long, Andrew Luciano, Andreas Menke, Otani Naoki, Eugene Park, Nicholas Phan, Neggy Rismanchi, Tomoko Sengoku.*

LOCAL TRANSPORTATION

TO TAMPA INTERNATIONAL AIRPORT

Saddlebrook Resort is providing a shuttle service to Tampa International Airport (TPA) for $19.00 per person each way. For reservations, please call extension 4455. Please plan to depart 2 hours or more prior to your flight departure time. Shuttles to the airport depart on the hour except for the first shuttle, which is 4:30am. Transportation charges will be billed to your room account at Saddlebrook. *Children 17 & under traveling with an adult are free.*

Cabs are also available to the airport, however the average one way fare is approx. $75.00 USD.

TO ORLANDO

Bus departs at 1:30 p.m. from the Royal Palm Mall area. *Advance reservations required.*

For delegates who will be attending the 31st Annual Meeting of the Society for Neuroscience, held on Nov. 2-7 in Orlando, we are providing One Way bus transportation to Orlando for $25.00 pp. Even if you will not be attending the Orlando conference, you may wish to take advantage of this opportunity to spend a day at Walt Disney World or one of the many other attractions in the area before returning home.

RENTAL CARS

Enterprise rent-a-car

We have negotiated special discounted rates with Enterprise Rent-a-Car for NINTS 2002 attendees, including:

- Sunset Special 50% off rentals beginning between 4:00-5:30 pm and ending by 8:00 am the following day.
- $15.99 a Day Weekend Rate Applies to a compact car rented from Friday through Monday for a 3 day total of $47.97 with 300 free miles included.
- Complimentary Pick Up or Delivery available at Tampa International Airport and Saddlebrook Resort

If you have a need for a rental car while in Tampa, please make your reservations by calling Enterprise at 813-949-7458 (local) or 813-282-1680 (Tampa airport). If you need further assistance, please contact Marta Apostulu at 727-539-0702 x. 211. To receive your discounted rate, please refer to Account #686876 when calling to make a reservation.

PARKING FACILITIES

Valet parking charges are currently $5.00 for day parking and $10.00 for overnight parking. Self parking is also available on a complimentary basis.

LOCAL AREA BUSINESSES

BANKS

1.1 miles Sun Trust Bank
5310 County Road 581
Wesley Chapel, FL
813-907-1335

1.2 miles Southtrust Bank
5227 County Road 581
Wesley Chapel, FL
813-973-2265

6.0 miles Community Bank
19910 Bruce B Downs Blvd
Tampa, FL
813-991-9206

6.5 miles First Union National Bank
8902 Regents Park Dr
Tampa, FL
813-276-4449

MOVIE THEATERS

7.7 miles Muvico Theaters
18002 Highwoods Preserve Pkwy
Tampa, FL
813-558-9755

8.9 miles Zephyrhills Cinema 6
6848 Gall Blvd
Zephyrhills, FL
813-782-2222

GROCERY STORES

1.1 miles Publix Supermarkets Inc
5400 County Road 581
Wesley Chapel, FL
813-907-1699

1.1 miles Winn-Dixie
5351 Village Mart
Wesley Chapel, FL
813-973-3000

PHARMACIES

1.0 miles Walgreens
28115 State Road 54
Zephyrhills, FL
813-973-2095

6.7 miles Eckerd Drug
8809 New Tampa Blvd
Tampa, FL
813-632-8989

SHOPPING CENTERS

1.0 miles Bealls Outlet
5417 Village Market St
Zephyrhills, FL
813-994-3550

6.0 miles Wal-Mart Supercenter
19910 Bruce B Downs Blvd
Tampa, FL
813-994-6543

7.3 miles Dollar General
36524 State Road 54
Zephyrhills, FL
813-780-6808

7.3 miles K Mart
22920 State Road 54
Lutz, FL
813-949-6303

PLACES OF WORSHIP

0.5 miles Atonement Lutheran Church
29617 State Road 54
Wesley Chapel, FL
813-973-2211

0.5 miles First Baptist Church of Wesley Chapel
29716 State Road 54
Wesley Chapel, FL
813-973-7185

1.8 miles Faith Baptist Church
6300 Oakley Blvd
Wesley Chapel, FL
813-907-9462

4.5 miles Wesley Chapel Seventh Day
33420 State Road 54
Wesley Chapel, FL
813-788-1550

4.5 miles Trinity United Methodist Church
33425 State Road 54
Wesley Chapel, FL
813-788-2898

7.1 miles Our Lady of the Rosary Church
2348 Collier Pkwy
Land O Lakes, FL
813-949-4565

SUNDAY, OCTOBER 27TH

	Royal Palm Foyer	Grand Pavilion	Executive Boardroom	Boardroom Two
12:00				
12:30				
1:00				
1:30				
2:00	**Guest Check-In**		INTS Executive Committee & Scientific Advisory Board Meeting	
2:30				
3:00	**&**			NNS Officer Meeting
3:30				
4:00	**Credentials Desk/ Registration Area Open**			
4:30				
5:00				
5:30		**Opening Ceremony**		
6:00				
6:30		**Welcome Reception** *(poolside)*		
7:00				
7:30				

NAME BADGES

Name badges are required for access to all sessions, meals and evening events included with registration.
Please wear your name badge at all times.
In addition, please be sure to bring your ticket with you for admittance to ticketed events.

MONDAY, OCTOBER 28TH

	Royal Palm Ballroom & Foyer	Grand Pavilion	Pegasus Ballroom	Royal Palm East 1-2-3
7:00	Credentials Desk Opens			
7:30	Continental Breakfast			
8:00		**General Session 1** Bedside to the Laboratory		
8:30				
9:00				
9:30				
10:00	Coffee Break *Provided by Pharmos*			
10:30	**Poster Session 1** & Visit Exhibits			
11:00				
11:30				
12:00			**Pharmacia Luncheon**	
12:30				
1:00		**General Session 2** Cell Death & Survival After CNS Injury		
1:30				
2:00				
2:30	Coffee Break			
3:00	**Poster Session 2** & Visit Exhibits			
3:30				
4:00				
4:30		**Breakout Session 1** Bedside to the Laboratory	**Breakout Session 2** Cell Death & Survival after CNS Injury	**Free Communications Session 1**
5:00				
5:30				
6:00				
6:30				
7:00 to 10:00	*Ticketed Event:* **An Evening in the Tropics Dinner**, Poolside			

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PROGRAM AT A GLANCE

TUESDAY, OCTOBER 29TH

	Royal Palm Ballroom & Foyer	Grand Pavilion	Pegasus Ballroom	Royal Palm East 1-2-3
7:00	Credentials Desk Opens			
7:30	Continental Breakfast *Provided by Aventis*			
8:00		**General Session 3** Guidelines and Management in CNS Injury		
8:30				
9:00				
9:30				
10:00	Coffee Break *Provided by Aventis*			
10:30	**Poster Session 3** & Visit Exhibits			
11:00				
11:30				
12:00			*Ticketed Event:* **Women in Neurotrauma Luncheon**	
12:30				
1:00		**General Session 4** Age & Gender Differences After CNS Injury		
1:30				
2:00				
2:30	Coffee Break *Provided by Aventis*			
3:00	**Poster Session 4** & Visit Exhibits			
3:30				
4:00				
4:30		**Breakout Session 3** Guidelines and Management in CNS Injury	**Breakout Session 4** Age & Gender Differences After CNS Injury	**Free Communications Session 2**
5:00				
5:30				
6:00 to 7:00	*Ticketed Event:* **Women in Neurotrauma Reception**, Little Club Patio			
7:00 to 12:00	*Ticketed Event:* **Ybor City / Columbia Dinner**			

NAME BADGES

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PROGRAM AT A GLANCE

WEDNESDAY, OCTOBER 30TH

	Royal Palm Ballroom & Foyer	Grand Pavilion	Pegasus Ballroom	Pegasus South
7:00	Credentials Desk Opens			
7:30	Continental Breakfast			
8:00		**General Session 5** Functional Recovery After CNS Injury		
8:30				
9:00				
9:30				
10:00	Coffee Break			
10:30		**Breakout Session 5** Functional Recovery After CNS Injury	**Free Communications Session 3**	
11:00				
11:30				
12:00	**Poster Session 5** & Visit Exhibits			*Ticketed Event:* **NNS Business Luncheon Meeting**
12:30				
1:00				
1:30 to 6:30	*Ticketed Event:* **Busch Gardens Excursion**			
6:30 to 10:30	*Ticketed Event:* **Florida Aquarium / Starship Dining Yacht**			

NAME BADGES

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In addition, please be sure to bring your ticket with you for admittance to ticketed events.

PROGRAM AT A GLANCE

THURSDAY, OCTOBER 31ST

	Royal Palm Ballroom & Foyer	Grand Pavilion	Pegasus Ballroom	Royal Palm East 1-2-3
7:00	Credentials Desk Open			
7:30	Continental Breakfast			
8:00		**General Session 6** Stem Cells & Neurotransplantation		
8:30				
9:00				
9:30				
10:00	Coffee Break			
10:30	**Poster Session 6** & Visit Exhibits			
11:00				
11:30				
12:00			*Optional Ticketed Lunch*	
12:30				
1:00		**General Session 7** Plasticity & Regeneration		
1:30				
2:00				
2:30	Coffee Break			
3:00		**Breakout Session 6** Stem Cells & Neurotransplantation	**Breakout Session 7** Plasticity & Regeneration	**Free Communications Session 4**
3:30				
4:00				
4:30		**Abstract Awards Presentation**		
5:00				
5:30				
6:00				
6:30				
7:00				
7:30 to 10:30		**Masquerade Gala Reception and Dinner** *(costumes optional)*		

NAME BADGES

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Please wear your name badge at all times.
In addition, please be sure to bring your ticket with you for admittance to ticketed events.

PROGRAM AT A GLANCE

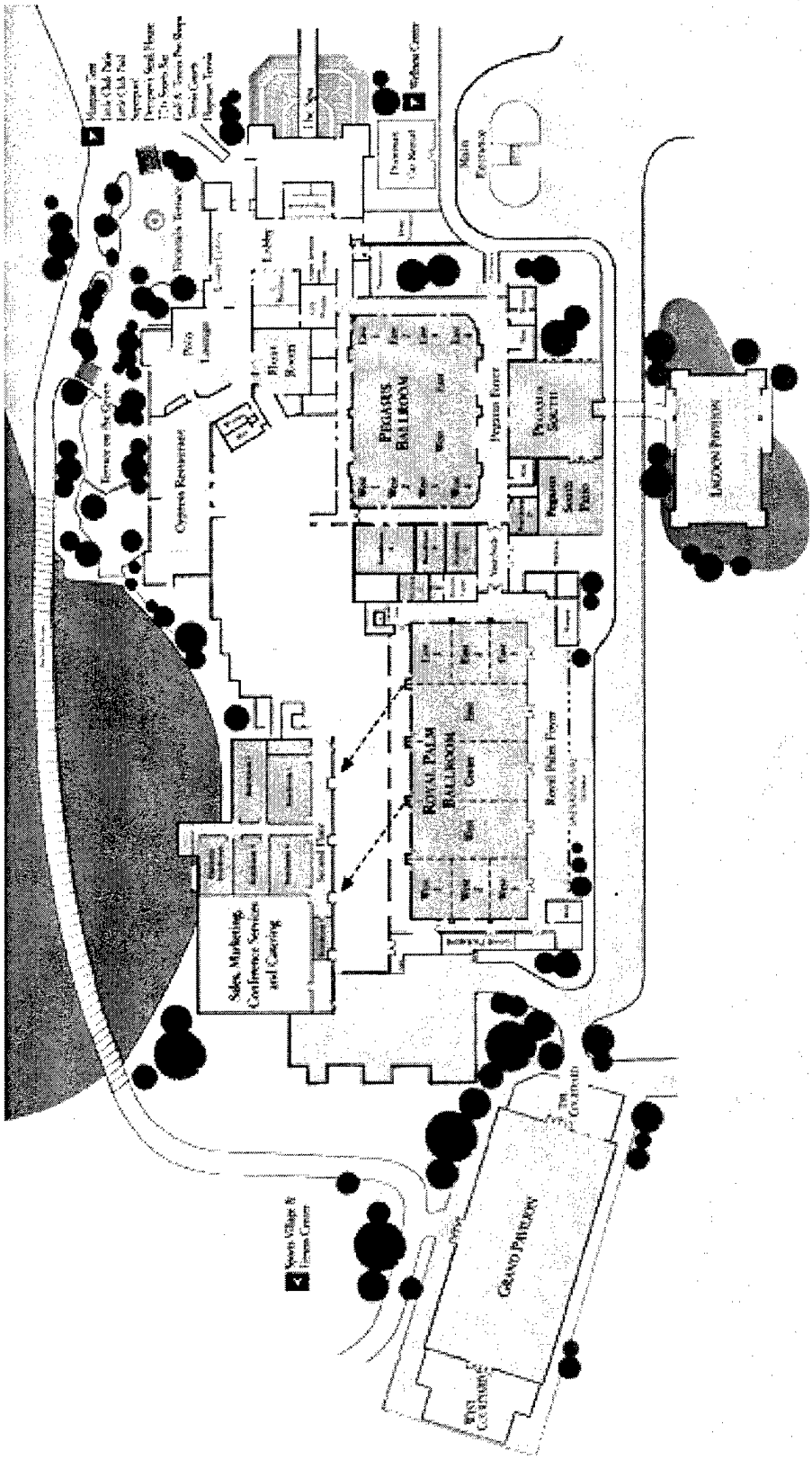
FRIDAY, NOVEMBER 1ST

	Royal Palm Ballroom & Foyer	Grand Pavilion	Pegasus Ballroom	Royal Palm East 1-2-3
7:00	Credentials Desk Opens			
7:30	Continental Breakfast			
8:00		**General Session 8** Inflammatory/Immune Response to CNS Injury		
8:30				
9:00				
9:30				
10:00	Coffee Break			
10:30		**General Session 9** Genomics & Proteomics: Where Do We Go From Here?		
11:00				
11:30				
12:00				
12:30				
1:00	*Ticketed Event:* **Bus to Orlando Departs**, Royal Palm Mall			

NAME BADGES

Name badges are required for access to all sessions, meals and evening events included with registration. Please wear your name badge at all times.
In addition, please be sure to bring your ticket with you for admittance to ticketed events.

SADDLEBROOK RESORT MAP



N-INTS 2002 SCIENTIFIC PROGRAM

SUNDAY, OCTOBER 27th *(Daylight Savings Time ends)*		LOCATION
All day	*Guest Check-In*	*Royal Palm Foyer*
12:00 PM	Credentials Claiming Opens	Royal Palm Foyer
2:00-5:00	INTS Executive Committee & Scientific Advisory Board Meeting	Executive Boardroom
3:00-5:00	NNS Officer Meeting	Boardroom Two
5:30-6:30	**OPENING CEREMONY** Dr. Thomas Genarelli, President, INTS Dr. Linda Noble, President, NNS *Program Chairs:* Doug Anderson, W. Dalton Dietrich and Linda Noble *Keynote Speakers:* Marilyn Anderson, Alexander Rabchevsky, Senator Rod Smith **PRESENTATION OF THE AMERITEC PRIZE FOR PARALYSIS RESEARCH** 2002 Award Recipient: Dr. Stephen Strittmatter, Yale University	Grand Pavilion
6:30	**WELCOME RECEPTION**	Poolside

MONDAY, OCTOBER 28th - DAY 1		LOCATION
7:00 AM	Credentials Claiming Opens	Royal Palm Foyer
7:30-8:00	Continental Breakfast	Royal Palm Ballroom
8:00-10:00	**GENERAL SESSION 1: BEDSIDE TO THE LABORATORY** *Co-Chairs: Russ Nockels, Alexander Rabchevsky* **TBI: History and Challenges for the Future** - Graham Teasdale **History and Challenges for the Future in SCI Research: Closing The Gap Between Basic Science and Clinical Practice** - Anders Holtz **Novel Approaches to the Clinical Investigation of CNS Injury** - Susan Horne	Grand Pavilion
10:00-10:15	Refreshment Break – *Provided by Pharmos Corporation*	Royal Palm Ballroom
10:15-11:45	**POSTER SESSION 1** (P133-P208) **& VISIT EXHIBITS**	Royal Palm Ballroom
11:45-1:00	**DELEGATE LUNCHEON** - *Provided by Pharmacia*	Pegasus Ballroom
1:00-2:45	**GENERAL SESSION 2: CELL DEATH AND SURVIVAL AFTER CNS INJURY** *Co-Chairs: Jackie Bresnahan, Robert Clark* **Overview of Cell Death Mechanisms After CNS Injury** - Tracy McIntosh **Mitochondrial and Nuclear DNA Damage** - Ella Englander **Molecular Pathways to CNS Neuronal Apoptosis Involving Nitrosative and Oxidative Stress** - Stuart Lipton	Grand Pavilion
2:45:3:00	Refreshment Break – *Provided by Pharmos Corporation*	Royal Palm Ballroom
3:00-4:30	**POSTER SESSION 2** (P209-P284) **& VISIT EXHIBITS**	Royal Palm Ballroom

MONDAY, OCTOBER 28th - DAY 1

		LOCATION
4:30-6:00	**BREAKOUT SESSION 1: BEDSIDE TO THE LABORATORY** *Co-Chairs: John Povlishock, Ed Wirth* **Chronic Complications of SCI** - Paul Muizelaar **Mild Traumatic Brain Injury** - David Hovda **Conference Report of TBI Clinical Trials** - Mary Ellen Michel	Grand Pavilion
4:30-6:00	**BREAKOUT SESSION 2: CELL DEATH AND SURVIVAL AFTER CNS INJURY** *Co-Chairs: Ed Hall, Kathy Saatman* **DNA Damage & Repair** – Jun Chen **Oxidative Stress** - Joe Beckman **Mitochondrial Dysfunction** - Pak Chan	Pegasus Ballroom
4:30-6:00	**FREE COMMUNICATIONS SESSION 1** *Co-Chairs: Ed Dixon, Lynne Weaver*	Royal Palm East (1-2-3)
4:30-4:45	**P107. Local Treatment With Phosphocreatine Improves Injury-Induced Metabolic And Electrophysiological Changes After TBI** *Oscar L. Alves, Thomas M. Reeves, Ross Bullock*	
4:45-5:00	**P108. Heme Oxygenase-2 Prevents Lipid Peroxidation-Mediated Cell Loss And Promotes Functional Recovery After Traumatic Brain Injury** *EF Chang*, T Igarashi, RJ Wong, HJ Vreman, DK Stevenson, LJ Noble.*	
5:00-5:15	**P113. Activated EGFR Signaling And Transplanted Neural Stem Cell Motility** *John A. Boockvar, Joost Schouten, Saori Shimuzo, Rachel C. Hoover, Donald M. O'Rourke, Tracy K. McIntosh.*	
5:15-5:30	**P123. Quantitative Diffusion Weighted Imaging Analyis Of Cell-Permeant Calcium Buffer Induced Neuroprotection After Cortical Devascularization In Rats** *Brenda Bartnik, Igor Spigelman and André Obenaus*	
5:30-5:45	**P128. Olfactory Ensheathing Cells Promote Robust Axon Growth Following Compressive Spinal Cord Injury** *Boyd, JG, Lee, J, Skihar, V, Doucette R, and Kawaja, MD.*	
5:45-6:00	**P318. Age Related Effects Of Acute Nmda Blockade On Functional Outcome After Controlled Cortical Impact In Immature Rats** *PD Adelson*, CE Dixon, DS Davis, DJ Santone, AS Gordon, LW Jenkins, PM Kochanek.*	

TUESDAY, OCTOBER 29th - DAY 2

		LOCATION
7:00 AM	Credentials Claiming Opens	Royal Palm Foyer
7:30-8:00	Continental Breakfast *Provided by an unrestricted grant from Aventis Pharmaceuticals*	Royal Palm Ballroom
8:00-10:00	**GENERAL SESSION 3: GUIDELINES IN MANAGEMENT OF CNS INJURY** *Co-Chairs: Tom Genarelli, Paul Vespa* **Management Controversies in Traumatic Injury** - Ross Bullock **Critical Care in TBI** - Elisabeth Ronne-Engstrom **Critical Care in SCI** - Michael Fehlings	Grand Pavilion
10:00-10:15	Refreshment Break *Provided by an unrestricted grant from Aventis Pharmaceuticals*	Royal Palm Ballroom
10:15-11:45	**POSTER SESSION 3** (P285-P360) **& VISIT EXHIBITS**	Royal Palm Ballroom

TUESDAY, OCTOBER 29th - DAY 2

		LOCATION
11:45-1:00	**SPECIAL WOMEN IN NEUROTRAUMA LUNCHEON** *(Optional Ticketed Lunch)* **Challenges We Have Met –** Elaine Aparecida Del Bel, Lisa McKerracher, Lisa Schnell, Esther Shohami	Pegasus Ballroom
1:00-2:45	**GENERAL SESSION 4: AGE AND GENDER DIFFERENCES AFTER CNS INJURY-CLINICAL CONSIDERATIONS** *Co-Chairs: Helen Bramlett, Ann-Christine Duhaime* **Gender** - Patricia Hurn **Pediatric** - Donna Ferreiro **Age and Outcome** - Andrew Maas	Grand Pavilion
2:45:3:00	Refreshment Break *Provided by an unrestricted grant from Aventis Pharmaceuticals*	Royal Palm Ballroom
3:00-4:30	**POSTER SESSION 4** (P361-P436) **& VISIT EXHIBITS**	Royal Palm Ballroom
4:30-6:00	**BREAKOUT SESSION 3: GUIDELINES IN MANAGEMENT OF CNS INJURY** *Co-Chairs: David Adelson, David Graham* **Management of Acute Brain Injury: New Directions -** Geoff Manley **Clinical Care in Severe TBI -** Claudia Robertson **Population Based Study on Risk Factors and Quality of Management in TBI** - Alex Baethmann	Grand Pavilion
4:30-6:00	**BREAKOUT SESSION 4: AGE & GENDER DIFFERENCES AFTER CNS INJURY** *Co-Chairs: Sean Grady, Stuart Hoffman* **Metabolic changes in the Developing Brain after TBI** - Mayumi Prins **Gender and TBI** - Robin Roof **Oxidative Injury and Gender Differences After TBI**- Takuji Igarashi	Pegasus Ballroom
4:30-6:00	**FREE COMMUNICATIONS SESSION 2** *Co-Chairs: Nariyuki Hayashi, Mary Eaton*	Royal Palm East (1-2-3)
4:30-4:45	**P105. Effects Of Injury Severity On Regional And Temporal Caspase-12 mRNA and Protein Expression Levels After Traumatic Brain Injury In Rats** *S.F. Larner*, B.R. Pike, D. McKinsey, R.L. Hayes*	
4:45-5:00	**P115. Voluntary Exercise Therapy After TBI: A Critical Window Of Opportunity** *G.S. Griesbach*, R. Molteni, F. Gomez-Pinilla & D.A. Hovda*	
5:00-5:15	**P116. UP-Regulation Of The Cell Cycle/ Inhibitor Of Apoptosis Protein Survivin In Astrocytes And Neurons After TBI In Rats.** *E.A. Johnson*. B.R. Pike. P. Tolentino. R.L. Hayes & J. Pineda*	
5:15-5:30	**P120. Co-Accumulation Of Amyloid-Beta, Beta-Secretase, And Presenilin-1 In Cultured Axons Following Stretch Injury** *A. Iwata*, X.H. Chen, B.J. Pfister, D.F. Meaney, and D.H. Smith*	
5:30-5:45	**P126. Rapid Functional Recovery After Thoracic Spinal Cord Injury In Young Rats** *K.M. Brown*. B.B. Wolfe. and J.R. Wrathall*	
5:45-6:00	**P313. Treatment Of Cold Injury-Induced Brain Edema With A Nonspecific Matrix Metalloproteinase Inhibitor MMI270 In Rats** *Nobuyuki Kawai*, Masanobu Okauchi, Seigo Nagao*	

WEDNESDAY, OCTOBER 30th - DAY 3

		LOCATION
7:00 AM	Credentials Claiming Opens	Royal Palm Foyer
7:30-8:00	Continental Breakfast	Royal Palm Ballroom
8:00-10:00	**GENERAL SESSION 5: FUNCTIONAL RECOVERY AFTER CNS INJURY** *Co-Chairs: Michele Basso, Kyoung-Suok Cho* **Outcome Measures and Human TBI -** Jennie Ponsford **Recovery After Traumatic Brain Injury: Animal Studies** - Tim Schallert **Recovery After Spinal Cord Injury: Human & Animal Studies -** Susan Harkema	Grand Pavilion
10:00-10:15	Refreshment Break	Royal Palm Ballroom
10:15-11:45	**BREAKOUT SESSION 5: FUNCTIONAL RECOVERY AFTER CNS INJURY** *Co-Chairs: Angelika Mautes, Roi Ann Wallis* **Bladder Function After SCI** - Jean Wrathall **Brain Injury-Induced Epileptogenesis: Animal Studies** - Doug Coulter **Inflammation After TBI** - Cristina Morganti-Kossmann	Grand Pavilion
10:15-11:45	**FREE COMMUNICATIONS SESSION 3** *Co-Chairs: Peter Blumbergs, Linda Phillips*	Pegasus Ballroom
10:15-10:30	**P104. Quantitative Analysis of Neurofilament Compaction and Axonal Transport Following Diffuse Traumatic Brain Injury** *C. R. Marmarou,*S.A Walker, J.R. Stone, E. Suehiro, Y. Ueda, R.H. Singleton and J. T. Povlishock*	
10:30-10:45	**P110. Temporal and Spatial Profile of Phosphorylated Mitogen-Activated Protein Kinase Pathways Following Lateral Fluid Percussion Brain Injury in Rats** *Naoki Otani*, Hiroshi Nawashiro, Katsuji Shima*	
10:45-11:00	**P114. Inhibition of NOGO-A Improves Recovery of Neuromotor and Cognitive Function Following Experimental Traumatic Brain Injury in Rats** *PM Lenzlinger*, FM Bareyre, M Motta, S Shimizu, A Luginbuhl, RC Hoover, H Thompson, A Clause, KE Saatman, ME Schwab, and TK McIntosh.*	
11:00-11:15	**P122. Relationship Of 40kD, 10kD, AND 3kD Fluorescent Indicators Of Altered Axolemmal Permeability To Impaired Axoplasmic Transport In Traumatic Axonal Injury.** *J.R. Stone*, D.G. Rubin, A.O. Dialo, D.O. Okonkwo, G.A. Helm.*	
11:15-11:30	**P127. Beneficial Effect of an Early Anti-Inflammatory Strategy After Acute Spinal Cord Injury: Comparison to the Efficacy of Methylprednisolone** *D. Gris*, G.A. Dekaban and L.C. Weaver.*	
11:30-11:45	**P519. Caspase Inhibition Attenuates Mitochondrial Release of Cytochrome C and Apoptosis-Inducing Factor After Traumatic Brain Injury in Rats** *Paula D. Nathaniel*, Xiaopeng Zhang, Patrick M. Kochanek, C. Edward Dixon, Robert S. B. Clark.*	
11:45-1:15	**POSTER SESSION 5** (P437-P512) **& VISIT EXHIBITS**	Royal Palm Ballroom
11:45-1:15	**NATIONAL NEUROTRAUMA SOCIETY BUSINESS MEETING** *(All NNS Members are invited to attend. Ticketed Lunch optional)*	Pegasus South
1:15 pm	*Free Afternoon*	

THURSDAY, OCTOBER 31st - DAY 4

		LOCATION
7:00 AM	Credentials Claiming Opens	
7:30-8:00	Continental Breakfast	Royal Palm Ballroom
8:00-10:00	**GENERAL SESSION 6: STEM CELLS AND NEUROTRANSPLANTATION** *Co-Chairs: Katsuji Shima, Bjoern Sheffler* **History of Transplantation** - Paul Reier **Endogenous Stem Cells as a Transplant Source** - Alain Privat **Adult Stem Cells, In Vitro and In Vivo** - Arlene Chiu	Grand Pavilion
10:00-10:15	Refreshment Break	Royal Palm Ballroom
10:15-11:45	**POSTER SESSION 6** (P513-P587) **& VISIT EXHIBITS**	Royal Palm Ballroom
11:45-1:00	Lunch *(on your own)* *- OR -* Lunch at Saddlebrook *(Ticketed Event)*	 Pegasus West
1:00-2:45	**GENERAL SESSION 7: PLASTICITY AND REGENERATION** *Co-Chairs: Mary Bunge, Dana McTigue* **CNS Plasticity -** Thomas Woolsey **Factors that Influence Axonal Outgrowth** - Lisa Schnell **Spinal Transplants: What are the Limitations on Repair?**- Marion Murray	Grand Pavilion
2:45:3:00	Refreshment Break	Royal Palm Ballroom
3:00-4:30	**BREAKOUT SESSION 6: STEM CELLS AND NEUROTRANSPLANTATION** *Co-Chairs: Bruce Lyeth, Dena Howland* **Fate of Transplanted Stem Cells -** Scott Whittemore **Regulation of Neuronal Differentiation in Multipotent Human Neural Stem Cells** - Angelo Vescovi **Olfactory Ensheathing Glia and CNS Injury** - Osamu Honmou	Grand Pavilion
3:00-4:30	**BREAKOUT SESSION 7: PLASTICITY AND REGENERATION** *Co-Chairs: Harry Goshgarian, Hans Kierstead* **Genetic Models** - Phil Horner **Functional and Dysfunctional Plasticity After CNS Neurotrauma** - Claire Hulsebosch **Use of Transplantation Approaches to Enhance Regeneration** - Martin Oudega	Pegasus Ballroom
3:00-4:30	**FREE COMMUNICATIONS SESSION 4** *Co-Chairs: Peter Reilly, Natalie Compagnone*	Royal Palm West (1,2,3)
3:00-3:15	**P106. 5.6-Epoxyeicosatrienoic Acid - Mediated Ca2+ Signaling Is Enhanced In Microglia Activated By Exposure To Soluble Factors From Traumatically Injured Astrocytes.** *S. Merchant*, S. Forde, R. Fry, B. Balleste, C. Liang, J.R. Falck, B. A. Rzigalinski.*	
3:15-3:30	**P125. Differential Gene Expression Profiling In The Embryonic And Adult-Injured Spinal Cords** *Paul Gris* and Arthur Brown.*	

Continued on next page...

THURSDAY, OCTOBER 31st - DAY 4

		LOCATION
3:00-4:30	**FREE COMMUNICATIONS SESSION 4** *(continued)*	Royal Palm West 1-2-3
3:30-3:45	**P131. NOGO-66 Receptor Antagonist Peptide Promotes Axonal Regeneration and Functional Recovery After Spinal Cord Injury** *Shuxin Li*, Tadzia GrandPré and Stephen M. Strittmatter.*	
3:45-4:00	**P333. Mild Fluid Percussion Injury Lowers The Threshold To Kainic Acid-Induced Seizures Which In Turn Elicit Recurrent Increases In Glutamate And Energy Demand In Vulnerable Tissue** *P Vespa, E Roncati Zanier, E Shieh, D Hovda.*	
4:00-4:15	**P375. The Potential Role Of The Chemokines MCP-1 AND IL-8 As Well As ICAM-1 In Traumatic Brain Injury** *Mario Rancan*, Thomas Kossmann, Maria Cristina Morganti-Kossmann.*	
4:15-4:30	**P545. Injury-Induced Changes In NMDA Receptor Subunit Composition Contribute To Prolonged Calcium-45 Accumulation In Intact Cortex** *C.L. Osteen*, C.C. Giza, and D.A. Hovda.*	
4:40-6:00	**ABSTRACT AWARDS PRESENTATION** Top Abstract Student Poster Competition Awards Women in Neurotrauma Award Outstanding Young Investigator (ICCP) Awards	Grand Pavilion
7:30-10:30	**MASQUERADE GALA RECEPTION AND DINNER** *(Costumes optional)*	Grand Pavilion

FRIDAY, NOVEMBER 1ST- DAY 5

		LOCATION
7:00 AM	Credentials Claiming Opens	Royal Palm Foyer
7:30-8:00	Continental Breakfast	Royal Palm Ballroom
8:00-10:00	**GENERAL SESSION 8: THE INFLAMMATORY/IMMUNE RESPONSE TO CNS INJURY: THERAPEUTIC OPPORTUNITIES** *Co-Chairs: John Bethea, Patrick Kochanek* **Inflammatory Responses to CNS Injury** - Phillip Popovich **Protective Autoimmunity in CNS Injury** - Michal Schwartz **Cytokines: Pro and/or Anti-Inflammatory Mediators** - Esther Shohami	Grand Pavilion
10:00-10:15	Refreshment Break	Royal Palm Ballroom
10:15-12:15	**GENERAL SESSION 9: GENOMICS & PROTEOMICS-WHERE DO WE GO FROM HERE?** *Co-Chairs: Alan Faden, Ramesh Raghupathi* **The Use of Multiple Proteomic Approaches in the Study of TBI** - Larry Jenkins **Applications of Proteomics to the Study of Traumatic Brain Injury: Opportunities and Challenges** - Ron Hayes **Genomics: Taking The First Step. Spinal Cord Injury** - Ron Hart	Grand Pavilion
	FINAL SUMMARY & ADJOURN	

P101. REGIONAL HYPERGLYCOLYSIS IS CHARACTERIZED BY DECREASED GLUCOSE TRANSPORT AND PRESERVED HEXOKINASE ACTIVITY FOLLOWING TRAUMATIC HEAD INJURY.

N.Hattori[1]. SC.Huang[1]. HM.Wu[1]. WH Liao[1]. TC Glenn[2]. PM.Vespa[2]. M.Phelps[1]. DA. Hovda[1]. [2]. M.Bergsneider[2]. ([1]Dept. of Molecular and Medical Pharmacology. [2]UCLA Brain Injury Research Center. David Geffen School of Medicine at UCLA. Los Angeles. CA).*

P102. TOPICAL L-ARGININE, BUT NOT NITRIC OXIDE DONOR, RESTORES CEREBROVASCULAR PRESSURE AUTOREGULATION FOLLOWING TRAUMATIC BRAIN INJURY IN RATS: POSSIBLE ROLE OF ENDOTHELIAL NITRIC OXIDE SYNTHASE.

Candace V. Campos, MD, Fangyi Zhang, MD, Minnette G. Son, MD, Farrokh R. Farrokhi, MD, Shane M. Sprague, Georgina Saravia, Dennis G. Vollmer, MD. (UTHSCSA-Neurosurgery, San Antonio, TX US).*

P103. TUMOR NECROSIS FACTOR RECEPTOR FAMILY MEMBERS MEDIATE POSTTRAUMATIC CELL DEATH AFTER CONTROLLED CORTICAL IMPACT IN MICE

Michael J. Whalen, Jianhua Qiu, Deirdra McCarthy, and Michael A. Moskowitz. (Massachusetts General Hospital, Boston, MA US).*

P104. QUANTITATIVE ANALYSIS OF NEUROFILAMENT COMPACTION AND AXONAL TRANSPORT FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY

*C. R. Marmarou, * S.A Walker, J.R. Stone, E. Suehiro, Y. Ueda, R.H. Singleton and J. T. Povlishock. (Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia US).*

P105. EFFECTS OF INJURY SEVERITY ON REGIONAL AND TEMPORAL CASPASE-12 mRNA AND PROTEIN EXPRESSION LEVELS AFTER TRAUMATIC BRAIN INJURY IN RATS

S.F. Lamer, B.R. Pike, D. McKinsey, R.L. Hayes. (McKnight Brain Institute of University of Florida, Gainesville FL, US).*

P106. 5.6-EPOXYEICOSATRIENOIC ACID - MEDIATED Ca2+ SIGNALING IS ENHANCED IN MICROGLIA ACTIVATED BY EXPOSURE TO SOLUBLE FACTORS FROM TRAUMATICALLY INJURED ASTROCYTES.

S. Merchant[1] S. Forde[1]. R. Fry[1]. B. Ballester[1]. C. Liang[1]. J.R. Falck[2]. and B. A. Rzigalinski[1]. ([1]University of Central Florida, Gainesville FL; [2]University of Texas. Southwestem Medical Center, Dallas. TX USA).*

P107. LOCAL TREATMENT WITH PHOSPHOCREATINE IMPROVES INJURY-INDUCED METABOLIC AND ELECTROPHYSIOLOGICAL CHANGES AFTER TBI.

Oscar L. Alves, Thomas M. Reeves, Ross Bullock (Medical College of Virginia, Virginia Commonwealth University, Richmond, VA USA).

P108. HEME OXYGENASE-2 PREVENTS LIPID PEROXIDATION-MEDIATED CELL LOSS AND PROMOTES FUNCTIONAL RECOVERY AFTER TRAUMATIC BRAIN INJURY

EF Chang, T Igarashi, RJ Wong, HJ Vreman, DK Stevenson, LJ Noble. (University of California San Francisco, San Francisco, California US).*

P109. GAS CHROMATOGRAPHY AND MASS SPECTROMETRY ASSESSMENT OF F2–ISOPROSTANE LEVELS IN CSF AFTER TRAUMATIC BRAIN INJURY IN RATS

Andrea Gabrielli. Brian R. Pike. Ronald L. Hayes. A. Joseph Layon. Ahamed H. Idris (Center for Traumatic Brain Injury Studies, EF & WL McKnight Brain Institute of the University of Florida. Gainesville, FL. USA).*

P110. TEMPORAL AND SPATIAL PROFILE OF PHOSPHORYLATED MITOGEN-ACTIVATED PROTEIN KINASE PATHWAYS FOLLOWING LATERAL FLUID PERCUSSION BRAIN INJURY IN RATS

Naoki Otani, Hiroshi Nawashiro, Katsuji Shima. (National Defense Medical College, Tokorozawa, Saitama JP).

P111. TRANSPLANTATION OF NGF-EXPRESSING NT2N NEURONS ATTENUATES A LEARNING DEFICIT FOLLOWING CONTROLLED CORTICAL IMPACT BRAIN INJURY IN MICE

Deborah J. Watson[1]. Luca Longhi[2,3]+. Scott Fujimoto[2,4]. Adam Lungibuhl[2]. Carl T Fulp[2]. Nicolas Royo[2]. Chen Zhang[2]. Kathryn E. Saatman[2]. John H. Wolfe[1].Tracy K. McIntosh[2,4]. ([1]Neurology, Children's Hospital of Philadelphia, PA. [2]Departments of Neurosurgery, University of Pennsylvania. [3]Anesthesia and Critical Care Medicine, Ospedale Maggiore Policlinico, IRCCS. Milan, Italy. [4]Veteran Administration Medical Center, University of Pennsylvania.).*

P112. NEURAL PROGENITOR CELL TRANSPLANTS SHOW LONG-TERM SURVIVAL AND ENHANCE BEHAVIORAL RECOVERY IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY

Deborah A. Shear[1], Matthew C. Tate[2], David R. Archer[3], Stuart W. Hoffman[5,6], Verne D. Hulce[4], Michelle C. LaPlaca[2], and Donald G. Stein [1,5,6]. ([1]Dept of Psychology. [5] Neurology. [6]Emergency Medicine. [3] Pediatrics. Emory University; [2]Dept. of Biomedical Engineering. Georgia Tech/Emory. Atlanta. GA. [4]Field Neurosciences Institute. Saginaw. MI).*

P113. ACTIVATED EGFR SIGNALING AND TRANSPLANTED NEURAL STEM CELL MOTILITY

John A. Boockvar, M.D., Joost Schouten, M.D., Saori Shimuzo, M.D., PhD., Rachel C. Hoover, B.S., Donald M. O'Rourke, M.D., Tracy K. McIntosh, M.D. (University of Pennsylvania, Philadelphia, PA US).

P114. INHIBITION OF NOGO-A IMPROVES RECOVERY OF NEUROMOTOR AND COGNITIVE FUNCTION FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY IN RATS
PM Lenzlinger[*1,2], FM Bareyre[3], M Motta[1], S Shimizu[1], A Luginbuhl[1], RC Hoover[1], H Thompson[1], A Clause[1], KE Saatman[1], ME Schwab[3], and TK McIntosh[1]. ([1]Dept. of Neurosurgery, University of Pennsylvania, Philadelphia PA, USA; [2]Div. of Trauma Surgery, University Hospital, Zurich, Switzerland. [3]Brain Research Institute, University and Swiss Federal Institute of Technology, Zurich, Switzerland).*

P115. VOLUNTARY EXERCISE THERAPY AFTER TBI: A CRITICAL WINDOW OF OPPORTUNITY.
G.S. Griesbach, R. Molteni, F. Gomez-Pinilla & D.A. (Hovda Brain Injury Res. Ctr. UCLA, Los Angeles, CA US).*

P116. UP-REGULATION OF THE CELL CYCLE/ INHIBITOR OF APOPTOSIS PROTEIN SURVIVIN IN ASTROCYTES AND NEURONS AFTER TBI IN RATS.
E.A. Johnson[1]. B.R. Pike. P. Tolentino[3]. R.L. Hayes & J. Pineda[1,2].(Center for Traumatic Brain Injury Studies, U of FL McKnight Brain Institute. Gainesville. FL USA [1]Dept. of Neuroscience. [2]Division of Pediatric Critical Care Medicine. [3]Dept. of Neurosurgery).*

P117. A SUBPOPULATION OF MITOTICALLY-ACTIVE CELLS MIGRATE ECTOPICALLY FROM THE ANTERIOR SUBVENTRICULAR ZONE FOLLOWING EXPERIMENTAL BRAIN INJURY
C.T. Fulp[1], S. Shimizu[1], J.E. Davis[1], T.K McIntosh[1,2]. ([1]The Head Injury Center and Dept. of Neurosurgery, Univ. of Penn. School of Medicine. [2]Veteran's Administration Medical Center. Philadelphia. PA).*

P118. GENDER DIFFERENCES IN COGNITIVE RECOVERY AFTER INTERVENTION WITH ENVIRONMENTAL ENRICHMENT FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY
**Sokoloski J[1], Kline AE[1], Zafonte RD[1], Dixon CE[2], Wagner AK[1] ([1]Dept. Physical Medicine and Rehabilitation, [2]Dept. Neurological Surgery, University of Pittsburg, Pittsburgh, PA US).*

P119. EXTRACELLULAR SIGNAL-RELATED KINASE/MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION IS CRITICAL FOR ASTROCYTE PROCESS EXTENSION AND MIGRATION IN THE SETTING OF BRAIN INJURY
W. Shawn Carbonell and James W. Mandell. (University of Virginia, Charlottesville, VA US).*

P120. CO-ACCUMULATION OF AMYLOID-BETA, BETA-SECRETASE, AND PRESENILIN-1 IN CULTURED AXONS FOLLOWING STRETCH INJURY
A. Iwata[1], X.H. Chen, B.J. Pfister, D.F. Meaney, and D.H. Smith. (Univ.of Pennsylvania, Philadelphia, PA.

P121. IDENTIFICATION OF MULTIPLE DISTINCT PATHOLOGIC NEURONAL PHENOTYPES WITHIN DIFFUSELY INJURED BRAIN
Richard H. Singleton and John T. Povlishock. (Medical College of Virginia/VCU, Richmond, VA US).*

P122. RELATIONSHIP OF 40kD, 10kD, AND 3kD FLUORESCENT INDICATORS OF ALTERED AXOLEMMAL PERMEABILITY TO IMPAIRED AXOPLASMIC TRANSPORT IN TRAUMATIC AXONAL INJURY.
J.R. Stone, D.G. Rubin, A.O. Dialo, D.O. Okonkwo, G.A. Helm. (University of Virginia, Charlottesville, VA US).*

P123. QUANTITATIVE DIFFUSION WEIGHTED IMAGING ANALYIS OF CELL-PERMEANT CALCIUM BUFFER INDUCED NEUROPROTECTION AFTER CORTICAL DEVASCULARIZATION IN RATS
Brenda Bartnik [1,2], Igor Spigelman[3] and André Obenaus[1,2]. ([1] Department of Anatomy & Cell Biology. University of Saskatchewan. Saskatoon. SK. Canada; [2]Department of Radiation Medicine. Loma Linda University. Loma Linda. CA. USA; [3]Division of Oral Biology & Medicine. UCLA School of Dentistry. Los Angeles. CA. USA).

P124. SPINAL CORD OLIGODENDROGLIA EXPRESS ACTIVATED CASPASE-3 FOLLOWING K+ INDUCED DEPOLARIZATION AND NMDA EXPOSURE
S.A. Nottingham and J.E. Springer. (Anatomy and Neurobiology, Spinal Cord and Brain Injury Research Center, University of Kentucky Medical Center. Lexington. KY USA).*

P125. DIFFERENTIAL GENE EXPRESSION PROFILING IN THE EMBRYONIC AND ADULT-INJURED SPINAL CORDS
Paul Gris and Arthur Brown. (The John P. Robarts Research Institute, University of Western Ontario, London, Ontario CA).*

P126. RAPID FUNCTIONAL RECOVERY AFTER THORACIC SPINAL CORD INJURY IN YOUNG RATS
K.M. Brown. B.B. Wolfe. and J.R. Wrathall. (Georgetown University. Washington. DC. USA).*

P127. BENEFICIAL EFFECT OF AN EARLY ANTI-INFLAMMATORY STRATEGY AFTER ACUTE SPINAL CORD INJURY: COMPARISON TO THE EFFICACY OF METHYLPREDNISOLONE
D. Gris, G.A. Dekaban and L.C. Weaver. (Spinal Cord Injury Laboratory. BioTherapeutics Research Group. Robarts Research Institute. London. Ontario. Canada).*

P128. OLFACTORY ENSHEATHING CELLS PROMOTE ROBUST AXON GROWTH FOLLOWING COMPRESSIVE SPINAL CORD INJURY
Boyd, JG, Lee, J, Skihar, V, Doucette R, and Kawaja, MD. (Queen's University, Kingston, ON CA).

P129. CYCLIC AMP INDUCES FUNCTIONAL REGENERATION-ASSOCIATED GENES AND REPRESSES GAP-43
Jason B. Carmel[1], Marie A. Handler[2], Zixuan Cao[2], Wilfredo Mellado[2], Patricia Soteropoulos[3], Peter Tolias[3], Wise Young[1], Marie T. Filbin[2], Ronald P. Hart[1]. ([1]W. M. Keck Center for Collaborative Neuroscience, Rutgers University, Piscataway, NJ; [2]Department of Biological Sciences, Hunter College, CUNY, New York, NY; [3]Center for Applied Genomics, PHRI, Newark, NJ USA).*

P130. NOVEL SYNTHETIC GRAFTS THAT ARE BIOCOMPATIBLE AND PROMOTE AXONAL REGENERATION AFTER SPINAL CORD INJURY
Eve C. Tsai, Paul D. Dalton, Molly S. Shoichet, Charles H. Tator. (University of Toronto, Toronto, Ontario CA).*

P131. NOGO-66 RECEPTOR ANTAGONIST PEPTIDE PROMOTES AXONAL REGENERATION AND FUNCTIONAL RECOVERY AFTER SPINAL CORD
Shuxin Li, Tadzia GrandPré and Stephen M. Strittmatter. (Yale University, New Haven, CT US).*

P132. TRANSPLANTATION OF RODENT SKIN-DERIVED PRECURSOR CELLS ONTO RAT HIPPOCAMPAL SLICE CULTURE
Nao R. Kobayashi. Karl J.L. Fernandes, Amelie Rioux-Tache and Freda Miller. (Montreal Neurological Institute, McGill University, Montreal, QC. Canada).*

P133. PROGNOSTIC VALUE OF SPECT IN PATIENTS WITH POSTTRAUMATIC TRANSTENTORIAL HERNIATION
Martin Smrčka, M.D., Karel Máca, M.D., Vilém Juráň, M.D., Milan Vidlák, M.D., Vladimír Smrčka, M.D., Jiří Prášek, M.D., Roman Gál, M.D.. (Neurosurgery, University Hospital Brno, Brno, CZ).

P134. LEFT-RIGHT ASYMMETRY OF THE ESTIMATION OF CEREBRAL PERFUSION PRESSURE USING TRANSCRANIAL DOPPLER ULTRASONOGRAPHY IN HEAD INJURY: A PRELIMINARY REPORT.
Schmidt EA[1], Czosnyka M[1], Matta BF[2], Balestreri M[1], Piechnik SK[1], Steiner LA[1,2], Pickard JD[1], ([1]Academic Neurosurgery, [2]Department of Anaesthesiology Addenbrooke's Hospital, Cambridge, UK).*

P135. PERFUSION WEIGHTED MAGNETIC RESONANCE IMAGING (MRI) IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY.
Paul Mullins, Xao Di, Allan Faden. (University of New Mexico, Albuquerque, NM US).*

P136. EFFECTS OF EARLY AND LATE INFUSION OF NOREPINEPHRINE ON CEREBRAL BLOOD FLOW, BRAIN TISSUE OXYGENATION, AND BRAIN EDEMA FORMATION IN BRAIN-INJURED RATS
Stefan-Nikolaus Kroppenstedt, Ulrich-Wilhelm Thomale, Martin Griebenow, Oliver W. Sakowitz, Petra Mayr, John F. Stover, Andreas W. Unterberg. (Dept. of Neurosurgery, Berlin, Berlin DE).*

P137. EFFECTS OF HYPERHAES ON POSTTRAUMATIC CEREBRAL PERFUSION AND EDEMA FORMATION AFTER CONTROLLED CORTICAL IMPACT INJURY IN RATS.
Ulrich-Wilhelm Thomale, Martin Griebenow, Stefan-Nikolaus Kroppenstedt, John F. Stover, Andreas W. Unterberg. (Dept. Neurosurgery, Berlin, Berlin DE).*

P138. MAPPING FLOW-METABOLISM AND EVOLVING AXONAL INJURY AFTER EXPERIMENTAL BRAIN TRAUMA
Szu-Fu Chen, Hugh K. Richards, Piotr Smielewski, Peter Johnström, John D. Pickard, Neil G. Harris. ([1]Academic Neurosurgery Centre for Brain Repair. [2]Wolfson Brain Imaging Centre. & [3]Clinical Pharmacology. University of Cambridge. UK).*

P139. INTERPRETATION OF CEREBRAL LACTATE REDUCTION IN SEVERE HEAD INJURY. A MICRODIALYSIS STUDY.
**S Magnoni[1]. V Valeriani[1]. E Roncati Zanier[1]. S Rossi[1]. A Protti[1]. F Prada[2]. N Stocchetti[1]. ([1]Department of Anesthesia and Intensive Care and [2]Departement of Neurosurgery, Ospedale Maggiore Policlinico IRCCS, Milano).*

P140. AMYLOID BETA 1-42 AND TAU IN CEREBROSPINAL FLUID AFTER SEVERE HUMAN TRAUMATIC BRAIN INJURY
G. Franz. R. Beer. A. Kampfl. K. Engelhardt. E. Schmutzhard. H. Ulmer . and F. Deisenhammer. (Departments of Neurology and Biostatistics, University Hospital Innsbruck, Austria).*

P141. TEMPORAL AND SPATIAL PROFILE OF BID CLEAVAGE AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY
K. Engelhardt. R. Beer. G. Franz. S. Krajewski. J.C. Reed. B.R. Pike. R.L. Hayes. K.K. Wang. E. Schmutzhard. and A. Kampfl. (Department of Neurology, University Hospital Innsbruck, Austria; The Burnham Institute, La Jolla. California; McKnight Brain Institute of the University of Florida, Gainesville, Florida; Department of Neuroscience Therapeutics,. Pfizer Inc., Ann Arbor, Michigan. U.S.A).*

P142. TEMPORAL AND SPATIAL PROFILE OF CASPASE-6 EXPRESSION AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY
R. Beer[1]. G. Franz[1]. K. Engelhardt[1]. S. Krajewski[2]. J.C. Reed[2]. A. Buki[3]. T. Doczi[3]. N. Lettner[1]. E. Schmutzhard[1]. and A. Kampfl[1]. ([1]Department of Neurology, University Hospital Innsbruck, Austria; [2]The Burnham Institute. La Jolla. California. U.S.A.; [3]Department of Neurosurgery, Pecs University, Hungary).*

P143. MULTIDIMENSIONAL IMPAIRMENTS OF ATTENTION FOLLOWING PEDIATRIC TRAUMATIC BRAIN INJURY
Shelley C. Heaton[1,2], Danielle A. Becker[2], Eileen B. Fennell[1,2], Olivia Puyana[2], David Gribbins[2]
([1]Center for Traumatic Brain Injury Studies, Evelyn F & William L McKnight Brain Institute of the University of Florida; [2]Dept of Clinical & Health Psychology, University of Florida, Gainesville, FL).

P144. TEMPORAL PROFILE OF alfa-II-SPECTRIN BREAKDOWN PRODUCTS AFTER TRAUMATIC BRAIN INJURY IN IMMATURE RATS.
*Jose.A. Pineda**[2]. Jada M. Aikman[1]. Erik A. Johnson[1]. Brian R. Pike[1]. Barbara E. Osteen[1]. Tao Fan[1] and Ronald L. Hayes[1]. ([1]Center for Traumatic Brain Injury Studies. Evelyn F & William L McKnight Brain Institute of the University of Florida. [2]Division of Pediatric Critical Care Medicine. University of Florida Dept. of Pediatrics).*

P145. ZINC CHELATION ALTERS THE MOLECULAR PROFILE OF STRESS SIGNALING PATHWAYS IN TRAUMATIC BRAIN INJURY
H.L Hellmich, C. Frederickson, D.S. DeWitt, R. Saban, M. Parsley, R. Stephenson, D.S. Prough. (University of Texas Medical Branch, Galveston, Texas US).*

P146. EFFECTS OF INJURY SEVERITY ON REGIONAL AND TEMPORAL mRNA EXPRESSION LEVELS OF CALPAINS AND CASPASES AFTER TRAUMATIC BRAIN INJURY IN RATS
B.R. Pike, N.C. Ringger, D.M. McKinsey, P.J. Tolentino, S.M. DeFord, J.G. Brabham, and R.L. Hayes. (University of Florida, Gainesville, FL US).

P147. THE PREDICTIVE VALUE OF PROCALCITONIN AND S 100 B IN TRAUMATIC BRAIN INJURY
Linda E. Pelinka, MD, Albert Kroepfl, MD, PhD and Heinz Redl, PhD. (Ludwig Boltzmann Institute of Experimental and Clinical Traumatology, A-1200 Vienna, Austria).*

P148. CONTEXTUAL FEAR CONDITIONING TO ASSESS COGNITIVE DYSFUNCTION IN BRAIN INJURED MICE
Patrick T. Williams, Brent M. Witgen, Krishna P. Reddy, Jonathan Lifshitz, Ted Abel, M. Sean Grady, and Akiva S. Cohen. (Children's Hospital of Philadelphia, Folcroft, PA US).*

P149. A 4-AXES MODEL OF THE STRESS RESPONSE
Iliadis Charalampos and Alexandra Kunz. (Harvard University, Boston, MA US).*

P150. INDUCTION OF HIGH PURITY OLIGODENDROCYTE CULTURES FROM HUMAN EMBRYONIC STEM CELLS
Gabriel I. Nistor, Minodora O. Totoiu and Hans S. Keirstead. (Reeve-Irvine Research Center, Placentia, CA USA).*

P151. QUANTIFICATION OF DIFFUSION TENSOR IMAGING PREDICTS DIFFUSE AXONAL INJURY FOLLOWING TRAUMATIC BRAIN INJURY IN RATS
E Ozarslan, SM DeFord, TH Mareci, R Hayes. (Evelyn F & William L McKnight Brain Institute, Center for Traumatic Brain Injury Studies, Depts. of Physics, Neuroscience & Biochemistry, University of Florida,Gainesville, FL US).*

P152. RESPONSE OF NEURONS CULTURED IN TWO- AND THREE-DIMENSIONS TO DYNAMIC SHEAR DEFORMATION
D. Kacy Cullen and Michelle C. LaPlaca. (Department of Biomedical Engineering, Georgia Tech. Atlanta, GA. USA).

P153. ADAPTATION OF SENSORIMOTOR AND COGNITIVE TASKS FOR USE WITH MICE: EFFECTS OF CONTROLLED CORTICAL IMPACT INJURY AT VARIED INSULT LOCATIONS
Yelena K. Baskin, Annmarie. J. Bramwell, W. Dalton Dietrich and Edward J. Green. (Departments of Psychology and Neurological Surgery, University of Miami, Miami, FL USA).*

P154. PROGESTERONE IMPROVES BEHAVIORAL AND MORPHOLOGIC OUTCOMES AFTER TRAUMATIC BRAIN INJURY IN MALE C57BL4/6 MICE
Douglas W. Lowery, Joshua E. Logan, Deborah A. Shear, Stuart W. Hoffman, Donald G. Stein. (Emory University, Atlanta, Georgia US).*

P155. THE NEUROPROTECTIVE EFFECTS OF PROGESTERONE ARE ASSOCIATED WITH MODIFIED GENE EXPRESSION IN RAT CORTICAL IMPACT MODEL
Edward H. Pettus, David W. Wright, Stuart W. Hoffman, Donald G. Stein.* (Emergency Medicine, *Emory University, Atlanta, GA US).*

P156. ANESTHESIA AFFECTS GENDER-RELATED FUNCTIONAL OUTCOME FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY IN RAT
*Christine O'Connor, Ibolja Cernak and *Robert Vink. (**Department of Pathology, University of Adelaide, Adelaide SA, Australia; and Department of Neuroscience, Georgetown University, Washington DC, USA.

P157. A PARALLEL RANDOMIZED DOUBLE-BLIND MULTICENTRE CLINICAL TRIAL FOR THE EFFICACY AND SAFETY OF NALOXONE IN ACUTE TRAUMATIC BRAIN INJURY
Yuanli Zhao MD[1], Jiyao Jiang MD[2], Li Li MD[1], et al. on behalf of the National Naloxone Study Group. ([1]Beijing Neurosurgical Institute. [2]Shanghai Neurosurgical Institute).

P158. DOWNREGULATION OF AMYLOID PRECURSOR PROTEIN (APP) mRNA EXPRESSION FOLLOWING POST-TRAUMATIC CYCLOSPORIN-A ADMINISTRATION
**James J. Donkin[1]. Corinna Van Den Heuvel[1]. John W. Finnie[4]. Peter C. Blumbergs[3]. Tim Kuchel[4]. Barbara Koszyca[1]. Jim Manavis[3]. Nigel R. Jones[1]. Peter L. Reilly[2] and Robert Vink[1,3] (Departments of [1]Pathology and [2]Neurosurgery. University of Adelaide. and [3]Department of Neuropathology and the [4]Veterinary Division, Institute of Medical and Veterinary Science, Adelaide, Australia).*

P159. THE NEUROPROTECTIVE EFFECTS OF PROGESTERONE AND ALLOPREGNANOLONE AFTER CONTROLLED CORTICAL IMPACT IN RATS
Myriam J. Djebaili, Stuart W. Hoffman, Donald G. Stein. (Emory University, Atlanta, Georgia US).*

P160. PREGNENOLONE FACILITATES RECOVERY FOLLOWING TRAUMATIC BRAIN INJURY
Melissa A. Arellano, Robert M. Simkins, IV, Stuart W. Hoffman, Donald G. Stein. (Emory University, Atlanta, Georgia US).*

P161. STEROIDS IN SEVERE TBI: A META-ANALYSIS
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P198. POST-INJURY TREATMENT WITH Mn (III) TETRAKIS (4-BENZOIC ACID) PORPHYRIN IMPROVES FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD INJURY.
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P218. CSF ACCUMULATION OF CALPAIN-SPECIFIC aII-SPECTRIN BREAKDOWN PRODUCTS ARE ASSOCIATED WITH INJURY MAGNITUDE AND LESION VOLUME AFTER TRAUMATIC BRAIN INJURY IN RATS
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P220. RECOVERY OF SPEECH-SOUND PRODUCTION SKILLS IN YOUNG CHILDREN AFTER SEVERE TRAUMATIC BRAIN INJURY
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P221. EXPERIENCE-DEPENDENT LOSS OF PLASTICITY IS RESTORED AFTER DELAYED EXPOSURE TO AN ENRICHED ENVIRONMENT.
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P223. INFLUENCE OF APOE GENOTYPE ON SECONDARY INSULTS AFTER TRAUMATIC BRAIN INJURY
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P224. HIGH-DENSITY HIGH-THROUGHPUT TISSUE MICROARRAY PROFILING OF MOLECULAR ALTERATIONS IN EXPERIMENTAL TRAUMATIC BRAIN INJURY
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P225. GENE EXPRESSION FOLLOWING HUMAN TRAUMATIC BRAIN INJURY BY MICROARRAY ASSAY
**D. Michael, M. Byers and L.N. Irwin. (Depts. of Neurosurgery and Anatomy & Cell Biol., Wayne State Univ. Sch. of Medicine, Detroit, MI; and Dept. Biol. Sci., University of Texas at El Paso, El Paso, TX).*

P226. THE EXPERIMENTAL STUDY ON EXPRESSION AND ACTIVATION OF CASPASE 3 AFTER ACUTE BRAIN TRAUMA
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P227. CXC CHEMOKINES MAY CONTRIBUTE TO INFLAMMATION IN SUBARACHNOID HEMORRHAGE AND ITS CONSEQUENCES
Norihito Shirakawa, Takeharu Tonai. (National Zentsuji Hospital, Zentsuji, Kagawa JP).*

P228. SYSTEMIC ANTI-INFLAMMATORY REACTION AFTER BRAIN INJURY
Ch. Woiciechowsky, N. Daberkow, S. Rupprecht, H.D. Volk. (Department of Neurosurgery, Berlin, DE).

P229. TRAUMATIC BRAIN INJURY (TBI)-INDUCED SPASTICITY: MONOAMINE CHANGES AND POSSIBLE MECHANISMS.
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P230. SIMPLE MORPHOMETRY OF AXONAL SWELLINGS CANNOT BE USED IN ISOLATION FOR DATING LESIONS AFTER TRAUMATIC AXONAL INJURY
Gentleman SM[1], Leclercq PD[1], Stephenson MS[1], Murray LS[2], McIntosh TK[3], Graham DI[2]. ([1]Division of Neuroscience, Faculty of Medicine, Imperial College, London; [2]University of Glasgow, Glasgow, UK; and [3]Neurosurgery, University of Pennsylvania, Philadelphia. USA).

P231. COGNITIVE IMPAIRMENT WITH MENTAL FATIGUE DURING RECOVERY FROM NEUROTRAUMA. CELLULAR MECHANISMS FOCUSING ON ASTROGLIAL DYSFUNCTION IN GLUTAMATERGIC NEUROTRANSMISSION
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P232. DIFFERENTIAL PEPTIDE DISPLAY AND ANALYSIS IN CSF AND PLASMA FOLLWING TRAUMATIC BRAIN INJURY.
M.U. Schuhmann[1], G. Heine[2], M. Skardelly[1], H. Tammen[2], A. Appel[2], T. Brinker[3]. ([1]Hannover Medical School and [3]Nordstadt Hospital; [2]BioVisioN AG, Hannover, Germany).*

P233. TRAUMATIC BRAIN INJURY INDUCED SPASTICITY: NATURE, MAGNITUDE, AND TIME-COURSE
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P234. ESTROGEN REGULATION OF XIAP PROCESSING FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT
HM Bramlett[1], OF Alonso[1], G Lotocki[2], WD Dietrich[1], RW Keane[2]. (Neurotrauma Research Center, The Miami Project to Cure Paralysis, Depts. of Neurosurgery[1] and Physiology and Biophysics[2], University of Miami School of Medicine, Miami, FL USA).*

P235. NEURON-GLIA COMMUNICATION: METALLOTHIONEIN EXPRESSION IS RAPIDLY INCREASED BY ASTROCYTES IN RESPONSE TO NEURONAL INJURY
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P236. THE EFFECT OF CYCLOSPORIN A UPON MITOCHONDRIAL DYSFUNCTION AND ENERGETIC METABOLISM FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY
Stefano Signoretti[1]. Jana Dunbar[1], Angela M. Amorini[1], Barbara Tavazzi[3], Giuseppe Lazzarino[2], Roberto Vagnozzi[4], and Anthony Marmarou[1]. ([1]Virginia Commonwealth University, Richmond, Virginia; (2) University of Catania, Italy; [3]Department of Experimental Medicine and Biochemical Sciences and [4]Laboratory of Experimental Neurosurgery, University of Rome "Tor Vergata", Italy).*

P237. GENE DELIVERY OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) PRIOR TO TRAUMATIC BRAIN INJURY: DIFFERENTIAL EFFECTS ON ANATOMY AND BEHAVIOR
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P238. EXAMINATION OF THE ROLE OF N- AND P/Q-TYPE VOLTAGE SENSITIVE CALCIUM CHANNEL BLOCKERS IN TRAUMATIC BRAIN INJURY PRODUCED BY LATERAL FLUID PERCUSSION
LL. Lee, BD. Wiederhold, M. Zwienenberg-Lee, JP. Muizelaar, BG. Lyeth, RF. Berman. (University of California at Davis, Davis, CA US).*

P239. ASSESSING THE GLOBAL BURDEN OF PATHOLOGY IN HEAD INJURY USING 2-D PQ HISTOGRAMS AND DIFFUSION TENSOR IMAGING
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P240. REDUCTION IN THE FORMATION OF CEREBRAL EDEMA FOLLOWING FLUID PERCUSSION INJURY FROM FREE RADICAL SCAVANGER AND NSAID COMBINATION THERAPY
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*Morimichi Koshinaga *, Nobuhiro Moro, Masamichi Fukushima, Takeshi Suma, Yoichi Katayama. (Department of Neurological Surgery, Nihon University School of Medicine, Itabashi-ku, Tokyo JP).*

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P259. HEALTH-RELATED QUALITY OF LIFE AND POSTCONCUSSIONAL DISORDER SIX MONTHS FOLLOWING MILD TO MODERATE TRAUMATIC BRAIN INJURY
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P260. SCULL BASE MISSILE INJURIES
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P268. EARLY ANTI-INLFLAMMATORY TREATMENT ATTENUATES NEUROFILAMENT DEGRADATION AFTER SPINAL CORD INJURY.
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P269. EARLY SELECTIVE ANTI-INFLAMMATORY TREATMENT BLOCKS THE DEVELOPMENT OF CHRONIC PAIN AFTER SPINAL CORD INJURY.
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P270. EVALUATION OF CONDITIONS FOR CALPAIN INHIBITON IN THE RAT SPINAL CORD: EFFECTIVE POSTINJURY INHIBITION REQUIRES INTRASPINAL MICROINJECTION
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P271. TRANSPLANTATION OF NEUROTROPHIN-EXPRESSING FIBROBLASTS INTO CHRONIC CONTUSION CAVITIES
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P272. DELAYED GRAFTING OF FETAL SPINAL CORD TISSUE ENHANCES EARLY GRAFT SURVIVAL AND DEVELOPMENT IN THE INJURED ADULT RAT SPINAL CORD.
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P273. NMDA RECEPTOR ACTIVATION AS A BASIS FOR INCREASED VULNERABILITY: OLIGODENDROCYTES IN CONTUSED SPINAL CORD
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P274. INHERENT LOCOMOTOR DIFFERENCES IN MOUSE STRAINS IMPACT RECOVERY AFTER SPINAL CORD INJURY
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P275. FORMATION OF COLLAGENOUS SCAR IS A COMMON FEATURE FOLLOWING TRAUMATIC AND ISCHEMIC INJURIES TO THE CENTRAL NERVOUS SYSTEM
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P276. EFFECTS OF SEROTONERGIC DEPLETION IN BULBOSPINAL FIBERS ON LOCOMOTOR AND PUDENDAL REFLEXES IN INTACT AND CHRONIC SPINALLY CONTUSED RATS.
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P277. BILATERAL HYPEREXCITABILITY OF LUMBAR DORSAL HORN NEURONS FOLLOWING UNILATERAL THORACIC HEMISECTION-BASIS FOR "PHANTOM" NEUROPATHIC PAIN
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P279. VENTRAL FUNICULUS LESIONS OF THE SPINAL CORD PRODUCE LASTING SENSORY BUT NOT MOTOR DEFICITS
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P280. CELL PROLIFERATION AND SURVIVAL CHRONICALLY AFTER SPINAL CORD INJURY
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P281. HYDROGEN PEROXIDE ELEVATED BY SPINAL CORD INJURY INDUCES AND A METALLOPORPHYRIN ATTENUATES OXIDATIVE DAMAGE
D. Liu[2], F. Bao[1] ([1]Depts. of Neurology and [2]Human Biological Chemistry & Genetics, Univ. of Texas Medical Branch, Galveston, TX US).*

P282. ASSESSMENT OF POSSIBLE STRAIN-DEPENDENT DIFFERENCES IN MICE FOLLOWING SPINAL CORD INJURY
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P283. IS THERE AN ACQUIRED CHANNELOPATHY CONTRIBUTING TO AXONAL CONDUCTION DEFICITS FOLLOWING SPINAL CORD INJURY?
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P284. WHIPLASH INJURY OF THE NECK – CLINICAL SYNDROME OR MALINGERISM?
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P285. HETEROGENEITY OF REGIONAL CEREBRAL BLOOD FLOW FOLLOWING SEVERE HEAD INJURY
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P286. COMPARISON OF SINGLE VOXEL AND MULTIVOXEL MR SPECTROSCOPY IN PREDICTING 3 AND 6 MONTH NEUROLOGIC OUTCOME AFTER TRAUMATIC BRAIN INJURY
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P287. SIGNIFICANCE OF A REDUCED CEREBRAL BLOOD FLOW WITHIN 12 HOURS AFTER SEVERE HEAD INJURY.
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P288. SERIAL QUANTITATIVE PROTON SPECTROSCOPIC FINDINGS IN SEVERELY BRAIN INJURED PATIENTS CORRELATE WITH OUTCOMES
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P290. IMPROVED MRI DETECTION OF HEMORRHAGIC SHEARING INJURIES IN ADULTS USING SUSCEPTIBILITY WEIGHTED IMAGING (SWI): CORRELATION WITH SEVERITY AND OUTCOME.
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P291. CEREBRAL HEMODYNAMICS AFTER CORTICAL IMPACT INJURY IN THE eNOS KNOCKOUT MOUSE
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P292. BEHAVIORAL DEFICITS FOLLOWING LATERAL FLUID PERCUSSION INJURY IN THE RAT PUP DUE TO CELLULAR DYSFUNCTION AND NOT CELL DEATH.
G.G. Gurkoff, C.C. Giza and D.A. Hovda. (Interdepartmental Program in Neuroscience and Division Of Neurosurgery, UCLA School of Medicine, Los Angeles, CA USA).*

P293. CELLULAR LOCALIZATION AND ALTERATIONS OF INHIBITORS OF APOPTOSIS AFTER TRAUMATIC BRAIN INJURY.
George Lotocki[1], Ofelia Alonso[2], Beata Frydel[2], Jessie Truettner[2], W. Dalton Dietrich[2] and Robert W. Keane[1] (Departments of [1]Physiology and Biophysics and University of Miami School of Medicine, [2]Miami Project to Cure Paralysis, Miami, FL 33136. (University of Miami, Miami, FL US).

P294. THE EXPERIMENTAL OBSERVATION ON DELAY NEURONAL DEATH AFTER ACUTE BRAIN TRAUMA
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P295. AGE-DEPENDENT RESPONSE TO SCALED CORTICAL IMPACT IN THE PIGLET
Ann-Christine Duhaime, Loretta Grate, Jill Hunter, Jeff Golden, Susan Margulies. (Children's Hospital at Dartmouth, Lebanon, NH USA).*

P296. DIFFERENCES IN ICP-AND CARDIOVASCULAR RESPONSE IN DEVELOPING VERSUS ADULT RATS FOLOWING DIFFUSE TRAUMATIC BRAIN INJURY
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P297. MICROARRAY ANALYSIS OF CELL TRAFFICKING GENES AFTER TRAUMATIC BRAIN INJURY: THE EFFECTS OF HYPOTHERMIA AND HYPERTHERMIA
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P298. EXPRESSION OF P2 PURINERGIC RECEPTORS IN RAT CORTEX AFTER MODERATE TRAUMATIC BRIAN INJURY.
M.D. Tran[1], W.D. Dietrich[1,2], H.M. Bramlett[2], O.F. Alonso[2] and J.T. Neary[1,3]. ([1]Neuroscience Program, [2]The Miami Project to Cure Paralysis, Dept. Neurological Surgery, and [3]Research Service, VA Med. Ctr., Depts. Pathol. and Biochem. & Molec. Biol., Univ. Miami Sch. Medicine, Miami, FL USA).*

P299. THE INFLAMMATORY RESPONSE AFTER TRAUMATIC BRIAN INJURY IN MALE AND FEMALE RATS AS ASSESSED BY cDNA ARRAYS
JS Truettner, OF Alonso, WD Dietrich, HM Bramlett (Department of Neurological Surgery, the Neurotrauma Research Center, and Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL USA).*

P300. INTERLEUKIN-16 RELEASE FROM CD8-POSITIVE T LYMPHOCYTES FOLLOWING TRAUMATIC BRAIN INJURY.
Richard P. Shimonkevitz. Ph.D. and David Bar-Or. M.D. (Trauma Research Laboratory, Swedish Medical Center HealthONE, Englewood, CO, USA).*

P301. S100 BETA PROTEIN RESPONSE IN ASTROCYTES AFTER HUMAN BRAIN CONTUSION
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P302. HYPOTHERMIA REDUCES THE ACTIVITY OF NF-kB AFTER PARASAGITTAL FLUID-PERCUSSION BRAIN INJURY.
Chatzipanteli K.[1], Bethea J.[1], Alonso O.F.[1], & Dietrich. W.D.[1,2]. (The Miami Project to Cure Paralysis, Departments of Cell Biology and Anatomy[1] and Neurological Surgery[2], Univ. of Miami School of Medicine, Miami, FL USA).

P303. TRAUMATIC FRONTAL LOBE INJURY IN RATS CHRONICALLY AFFECTS T-MAZE ALTERNATION: EFFECTS OF CLYCLOSPORIN A.
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P304. LONGITUDINAL ANALYSIS OF THE DICHOTOMIZED GLASGOW OUTCOME SCALE SCORE
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P305. ASSESSMENT OF TRAUMATIC AXONAL INJURY IN THE CORPUS CALLOSUM: A COMPARISON OF THE CORONAL AND SAGITTAL PLANES.
Leclercq PD[1], Dani K[1], Graham DI[2], Gentleman SM[1], ([1]Division of Neuroscience, Imperial College London and [2]University Of Glasgow, UK).*

P306. LONGITUDINAL ANALYSIS OF THE DISABILITY RATING SCORE FOLLOWING TRAUMATIC BRAIN INJURY
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P307. GENDER SPECIFIC ACTIVITY AND FOOT-FAULT PERFORMANCE ON THE GRID TASK AFTER CASTRATIONS AND OVARECTOMIES
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P308. CEREBRAL AMYLOID ANGIOPATHY AND TRAUMATIC BRAIN INJURY: THE EFFECT OF APOLIPOPROTEIN E GENOTYPE
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P309. TETRAHYDROBIOPTERIN AND L-ARGININE AFTER CORTICAL IMPACT INJURY IN RATS
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P310. A META-ANALYSIS TO DETERMINE THE SIGNIFICANCE OF SKULL FRACTURE AS A RISK FACTOR FOR INTRACRANIAL PATHOLOGY IN THE PAEDIATRIC POPULATION
Joel Desmond, John Batchelor. (Manchester Royal Infirmary, Manchester, Manchester UK).*

P311. EFFECTS OF DIETARY CREATINE ON NEUROCHEMICAL MAKERS OF SECONDARY INJURY FOLLOWING TRAUMATIC BRAIN INJURY
H.S. Dhillon, L.M.Benjamin, T.J. Carbary, S.W. Scheff. (Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY USA).*

P312. THE PITFALL OF BRAIN HYPOTHERMIA MANAGEMENT IN NEUROTRAUMA PATIENTS
N. Hayashi. (Nihon University School of Medicine, Itabashi-Ku, JP).

P313. TREATMENT OF COLD INJURY-INDUCED BRAIN EDEMA WITH A NONSPECIFIC MATRIX METALLOPROTEINASE INHIBITOR MMI270 IN RATS
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P314. BONE MARROW STROMAL CELL TRANSPLANTED TO TRAUMATICALLY INJURED RODENT BRAINS MAY AID IN THEIR RECOVERY THROUGH PRODUCTION OF NERVE GROWTH FACTOR
**Yan Long[1], Xiaoqin Yuan[1], Linglong Zou[1], Holly Lu1, and Keyi Yang[1,3,4]. ([1]Neurosurgery, Center for Cell and Gene Therapy,. [2]Neurology,.Baylor College of Medicine; [3]PADRECC, Neurology Care line, VA Medical Center, Houston, TX USA).*

P315. THE SPECIAL CONSIDERATION OF MANAGEMENT OF VEGETATION IN BRAIN HYPOTHERMIA TREATMENT
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P316. EFFECTS OF HYPOTHERMIA AND ALKALIZING AGENTS ON BRAIN INJURIES IN RATS WITH ACUTE SUBDURAL HEMATOMAS
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P317. DIETARY CREATINE SUPPLEMENTATION ENHANCES COGNITIVE RECOVERY FOLLOWING EXPERIMENTAL RAT BRAIN INJURY
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P318. AGE RELATED EFFECTS OF ACUTE NMDA BLOCKADE ON FUNCTIONAL OUTCOME AFTER CONTROLLED CORTICAL IMPACT IN IMMATURE RATS
PD Adelson, CE Dixon, DS Davis, DJ Santone, AS Gordon, LW Jenkins, PM Kochanek. (University of Pittsburgh, Pittsburgh, PA US).*

P319. ENDOTHELIN-1 CONTRIBUTES TO AGE DEPENDENT G PROTEIN IMPAIRMENT AFTER BRAIN INJURY
William M. Armstead (University of Pennsylvania, Philadelphia, PA US).

P320. INCIDENCE AND PROGRESSION OF INTERCELLULAR CA2+ WAVES IN ASTROCYTES SURROUNDING AREAS OF MECHANICAL INJURY
WJ Miller, AE Grosvenor, DF Meaney. (Department of Bioengineering, University of Pennsylvania, Philadelphia, PA USA).*

P321. THE EFECTS OF VITAMIN B3 (NICOTINAMIDE) ON BEHAVIORAL OUTCOME FOLLOWING BILATERAL FRONTAL CORTEX CONTUSION INJURY IN THE RAT.
*M.R. Hoane * and S.L. Akstulewicz. (Brain Injury Laboratory, Department of Psychology and Program in Neuroscience, East Carolina University, Greenville, NC USA).*

P322. THE EFFECT OF AGE ON SENSORIMOTOR AND COGNITIVE REC0VERY FOLLOWING BILATERAL FRONTAL CORTEX CONTUSION INJURY IN THE RAT
L.A. Lasley, S.L. Akstulewicz, and M.R. Hoane. (Brain Injury Laboratory, Department of Psychology and Program in Neuroscience, East Carolina University, Greenville, NC USA).*

P323. SECONDARY CEREBRAL ISCHEMIA-INDUCED CA1 HIPPOCAMPAL CELL DEATH: LATERAL VS CENTRAL FLUID PERCUSSION INJURY IN RAT.
N. Aoyama, S.M. Lee, D.A. Hovda. (Brain Injury Research Center, Departments of Surgery/Neurosurgery and Molecular and Medical Pharmacology, UCLA Medical Center, Los Angeles, CA US).*

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Roumen Balabanov MD, Paula Dore-Duffy, Ph.D. (Wayne State University, Bloomfield Hills, Michigan US).*

P336. ACID-SENSING ION CHANNELS IN ACIDOSIS-INDUCED NEURONAL INJURY
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P337. OVEREXPRESSION OF RAT HEAT SHOCK PROTEIN 70 REDUCES NEURONAL INJURY AFTER TRANSIENT FOCAL ISCHEMIA, TRANSIENT GLOBAL ISCHEMIA, AND KAINIC ACID-INDUCED SEIZURE
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P339. ACCUMULATION OF CALPAIN AND CASPASE-3 CLEAVED aII-SPECTRIN BREAKDOWN PRODUCTS IN CSF AFTER MIDDLE CEREBRAL ARTERY OCCLUSION IN RATS
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P340. TISSUE-TYPE TRANSGLUTAMINASE EXPRESSION FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION
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Masami Nishio, Takamichi Yuguchi, Chihiro Akiyama, Toshiyuki Fujinaka, Yoshikazu Nakajima, Masaaki Taniguchi, Eiji Kohmura, Toshiki Yoshimine. (Department of Neurosurgery, Osaka University Medical School, Suita, Osaka JP).*

P342. DIFFERENTIAL EFFECTS OF HYPERBARIC OXYGENATION ON TISSUE NECROSIS AND ATP CONTENT FOLLOWING FOCAL CEREBRAL ISCHEMIA
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P343. SELECTIVE HIPPOCAMPAL CA1 NEURONAL ACIDOPHILIA (RED CELL CHANGE) IN CASES OF SUDDEN DEATH FROM TRAUMA
*Barbara Koszyca, *Peter C Blumbergs. (University of Adelaide and Neuropathology Laboratory, Institute of Medical and Veterinary Science, Adelaide, AU).*

P344. DNA MICOROARRAY ANALYSES OF GENE EXPRESSION CHANGES UNDERLYING CHRONIC CENTRAL PAIN IN SPINAL CORD INJURY
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P345. DATA MINING IN SCIGENES, THE DATABASE OF SPINAL CORD INJURY-RELATED GENES
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P347. CHARACTERIZATION OF A RAT CERVICAL CONTUSION MODEL
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P348. IMPLANTATION OF SKIN-ACTIVATED BLOOD-BORNE MONOCYTES TO SPINALLY CONTUSED RATS: RECOVERY OF MOTOR ACTIVITY AND REDUCED CYST FORMATION.
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P349. THE SERUM AND CEREBROSPINAL FLUID ELASTASE ACTIVITY DURING TREATMENT OF SPINAL CORD INJURY BY PERFTORAN
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P350. DOES MILD INTRAOPERATIVE HYPOTHERMIA LEAD TO INCREASED COMPLICATIONS IN ELECTIVE SPINAL SURGERY?
*Elizabeth J. Owen *, Lisa Silbert, James D. Guest. (University of Miami, Miami, Florida US).*

P351. CHRONIC CENTRAL PAIN IS ATTENUATED BY EXOGENOUS LEUKEMIA INHIBITORY FACTOR (LIF) AFTER SPINAL CORD INJURY (SCI).
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P352. ENHANCED REGENERATION INTO SCHWANN CELL BRIDGES IMPLANTED INTO THE COMPLETELY TRANSECTED SPINAL CORD FOLLOWING CYCLIC AMP INJECTION OR SUPERFUSION INTO THE STUMPS IS NOT ACCOMPANIED BY IMPROVED BEHAVIORAL RESTITUTION.
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P353. TRANSPLANT-MEDIATED REMYELINATION AND LOCOMOTOR RECOVERY OF THE MHV MODEL OF MULTIPLE SCLEROSIS
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P354. THE NEURONAL-SPECIFIC RNA-BINDING PROTEIN HUD IS UPREGULATED AND COLOCALIZED WITH GAP-43 MRNA IN THE FACIAL NUCLEUS OF THE MOUSE DURING REGENERATION.
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P355. THE ROLE OF OSP/CLAUDIN-11 IN OLIGODENDROCYTE PROGENITOR CELL MIGRATION FOLLOWING DEMYELINATION OF THE ADULT SPINAL CORD
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P356. LOCMOTOR TRAINING IN A RODENT MODEL OF INCOMPLETE SPINAL CORD INJURY
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P357. GENETICALLY TARGETED ASTROCYTE SCAR ABLATION RESULTS IN LIMITED, LOCAL GROWTH OF CORTICOSPINAL TRACT AXONS AFTER SPINAL CORD INJURY.
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P358. HP184, A COMBINED SODIUM AND POTASSIUM CHANNEL BLOCKER, IMPROVES LOCOMOTOR SCORES 35 DAYS AFTER A MODERATE SPINAL CORD INJURY IN THE RAT.
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P359. PRO-CYSTEINE COMPOUND (OTC) DECREASES THE NUMBER OF ACTIVATED MACROPHAGES/MICROGLIA FOLLOWING SPINAL CORD INJURY
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P360. THE ADMINISTRATION OF VARIOUS DOSES OF L-2 OXOTHIAZOLIDE CARBONATE TO PROMOTE RECOVERY FROM NEUROTRAUMA
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P361. CEREBRAL METABOLIC AND BLOOD FLOW DIFFERENCES BETWEEN TRAUMATIC HEAD INJURED PATIENTS: INFLUENCE OF COCAINE
JL Benae, TC Glenn, P Vespa, F Song, DF Kelly, DA Hovda, NA Martin. (UCLA Neurosurgery, Los Angeles, CA US).*

P362. ACUTE METABOLIC DEVIATION FROM NORMAL PREDICTS LONG TERM OUTCOME AFTER TBI
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P363. EXTRACELLULAR CALCIUM FLUCTUATIONS AFFECT VASCULAR TONE IN ISOLATED RAT MIDDLE CEREBRAL ARTERIES
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P364. CYCLOSPORIN A DOES NOT AMELIORATE THE ANAEROBIC GLYCOLYSIS RESPONSE TO VIBRISSA MOTOR CORTEX STIMULATION FOLLOWING TRAUMATIC BRAIN INJURY
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P365. OXYGEN, GLUCOSE AND LACTATE METABOLISM AS PREDICTORS OF OUTCOME AFTER TRAUMATIC BRAIN INJURY
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P366. PERICONTUSIONAL TISSUE DISPLAYS VULNERABILITY TO REDUCTION IN CEREBRAL PERFUSION PRESSURE WITHOUT MICRODIALYSIS EVIDENCE OF ISCHEMIA
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P367. SIMULTANEOUS QUANTITATIVE MEASUREMENTS OF GLUCOSE METABOLISM, CEREBRAL BLOOD FLOW AND ADENOSINE TRIPHOSPHATE (ATP) LEVELS FOLLOWING TRAUMATIC BRAIN INJURY.
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P368. DO NEURONS UTILIZE ALTERNATIVE FUELS ACUTELY FOLLOWING HUMAN TRAUMATIC BRAIN INJURY?
HM Wu, SC Huang, N Hattori, T Glenn, WH Liao, CL Yu, DA Hovda, ME Phelps, M Bergsneider. (Department of Molecular and Medical Pharmacology, Division of Neurosurgery, Brain Injury Research Center, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA).*

P369. MICROARRAY GENE EXPRESSION ANALYSIS OF POSTNATAL DAY 19 RAT CORTEX AFTER LATERAL FLUID PERCUSSION INJURY
C.C. Giza, H.H. Li, M.L. Prins and D.A. Hovda. (Divisions of Neurosurgery, Pediatric Neurology and Department of Medical and Molecular Pharmacology, UCLA, Los Angeles, CA USA).*

P370. AGE-RELATED MORPHOLOGIC CHANGES FOLLOWING TRAUMATIC BRAIN INJURY
Stephen W Scheff, Lisa M Benjamin. (Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY US).

P371. IN VIVO APPLICATION OF INOS ANTISENSE OLIGONUCLEOTIDES EXACERBATES HYPOPERFUSION AND UPREGULATES ENDOTHELIN-1 EXPRESSION FOLLOWING TRAUMATIC BRAIN INJURY.
J. Steiner[*1]; J.A. Rafols[1]; H.K. Park[2]; M.S. Katar[1]; T. Petrov[1]. ([1]Anatomy and Cell Biology, [2]Neurological Surgery, Wayne State University, Detroit, MI, USA).*

P372. MICROARRAY ANALYSIS OF MICROGLIA ACTIVATED BY SOLUBLE FACTORS FROM TRAUMATICALLY INJURED ASTROCYTES.
B. Ballester[*1], H. Mitchell[1], S. Forde[1], D. Bailey[1], A. Smith[2], N. Van[2], F. W. Morgan[2], J. V. Pattisapu[2], and B.A. Rzigalinski[1]. ([1]University of Central Florida. Dept. of Molecular Biology & Microbiology; [2]Orlando Regional Health Research Institute, Wade's Center for Hydrocephalus, Orlando, FL US).*

P373. APOLIPOPROTEIN E EPSILON 4 IN PEDIATRIC TRAUMATIC BRAIN INJURY: PHASE I - DIFFICULTIES IN OBTAINING APPROVALS AND PATIENT ENROLLMENT
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P374. THE ROLE OF CEREBRAL INFLAMMATION AFTER TRAUMATIC BRAIN INJURY - A CONCEPT REVISITED
Maria Cristina Morganti-Kossmann, Mario Rancan, Thomas Kossmann. (The Alfred Hospital/Monash University, Prahran, Melbourne, Victoria AU).

P375. THE POTENTIAL ROLE OF THE CHEMOKINES MCP-1 AND IL-8 AS WELL AS ICAM-1 IN TRAUMATIC BRAIN INJURY
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P376. INTRACRANIAL PRESSSURE DYNAMICS: CHANGES OF BANDWIDTH AS AN INDICATOR OF CEREBROVASCULAR TENSION
**Michael L. Daley[1], Massour Pourcyrous[2], Shelly Timmons[3] and Charles W. Leffler[4] ([1]Department of Electrical and Computer Engineering, The University of Memphis; [2]Departments of Pediatrics, Obstetrics/Gyn. and Physiology; [3]Department of Neurosurgery; and [4]Departments of Physiology and Pediatrics, The University of Tennessee Health Science Center, Memphis, TN USA).*

P377. THE ABBREVIATED INJURY SCALE IS A NEGLECTED TOOL IN TRAUMATIC BRAIN INJURY RESEACH
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P378. CORTICAL COMPACTION INJURY IN TRANSGENIC MICE TO STUDY THE ROLES OF REACTIVE ASTROCYTES
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P379. THE EFFECT OF POST-INJURY IRRADIATION ON NEURAL STEM CELL PROLIFERATION AND RECOVERY OF FUNCTION
Ann C. Rice, H. Benjamin Harvey, Robert J. Hamm, M. Ross Bullock. (Virginia Commonwealth University, Mechanicsville, VA US).*

P380. EFFECTIVENESS OF SEATBELTS TO PREVENT HEAD INJURY IN LATERAL VERSUS FRONTAL MOTOR VEHICLE IMPACTS
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P381. REGIONAL AND TEMPORAL PROFILE OF MITOTICALLY ACTIVE CELLS THROUGHOUT THE TRAUMATIZED BRAIN FOLLOWING BRAIN INJURY
C Urrea, B Frydel, OF Alonso, RW Keane, P Tsoulfas, WD Dietrich. (Departments of Neurological Surgery, Physiology & Biophysics, Neurotrauma Research Center, The Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL USA).*

P382. THERAPEUTIC HYPOTHERMIA PRESERVES ANTIOXIDANT DEFENSES AFTER TRAUMATIC BRAIN INJURY IN INFANTS AND CHILDREN
H Bayir, PD Adelson, VE Kagan, KL Janesko, RSB Clark, PM Kochanek. (Safar Center for Resuscitation Research, Children's Hospital of Pittsburgh, and Dept. of Environmental and Occupational Health, Univ. of Pittsburgh, Pittsburgh, PA USA).*

P383. SHORT-TERM EFFICACY IN THE TREATMENT OF BRAIN TRAUMA MAY NOT TRANSLATE INTO LONG-TERM IMPROVEMENTS
Kevin D. Browne, Matthew J. Leoni, Xiao-Han Chen, and Douglas H. Smith (Department of Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA USA).*

P384. CALPAIN MEDIATED SPECTRIN BREAKDOWN PRODUCTS IN THE CEREBROSPINAL FLUID OF SEVERELY HEAD INJURED PATIENTS
A. Buki. (Dept. Neurosurgery, Medical Fact., University of Pecs, Pecs, HU).

P385. TOPIRAMATE ATTENUATES TRAUMATIC BRAIN INJURY-INDUCED NEUROMOTOR DEFICITS IN RATS
R.C. Hoover[1], M. Motta[1], J.R. Rodriquez[1], S. Fujimoto[1], J. Davis[1], M.V. Patel[1], K.M. Soltesz[1], L.A. Samper[1], R. Twyman[2], H.S. White[3], T.K. McIntosh[1]. ([1]Univ. of Pennsylvania, Philadelphia, PA; [2]J & J Pharmaceutical Research & Development, LLC., Raritan, N.J.; [3]Univ. of Utah, Salt Lake City, UT USA).*

P386. ADENOSINE 2a RECEPTOR KNOCKOUT MICE ARE NEUROPROTECTED AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY.
K.L. Janesko, E.K. Jackson, L.W. Jenkins, V.A. Vagni, P. Shore, J.F. Chen, C.E. Dixon, M.A. Schwarzchild, R.S.B. Clark, P.M. Kochanek. (Safar Center for Resuscitation Research, Univ. of Pittsburgh, Pittsburgh, PA and Mass. General Hospital, Boston, MA).*

P387. THE THERAPEUTIC EFFICACY OF THE 5-HT1A RECEPTOR AGONIST 8-OH-DPAT IN TRAUMATICALLY BRAIN-INJURED RATS IS NOT MEDIATED BY CONCOMITANT HYPOTHERMIA.
A.E. Kline, J.L. Massucci, R.D. Zafonte, and C.E. Dixon. (University of Pittsburgh, Pittsburgh, PA , US).*

P388. TEMPORAL AND REGIONAL ALTERATIONS IN ENDOGENOUS GDNF EXPRESSION AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY
S. Shimizu, N.C. Royo, K.E. Saatman, T.K. McIntosh. (University of Pennsylvania, Philadelphia, PA US).*

P389. DOWNREGULATION OF MATRIX METALLOPROTEINASE-9 AND ATTENUATION OF EDEMA VIA INHIBITION OF ERK MAP KINASE
Mori Tatsuro. (Nihon University School of Medicine, Tokyo, Japan, Meguro-ku, Tokyo JP).

P390. TREATMENT WITH THE IRREVERSIBLE, CELL PERMEABLE CASPASE-9 INHIBITOR III, PROVIDES PROTECTION AGAINST CA1 TRAUMATIC NEURONAL INJURY IN THE HIPPOCAMPAL SLICE
Roi Ann Wallis, Kimberly L. Panizzon. (VA GLAHS and UCLA, North Hills, CA US).*

P391. THE EFFECTS OF MEK INHIBITOR U0126 FOLLOWING TRAUMATIC BRAIN INJURY IN RATS
Naoki Otani, Hiroshi Nawashiro, Katsuji Shima. (Department of Neurosurgery, National Defense Medical College, Tokorozawa, Saitama JP).

P392. CHANGES IN DARPP-32 PROTEIN EXPRESSION FOLLOWING CONTROLLED CORTICAL IMPACT
Margaret S. Wilson, Youming Li, X. Ma and C. Edward Dixon. (Department of Neurosurgery, University of Pittsburgh, PA USA).*

P393. SYNAPTOSOMAL DOPAMINE UPTAKE IN RAT STRIATUM FOLLOWING CONTROLLED CORTICAL IMPACT
Margaret S. Wilson, X. Ma, Ian J. Reynolds and C. Edward Dixon. (Departments of Neurosurgery and Pharmacology, University of Pittsburgh, Pittsburgh, PA US).*

P394. MORPHOLOGICAL, DYNAMIC AND CYTOSKELETAL PROPERTIES UNDERLYING NEURITE DEVELOPMENT AND AXONAL SPROUTING FOLLOWING LOCALISED TRANSECTION OF CORTICAL AXONS IN VITRO
Jyoti A Chuckowree and James C Vickers (NeuroRepair Group, University of Tasmania, Hobart, Tasmania, AU).*

P395. CLINICAL BIOMECHANICS OF PENETRATING BRAIN TRAUMA
Cheryl A. Muszynski, Frank A. Pintar, Narayan Yoganandan, Thomas A. Genarelli. (Medical College of Wisconsin, Milwaukee, WI USA).*

P396. LOCALISATION OF ALPHA-SYNUCLEIN FOLLOWING AXONAL TRANSECTION: IMPLICATIONS FOR REGROWTH AND REPAIR
Marian C Quilty, Wei-Ping Gai[1]. Adrian K West, James C Vickers (University of Tasmania, Hobart, Tasmania; and [1]Flinders University, Adelaide, Australia).*

P397. REACTIVE AND REGENERATIVE NEURONAL CYTOSKELETAL ALTERATIONS FOLLOWING ACUTE LOCALIZED INJURY TO THE RAT NEOCORTEX.
James C. Vickers, Jyoti A. Chuckowree and Sandrine Chopin. (NeuroRepair Group, University of Tasmania, Hobart, Tasmania AU).*

P398. LONG-TERM ACCUMULATION OF AMYLOID-BETA, BETA-SECRETASE AND PRESENILIN-1, AND CASPASE-3 IN DAMAGED AXONS FOLLOWING BRAIN TRAUMA.
XH.Chen[1], R.Siman[2], A.Iwata[1], JQ.Trojanowski[4], DF.Meaney[3], DH.Smith[1]. (Depts. of [1]Neurosurgery, [2]Pharmacology, [3]Bioengineering and [4]Pathology and Laboratory Medicine, Univ. of Pennsylvania, Philadelphia, PA USA).*

P399. DELAYED DISRUPTION IN AXONAL TRANSPORT FOLLOWING LATERAL FLUID PERCUSSION BRAIN INJURY IN RATS
A.K. Clouse[1], M. Motta[1], R.C. Hoover[1], R. Siman[2], K.E. Saatman[1] (Depts. of [1]Neurosurgery and [2]Pharmacology, University of Pennsylvania, Philadelphia, Pennsylvania US).*

P400. INCREASED APPARENT DIFFUSION COEFFICIENT IN NORMAL APPEARING WHITE MATTER
Mr. Pablo Goetz MRCS(eng)[1,2], Dr. Andrew Blamire PhD[1], Dr. Bheeshma Rajagopalan DPhil[1], Mr. Tom Cadoux-Hudson DPhil, FRCS[1,2], Prof. Peter Styles DPhil[1]. ([1]MRC Biochemical and Clinical Magnetic Resonance Spectroscopy Unit, Department of Biochemistry, University of Oxford, UK; [2]Department of Neurosurgery, Radcliffe Infirmary, Oxford, UK).

P401. TEMPORAL VULNERABITY TO REPETITIVE EXPERIMENTAL BRAIN INJURY: LONG TERM SEQUELAE OF MULTIPLE CONCUSSIONS.
L. Longhi[1,3]. S. Fujimoto[1], J. Davis[1], A. McMillan[1], H.L. Laurer[1], K.E. Saatman[1], R. Raghupathi[1], N. Stocchetti[3] and T.K. McIntosh[1,2]. (Departments of [1]Neurosurgery, Univ. of Pennsylvania, [2]Veterans Administration Medical Center, Philadelphia, PA; and [3]Anesthesia and Critical Care Medicine, Ospedale Maggiore Policlinico, IRCCS, Milan, Italy).*

P402. NET CONTRACTILE FORCES OF THE ACTOMYOSIN NETWORK POWER THE DELAYED ELASTIC RESPONSE OF THE AXONAL CYTOSKELETON FOLLOWING STRETCH INJURY
JS Rangan, DF Meaney, DH Smith. (Department of Bioengineering, University of Pennsylvania, Philadelphia, PA US).*

P403. THE INCREASED INTRACRANIAL PRESSURE AS AN IMPOTRANT NEGATIVE INDICATOR OF SEVERE HEAD INJURY OUTCOME
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P409. REPEATED MILD INJURY CAUSES CUMULATIVE DAMAGE TO HIPPOCAMPAL CELLS
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P426. REGIONAL ENERGY METABOLISM FOLLOWING SHORT-TERM NEURAL STEM CELL TRANSPLANTATION INTO THE INJURED SPINAL CORD
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P427. VACCINATION THERAPY IN RAT SPINAL CORD INJURY
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P428. EFFECTS OF INOSINE ON RAT SPINAL CORD INJURY.
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P429. DEPLETION OF NORADRENERGIC FIBERS ATTENUATES HINDLIMB LOCOMOTOR RECOVERY FOLLOWING THORACIC CONTUSION INJURY
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P430. TRANSPLANTATION OF OLFACTORY ENSHEATHING GLIA CELLS GENETICALLY MODIFIED TO SECRETE THE NEUROTROPHINS BDNF AND NT-3 MEDIATES ENHANCED RECOVERY OF HIND LIMB FUNCTION IN RUBROSPINAL TRACT LESIONED RATS
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P431. PROLONGED SPINAL CORD EDEMA IN ACUTE CERVICAL CORD INJURY
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P432. HP184 IMPROVES LOCOMOTOR PERFORMANCE IN RATS WITH MILD ESTABLISHED SPINAL CORD COMPRESSION INJURY
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P433. SRC FAMILY KINASE INHIBITOR PP1 IMPROVES MOTOR FUNCTION AFTER SPINAL CORD CONTUSION IN RATS.
Chihiro Akiyama, Takamichi Yuguchi, Masami Nishio, Toshiyuki Fujinaka, Masaaki Taniguchi, Yoshikazu Nakajima, Eiji Kohmura and Toshiki Yoshimine. (Department of Neurosurgery, Osaka University Medical School, Suita, Osaka JP).*

P434. EXPERIENCE OF ANTERIOR RECONSTRUCTION WITH KANEDA SR IN THE TREATMENT OF THORACOLUMBAR BURST FRACTURE

Kyoung-S Cho, Choon-K Park, Pil-W Huh, Jae-K Kim, Do-S Yoo, Dal-S Kim, CK Park, Moon-C Kim. (Dept of Neurological Surgery, Uijongbu St. Mary's Hospital, The Catholic University of Korea College of Medicine, Uijongbu, Korea).*

P435. S-100BETA LEVELS AND MYELOPEROXIDASE ACTIVITY AFTER SPINAL CORD INJURY IN THE RAT.

E. Schultke, R.W. Griebel, H. Kamencic, V. M. Skihar, B.H.J. Juurlink. (Department of Anatomy & Cell Biology and Division of Neurosurgery, University of Saskatchewan, Saskatoon, CA).*

P436. EFFECTS OF HP184 ON C-FIBER MEDIATED HYPERREFLEXIVE BLADDER CONTRACTIONS INDUCED BY EITHER ACUTE SCI OR IRRITATION

Kazumasa Torimoto, Macrina Xavier, Kara E. Dally, Pradeep Tyag, Craig P. Smith, William C. de Groat, and Matthew O. Fraser. (University of Pittsburgh School of Medicine, Pittsburgh, PA; and Aventis Pharmaceuticals, Inc., Bridgewater, NJ, USA).*

P437. MITOCHONDRIAL FUNCTION AS MEASURED BY REDOX POTENTIAL IS REDUCED FOLLOWING LATERAL FLUID PERCUSSION BRAIN INJURY.
Wilson P. Daugherty, Dong Sun, M. Ross Bullock. (Medical College of Virginia /VCU, Richmond, VA USA).

P438. GLYCOGEN LEVELS IN CORTEX AND HIPPOCAMPUS INCREASE 24 HOURS AFTER LATERAL FLUID-PERCUSSION BRAIN INJURY.
JC Friedland[*1], T Otori, H Muramatsu, R Raghupathi, TK McIntosh, FA Welsh. (Departments of Neurosurgery and Pharmacology[1]; University of Pennsylvania School of Medicine, Philadelphia, PA USA).*

P439. THE EFFECTS OF DELAYED BUT PROLONGED HYPOTHERMIA ON THE PIAL VASCULAR RESPONSE AFTER TRAUMATIC BRAIN INJURY IN RATS
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P440. CHANGES IN CEREBRAL PERFUSION AND HIGH ENERGY-RELATED METABOLITES IN RESPONSE TO FLUID PERCUSSION INJURY
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P441. PERIVASCULAR NERVE DAMAGE IN THE CEREBRAL CIRCULATION FOLLOWING TRAUMATIC BRAIN INJURY
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P442. HYPOTHERMIC CEREBROVASCULAR PROTECTION IS RELATED TO THE RATE OF POST HYPOTHERMIC REWARMING
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P443. STUDY OF MILD HYPOTHERMIA ON PBTO2 AND BT PATIENTS WITH SEVERE HEAD INJURY
Professor Shuyuan Yang. (Huanhu Hospital, Tianjin, Tianjin CN).

P444. APOPTOTIC CELL DEATH FOLLOWING IN VITRO TRAUMATIC INJURY IS INDEPENDENT OF CALCIUM INFLUX
T. A. Lusardi, R. Raghupathi, D. F. Meaney. (University of Pennsylvania, Philadelphia, PA US).*

P445. REPEATED RAPID ACCELERATIONS PRODUCE INCREASED AXONAL INJURY IN THE IMMATURE BRAIN
Mehrdad F. Mehr, Ramesh Raghupathi, Mark A. Halfaer, Susan S. Margulies. (Univ of PA, Phildelphia, PA US).*

P446. NEURONAL LOSS FROM BRAIN NUCLEI AFTER HUMAN BLUNT HEAD-INJURY
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P447. STEREOLOGICAL COMPARISON OF REGIONAL HIPPOCAMPAL CELL LOSS IN INBRED MOUSE STRAINS FOLLOWING FLUID PERCUSSION INJURY
B.M. Witgen[1], J. Lifshitz[1], H. Evcimen[3], A.S. Cohen[2,3], M.S. Grady[1] ([1]Head Injury Center, Dept. of Neurosurgery and [2]Dept. of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA, [3]Department of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA USA).

P448. AGE-ASSOCIATED MITOCHONDRIAL DNA DELETIONS AND OXIDATION ARE NOT EVIDENT CHRONICALLY FOLLOWING EXPERIMENTAL BRAIN INJURY IN THE RAT
Jonathan Lifshitz[1], Paolo A. Marciano[1], and Tracy K. McIntosh[1,2] ([1]Head Injury Center, Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA 19104 and [2]Veterans Administration Medical Center, Philadelphia, PA USA).

P449. LONG-TERM PRION PROTEIN ACCUMULATION IN DAMAGED AXONS FOLLOWING INERTIAL BRAIN INJURY IN THE PIG.
Douglas H. Smith[1], Akira Iwata[1], David F. Meaney[2], and Xiao-Han Chen[1]. ([1]Departments of Neurosurgery and [2]Bioengineering, University of Pennsylvania, Philadelphia, PA USA).*

P450. TRANSCRIPTIONALLY PROFILING THE EFFECTS OF CHRONIC METHYLPHENIDATE TREATMENT IN RATS AFTER TRAUMATIC BRAIN INJURY
C.E. Dixon[1], H.Q. Yan[1], L. Shao[1], X. Ma[1], D.J. Hollins[2], L.W. Jenkins[1] and D. Marion[1]. ([1]Dept. Neurological Surgery, [2]Dept. of Neurobiology, University of Pittsburgh, Pittsburgh, PA USA).*

P451. ASSOCIATIONS BETWEEN DOPAMINE TRANSPORTER GENOTYPE AND CEREBRAL SPINAL FLUID DOPAMINE LEVELS AFTER SEVERE TRAUMATIC BRAIN INJURY
X. Ma[1], Y. Conley[3], R.D. Zafonte[2], C.E. Dixon[1], M. Kerr[4], R. Ferrell[3], D. Marion[1], A.K. Wagner[2] ([1]Dept. Neurological Surgery, [2]Dept. Physical Medicine and Rehabilitation, [3]Dept. Human Genetics, [4]School of Nursing, University of Pittsburgh, Pittsburgh, PA US).

P452. DOPAMINE TRANSPORTER GENOTYPE IS ASSOCIATED WITH FUNCTIONAL AND NEUROPSYCHOLOGICAL OUTCOME FOLLOWING TRAUMATIC BRAIN INJURY
Zafonte RD[1], Conley Y[2], Dixon CE[3], Beers S[4], Marion D[3], Ferrell R[2], Kerr M[5], Wagner AK[1] ([1]Dept Physical Medicine and Rehabilitation, [2]Dept. Human Genetics, [3]Dept. Neurological Surgery, [4]Dept. Psychiatry, [5]School of Nursing, University of Pittsburgh, Pittsburgh, PA US).*

P453. MICROGLIAL CHEMOTAXIS IS REGULATED BY ATP AND ADP RELEASED BY TRAUMATICALLY INJURED ASTROCYTES.
C. Liang[1], D. Bailey[1], R. Fry[1], S. Merchant[1], L. Webb[2], E. Ellis[2], and B.A. Rzigalinski[1]. ([1]Univ. of Central Florida, Dept. of Molecular, Biology. & Microbiology, Orlando, FL; [2]Virginia Commonwealth Univ., Dept. of Pharmacology & Toxicology, Richmond, VA USA).*

P454. MILD OR MODERATE TRAUMATIC BRAIN INJURY: BEHAVIORAL AND HISTOPATHOLOGICAL OUTCOMES IN MICE
K.J. Feeko; K.E. Saatman; R. Raghupathi. (Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA USA).*

P455. A CONFOCAL MICROSCOPIC EXAMINATION OF THE EFFECTS OF STRAIN (STRETCH) ON CULTURED NEURONS AND ASTROCYTES
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P456. PROTEIN EXTRAVASATION, REACTIVE ASTROGLIOSIS, AND NEURONAL DAMAGE FOLLOWING MILD OR MODERATE TRAUMATIC BRAIN INJURY IN MICE.
RL Pape[1], KJ Feeko[1], JW Huh[2], AK Clouse[1], R Raghupathi[1], KE Saatman[1]. (Departments of [1]Neurosurgery, University of Pennsylvania; and [2]Anesthesiology and Critical Care, The Children's Hospital of Philadelphia, Philadelphia, PA USA).*

P457. TRAUMATIC AXONAL INJURY DIFFERENTIALLY IMPAIRS FAST- VS. SLOW-CONDUCTING CORPUS CALLOSUM FIBERS.
T.M. Reeves, L.L. Phillips, J.T. Povlishock. (Department of Anatomy and Neurobiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA US).*

P458. REGIONAL SPECIFIC ALTERATIONS IN NERVE GROWTH FACTOR (NGF) & NEUROTROPHIN-4/5 (NT-4/5) AFTER TRAUMATIC BRAIN INJURY IN RATS.
N.C. Royo[1], S. Shimizu[1], K.E. Saatman[1], T.K. McIntosh[1,2]. ([1]Department of Neurosurgery. University of Pennsylvania, Philadelphia, PA; [2]Veterans Administration Medical Center, Philadelphia, PA USA).*

P459. NON-INVASIVE ASSESSMENT OF ICP FROM CEREBRAL BLOOD FLOW VELOCITY AND ARTERIAL BLOOD PRESSURE USING A FUZZY PATTERN CLASSIFICATION METHOD
B. Schmidt[1], S.F. Bocklisch[2], M. Päßler[2], M. Czosnyka[3], J.J. Schwarz[2], J. Klingelhöfer[1]. ([1]Dept. of Neurology, Chemnitz Medical Centre, Chemnitz, Germany; [2]Dept. of Systems Theory, Technical University, Chemnitz, Germany; [3]Academic Neurosurgical Unit, Addenbrooke's Hospital, Cambridge, UK).*

P460. EARLY ONSET OF OXIDATIVE STRESS IN HUMAN TRAUMATIC BRAIN INJURY MAY BE RESPONSIBLE FOR FAILURES OF FREE-RADICAL SCAVENGER PHARMACOLOGICAL THERAPIES
Angela M. Amorini[1], Luciano Cristofori[2], Barbara Tavazzi[3], Roberta Gambin[2], Carlo Vivenza[2], Roberto Vagnozzi, Giovanna Fazzina[4], Giuseppe Lazzarino[4]. ([1]Medical College of VA, Richmond, VA; [2]Univ. Hospital of Verona. Italy; [3]Inst. Biochem.and Clin. Biochem., Catholic Univ. Rome, Sacro Cuore, Italy; [4]Dept. Chem. Sci., Univ. Catania, Italy).*

P461. RAPID UPREGULATION OF PHOSPHORYLATED-ERK SUGGESTS A ROLE FOR THE MITOGEN ACTIVATED PROTEIN KINASE PATHWAY IN TRAUMATIC BRAIN INJURY
H. Lundqvist, F. Clausen, A. Lewén, L. Hillered Department of Neuroscience, Section of Neurosurgery, Uppsala University Hospital, Uppsala, Sweden.. (Uppsala University, Section of Neurosurgery, Uppsala, SE).*

P462. EFFECT OF DURATION OF HYPOTHERMIA FOLLOWING CONTROLLED CORTICAL IMPACT IN IMMATURE RATS
DS Davis, KS Stevenson, DJ Santone, AS Gordon, CE Dixon, PM Kochanek, LW Jenkins, PD Adelson. (University of Pittsburgh, Pittsburgh, PA US).*

P463. DELAYED TREATMENT WITH ANIRACETAM IMPROVES COGNITIVE RECOVERY AFTER TRAUMATIC BRAIN INJURY IN RATS
Anya Baranova, Katharine C. Eakin, Robert J. Hamm. (Virginia Commonwealth University, Richmond, VA US).*

P464. CASPASE INHIBITION AFTER TRAUMATIC BRAIN INJURY ALTERS AMYLOID PRECURSOR PROTEIN AND AMYLOID-BETA PRODUCTION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE
Milos D. Ikonomovic[1]. John R. Ciallella[2], William R. Paljug, Yetta I. Wilbur, Robert S.B. Clark, Dorothy G. Flood#, C. Edward Dixon, Patrick M. Kochanek, Donald W. Marion, and Steven T. DeKosky. ([1]University of Pittsburgh Medical Center and Safar Center for Resuscitation Research. Pittsburgh, PA; and [2]Cephalon. Inc., West Chester, PA).*

P465. ATTENUATION OF OXIDATIVE STRESS AFTER ACUTE BROMOCRIPTINE TREATMENT IN TRAUMATICALLY BRAIN INJURED RATS
J.L. Massucci[1], A.E. Kline[2], X. Ma[1] and C.E. Dixon[1] (Depts. of Neurosurgery[1] and Physical Medicine & Rehabilitation[2], University of Pittsburgh, Pittsburgh, PA USA).*

P466. INCREASED EXPRESSION OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) IN RAT BRAIN AFTER TRAUMATIC BRAIN INJURY
V. Rahimi-Movaghar[1]*, H.Q. Yan[2], X. Ma[2], D.W Marion[2] and C.E. Dixon[2] ([1]Dept. of Neurosurgery, Zahedan University of Medical Science, Zahedan, Iran; [2]Dept of Neurosurgery, Univ. of Pittsburgh, Pittsburgh, PA).

P467. EXPLORATORY STUDY OF ACUPUNCTURE TREATMENT ON TRAUMATIC BRAIN INJURY (TBI) IN RATS
H.Q. Yan, X. Ma, Y. Hao, Y. Li , D.W Marion and C.E. Dixon. (Department of Neurosurgery, University of Pittsburgh, Pittsburgh, PA USA).*

P468. CHRONIC IMPAIRMENT OF EXTRACELLULAR K+ HOMEOSTASIS FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT.
*Raimondo D'Ambrosio *, and David S Gordon. (Department of Neurological Surgery, University of Washington, Seattle, WA US).*

P469. THE mGluR1 ANTAGONIST AIDA REDUCES POST-TRAUMATIC EMPTYING OF CALCIUM STORES IN NEURONS AND ASTROCYTES
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P470. DIFFERENTIAL EFFECTS OF ACUTE AND CHRONIC EXERCISE ON PLASTICITY-RELATED GENES IN THE RAT HIPPOCAMPUS REVEALED BY MICROARRAY
Raffaella Molteni[2]. Zhe Ying[2]. Fernando Gomez-Pinilla[1,2] ([1]Neurosurgery Div., Brain Injury Research Center, UCLA and [2]Physiological Science Dept, UCLA, Los Angeles, CA USA).*

P471. THE USAGE EFFICIACY OF PROLONGED VENTRICULAR DRAINAGE AND APRICOT JUICE ON MANAGEMENT OF CNS INJURY
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P472. A HIGH-FAT SUCROSE DIET (HFS) EXACERBATED TRAUMATIC BRAIN INJURY (TBI) -INDUCED IMPAIRMENTS IN COGNITION AND NEURONAL PLASTICITY
Aiguo Wu[2], Raffaella Molteni[2], Zhe Ying[2], and F. Gomez-Pinilla[1,2] ([1]Division of Neurosurgery. UCLA Brain Injury Research Center, and [2]Department of Physiological Science, UCLA, Los Angeles, CA USA).

P473. IS MINOCYCLINE REGULATING GLUTAMATE TOXICITY AFTER TRAUMATIC BRAIN INJURY ?
S. Bele. A.Brawanski (Departement of Neurosurgery, University of Regensburg, Regensburg, Germany).*

P474. ALZHEIMER'S DISEASE PATHOLOGY IN SURVIVORS OF SEVERE BRAIN INJURY
Steven T. DeKosky[1], Kunihiro Uryu[2], John Q. Trojanowski[2], Virginia M-Y. Lee[2], John R. Ciallella[1], Robert S.B. Clark[3], Donald W. Marion[4], Milos D. Ikonomovic[1].(Depts. [1]Neurology and Psychiatry. [3]Critical Care Medicine. and [4]Neurosurgery, Univ. Pittsburgh Sch. Med., Pittsburgh, PA; [2]Dept. Pathology and Laboratory Med., Ctr. Neurodegenerative Disease Research,Philadelphia, PA USA).*

P475. ASSESSING COGNITIVE AND PSYCHOLOGICAL PATTERNS IN POST-TRAUMATIC HEADACHE FOLLOWING SEVERE BRAIN INJURY.
R. Formisano, M.G. Buzzi#, U. Bivona , M. Matteis, C. Caltagirone. (IRCCS Santa Lucia Foundation, Rome, Rome IT).*

P476. COMBINED MUSCARINIC AND NMDA RECEPTOR ANTAGONISM REDUCES HYPERGLYCEMIC EXACERBATION OF POSTTRAUMATIC CEREBRAL ISCHEMIC HYPERSENSITIVITY
LW Jenkins, CE Dixon, and PM Kochanek. (Univ of Pittsburgh, Pittsburgh, PA US).*

P477. BRAIN TISSUE PO2, INTRACRANIAL PRESSURE, ADENOSINE AND PURINE DEGRADATION PRODUCTS AFTER SEVERE HEAD INJURY IN ADULTS: A PRELIMINARY ANALYSIS
A.M. Puccio, P.M. Kochanek, E.K. Jackson, Z. Mi, S.R. Wisniewski, M.R. Fischer, D.W. Marion. (University of Pittsburgh Brain Trauma Research and Safar Centers, Pittsburgh, PA US).*

P478. MULTICENTER STUDY OF CONTINUOUS VS INTERMITTENT CEREBROSPINAL FLUID DRAINAGE AFTER SEVERE TRAUMATIC BRAIN INJURY IN CHILDREN: EFFECT ON BIOCHEMICAL MARKERS
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P479. DOWN REGULATION OF AQUAPORIN-4 IN AREA ADJACENT TO BRAIN INJURY IN A TRAUMATIC RAT BRAIN MODEL.
**Ming-Chieh Sun[1], M.B., Christopher R. Honey[2], D.Phil[1], M*ing-Chieh Sun[1]*, Christopher R. Honey[2], Norman L. M. Wong[3], Joseph K.C. Tsui[4]. ([1]Cardinal Tien Hospital and Fu-Jen Catholic University. Taiwan, [2]Divisions of Neurosurgery, [3]Nephrology, and [4]Neurology, University of British Columbia, Vancouver, British Columbia).

P480. GENDER INFLUENCES ON CEREBROSPINAL FLUID PATHOPHYSIOLOGY AFTER TRAUMATIC BRAIN INJURY
**A.K. Wagner[1], A. Fabio[2], A. Puccio[2], W. Li[2], R.D. Zafonte[1], D. Marion[2]. ([1]Dept. Physical Medicine and Rehabilitation; [2]Dept. Neurological Surgery, University of Pittsburgh, Pittsburgh, PA US).*

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P493. METHYLPREDNISOLONE INHIBITS INTERLEUKIN-1 AND INTERLEUKIN-6 PRODUCTION IN THE SPINAL CORD FOLLOWING COMPRESSION INJURY IN THE RAT
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P495. RECONSTRUCTION OF ANTERIOR COLUMN DEFICIT FOLLOWING CERVICAL SPINE INJURIE USING THE HARMS TITANIUM MASH CAGE
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P499. NOVEL ROLE OF PROSTACYCLIN IN COMPRESSION-INDUCED SPINAL CORD INJURY IN RATS.
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P500. LONGTERM RESULTS AFTER 36 MONTHS FOR CHRONIC BILATERAL NEUROMODULATION
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P501. THE INTRAVENOUS ADMINISTRATION OF AUTOLOGOUS BONE MARROW CELLS INTO THE RAT DEMYELINATED MODEL.
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P502. TRANSPLANTATION OF SCHWANN CELLS (WITH OR WITHOUT OLFACTORY ENSHEATHING GLIA) AFTER SPINAL CORD INJURY (SCI): CAN PRETREATMENT WITH THE NEUROPROTECTIVE STRATEGY OF CO-ADMINISTERED METHYLPREDNISOLONE AND INTERLEUKIN-10 ENHANCE RECOVERY?
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P503. DIFFERENTIAL RESPONSES OF PAIN AND SENSORY ABNORMALITIES TO I.V. BARBITURATES AND LIDOCAINE IN SPINAL CORD INJURED PATIENTS
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P504. FUNCTIONAL RECOVERY AFTER MODERATE CONTUSIVE INJURY IN THE MOUSE: ROLE OF DHEA IN IMPROVING BLADDER FUNCTION.
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P505. TARGETING THE RHO SIGNALING PATHWAY TO PROMOTE REPAIR AFTER SPINAL CORD INJURY
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P506. DIFFERENTIAL TEMPORAL EXPRESSION OF MATRIX METALLOPROTEINASES DURING WOUND HEALING IN A MURINE MODEL OF SPINAL CORD INJURY
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P507. THE EFFECT OF THE L-TYPE CALCIUM CHANNEL AGONIST, BAYK8644, ON REGENERATION OF CULTURED RAT SYMPATHETIC NEURONS
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P508. IDENTIFICATION OF PROLIFERATING EPENDYMAL CELLS IN THE RAT SPINAL CORD FOLLOWING TRAUMA
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P509. AXONAL PRESERVATION WITHIN DESCENDING VASOMOTOR PATHWAYS AND CARDIOVASCULAR CONTROL AFTER SPINAL CORD INJURY

J.C. Furlan[1], M.G. Fehlings[1], P. Shannon[1], M. Norenberg[2], and A.V. Krassioukov[1]. ([1]Krembil Neuroscience Center, University of Toronto, Canada; and [2]Miami Project to Cure Paralysis, Miami, FL, USA2, Toronto, ON CA).*

P510. ECG FINDINGS IN ACUTE SPINAL CORD INJURY IN HUMANS

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P511. CERVICAL SPINAL INJURY WITH ESOPHAGEAL RUPTURE-REPORT OF TWO CASES

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P512. HP184 IS NEUROPROTECTIVE AND IMPROVES LOCOMOTOR FUNCTION AFTER MODERATE ACUTE SPINAL CRUSH INJURY IN RATS

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P513. INFLUENCE OF CRANIOPLASTY ON CEREBRAL BLOOD FLOW AND CARDIAC FUNCTION
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P514. VOLUMETRIC PROTON MR SPECTROSCOPY OF MILD TRAUMATIC BRAIN INJURY
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P516. FUNCTIONAL MAGNETIC RESONANCE IMAGING AFTER ACUTE MILD TRAUMATIC BRAIN INJURY
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P517. PRIMARY AND DELAYED ISCHEMIA IN SEVERE TRAUMATIC BRAIN INJURY
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P519. CASPASE INHIBITION ATTENUATES MITOCHONDRIAL RELEASE OF CYTOCHROME C AND APOPTOSIS-INDUCING FACTOR AFTER TRAUMATIC BRAIN INJURY IN RATS.
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P520. TEMPORAL SEQUENCE OF POLY (ADP-RIBOSE) POLYMERASE EXPRESSION IN TRAUMATIC BRAIN INJURY IN HUMANS
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P521. INCREASED PHOSPHORYLATION AND NUCLEAR TO CYTOSOLIC TRANSLOCATION OF FORKHEAD TRANSCRIPTION FACTOR IN RAT CORTEX AND HIPPOCAMPUS AFTER TRAUMATIC BRAIN INJURY.
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P522. AGE-DEPENDENT SUSCEPTIBILITY TO OXIDATIVE STRESS AFTER TRAUMATIC BRAIN INJURY IN THE DEVELOPING BRAIN
Pichuan Fan[1,2], Toshihiro Yamauchi[1], Linda J. Noble[1], And Donna M. Ferriero[1] ([1]University of California, San Francisco, USA. [2]Chang Gung Children's Hospital, Taipei, Taiwan).

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P524. DIFFERENTIAL EXPRESSION OF GENES RELATED TO CELLULAR SIGNALING, SYNAPTIC FUNCTIONING AND ION CHANNELS POST-INJURY: A COMPARISON OF MODERATE AND SEVERE INJURY EFFECT ON GENE EXPRESSION IN HIPPOCAMPUS
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P525. GENE EXPRESSION PROFILING FOLLOWING TRAUMATIC BRAIN INJURY IN WILD TYPE AND ALZHEIMER TRANSGENIC MICE
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P526. GENE EXPRESSION PROFILES FOLLOWING MODERATE AND SEVERE BRAIN INJURY IN RATS: IMPLICATION OF ENERGY SHORTAGE AND CELLULAR VULNERABILITY IMMEDIATELY AFTER INJURY.
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P527. MICROARRAY GENE EXPRESSION ANALYSIS OF POSTNATAL DAY 26 RAT CORTEX AFTER LATERAL FLUID PERCUSSION INJURY
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P530. RELATIONSHIP OF APPROPRIATE HISTOLOGICAL PROCESSING TECHNIQUES AND ACCURATE EVALUATION OF EXPERIMENTAL BRAIN INJURY
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P531. CLINICORADIOLOGICAL CLASSIFICATION OF TRAUMATIC INTRACEREBRAL HEMATOMAS (THI).
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P532. CLINICAL CRITERIA OF SORTING, PROGNOSIS OF OUTCOMES AND TREATMENT OF THE PATIENTS WITH A CRANIOCEREBRAL TRAUMA AT STAGES OF MEDICAL EVACUATION
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P535. PRELIMINARY EXPERIENCE WITH DECOMPRESSIVE VENTRICULOSTOMY BY CONTIUOUS VENTRICULAR CEREBROSPINAL FLUID DRAINAGE IN POSTTRAUMATIC DIFFUSE BRAIN SWELLING.
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P536. CNS PROTECTION BY ANTI-OXIDANTS: PROMISING APPROACHES FOR HEAD TRAUMA IN A RAT MODEL
B.H.J. Juurlink[1], R.W. Griebel[2], H. Kamencic[1], E. Schültke[1,2], M. Zhao[3], G.-F. Tian[3]3, A.J. Baker[3].* (Department of Anatomy & Cell Biology[1] and Division of Neurosurgery[2], University of Saskatchewan, Saskatoon and [3]St. Michael's Hospital, Toronto, Canada).

P537. DELETERIOUS EFFECT OF SECONDARY INSULTS ADDED ON TRAUMATIZED ORGANOTYPIC CULTURE IS MORE PROMINENT IN MILD TO MODERATE THAN IN SEVERE DEGREES OF INJURY
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P539. NEUROPROTECTIVE EFFECTS OF AMINOGUANIDINE IN A RAT MODEL OF LATERAL FLUID-PERCUSSIVE BRAIN INJURY
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P542. ENHANCED GASTRIC TOLERANCE TO INDOMETHACIN FOLLOWING NEUROTRAUMA
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P543. THE ROLE OF ION TRANSPORTERS IN POSTTRAUMATIC CYTOTOXIC BRAIN EDEMA.
*Ankur Shah *, Jason S Fender, David K Kung, Samar S Hassouneh, and Raimondo D'Ambrosio. (Department of Neurological Surgery, University of Washington, Seattle, WA USA).*

P544. MODULATION OF NEURONAL AND GLIAL GROUP I MGLURS PREVENTS STRETCH-INDUCED ENHANCEMENT OF NMDA RECEPTOR CURRENT
Paul M. Lea IV[*1], Stephanie J. Custer[1], Stefano Vicini[1,2] and Alan I. Faden[1,3,4]. (Departments of Neuroscience[1], Physiology[2] & Pharmacology[3], Institute for Cognitive and Computational Sciences[4], Georgetown University Medical Center, Washington, DC US).*

P545. INJURY-INDUCED CHANGES IN NMDA RECEPTOR SUBUNIT COMPOSITION CONTRIBUTE TO PROLONGED CALCIUM-45 ACCUMULATION IN INTACT CORTEX
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P546. ASSESSMENT OF AGRIN EXPRESSION DURING TRAUMA-INDUCED SYNAPTIC PLASTICITY
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P547. MITOCHONDRIAL GENE EXPRESSION FOLLOWING TRAUMATIC BRAIN INJURY: ANALYSIS OF THE ND4 SUBUNIT OF COMPLEX I.
*LL Phillips *, LK Harris, RT Black, TM Reeves and JT Povlishock. (Dept. of Anatomy and Neurobiology, Medical College of VA, VA Commonwealth University, Richmond, Virginia US).*

P548. AGE-RELATED CELL PROLIFERATION IN THE RAT CNS FOLLOWING TRAUMATIC BRAIN INJURY
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P549. SEVERE HEAD INJURY MANAGEMENT IN LATVIA
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P550. TRAUMATIC SUBARACHNOID HEMORRHAGE, EVOLUTION AND PROGNOSIS.
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P551. NMDAR-PSD95 INTERACTION MEDIATES SECONDARY TRAUMATIC NEURONAL INJURY.
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P552. SYSTEMIC HEMORRHAGE AND THE TYPE OF RESUSCITATION IMPACTS HIPPOCAMPAL FUNCTION FOLLOWING BRAIN TRAUMA
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P553. EXPERIENCE OF DIAGNOSTICS AND TREATING OF A SEVERE TRAUMATIC BRAIN INJURY (STBI), COMBINED WITH OPENED DAMAGES OF A CHEST.
Dr. V. Banashkevich, Dr. A.Korobtsov, Dr.A.Lantuch, Dr.B.Sotnichenko. (Vladivostok State Medical University, Vladivostok, RU).*

P554. HEAD INJURED PATIENTS WHO TALK AND DETERIORATE: ANALYSIS OF 86 CASES REGISTERED ON THE JAPAN NEUROTRAUMA DATA BANK
Tatsuro Kawamata, Yoichi Katayama, and Japan Neurotrauma Data Bank Committee. (Japan Society of Neurotraumatology, Department of Neurological Surgery, Nihon University School of Medicine, Tokyo, JP).*

P555. SURGICAL COMPLICATIONS OF DECOMPRESSIVE CRANIECTOMY FOR HEAD INJURY
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P556. EARLY EDEMA FORMATION IN CEREBRAL CONTUSION: ULTRA-EARLY STUDY (<24 HOURS POST-TRAUMA) WITH DIFFUSION MRI AND ADC MAPPING
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Departments of Neurological Surgery[2] and Radiology[3], Yokohama Chuo Hospital, Kanagawa, JP).*

P557. DOES THE USE OF JUGULAR BULB OXYGEN SATURATION IMPROVE THE PROGNOSIS IN HEAD INJURED PATIENTS?
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P558. DIFFUSE AXONAL INJURY FOLLOWING FLUID PERCUSSION TRAUMATIC BRAIN INJURY IN THE RAT: CHARACTERIZATION AND CORRELATION BETWEEN ELECTROPHYSIOLOGICAL AND HISTOLOGICAL FEATURES.
N. Phan, E. Liu, M. Zhao, R.J. Moulton, M.G. Fehlings, and A.J. Baker. (Cara Phelan Centre for Trauma Research, St. Michael's Hospital, Toronto Western Research Institute, Institute of Medical Science, University of Toronto, Toronto, ON Canada).*

P559. DELAYED TRAUMATIC INTRACEREBRAL HEMATOMA AND COAGULOPATHY IN THE PATIENTS DIAGNOSED WITH A TRAUMATIC SUBARACHNOID HEMORRHAGE
*Satoshi Sawauchi *, Toshiaki Abe. (Jikei University School of Medicine, Tokyo, JP).*

P560. PRELIMINARY REPORT: TRAUMATIC COMA DATA BANK PROJECT IN JAPAN
Takeki Ogawa. (Jikei University School of Medicine, Tokyo, 105-8467 JP).

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N-INTS 2002 PROGRAM EVALUATION FORM

Please rate each speaker presentation by circling the appropriate letter in both columns.
(A=Excellent, B=Good, C=Fair, D=Poor)

	CONTENT	PRESENTATION
Alex Baethmann	A B C D	A B C D
Joe Beckman	A B C D	A B C D
Ross Bullock	A B C D	A B C D
Pak Chan	A B C D	A B C D
Jun Chen	A B C D	A B C D
Arlene Chiu	A B C D	A B C D
Doug Coulter	A B C D	A B C D
Ella Englander	A B C D	A B C D
Michael G. Fehlings	A B C D	A B C D
Donna Ferriero	A B C D	A B C D
Susan Harkema	A B C D	A B C D
Ron Hart	A B C D	A B C D
Ronald L. Hayes	A B C D	A B C D
Anders Holtz	A B C D	A B C D
Osamu Honmou	A B C D	A B C D
Susan Horn	A B C D	A B C D
Phillip Horner	A B C D	A B C D
David Hovda	A B C D	A B C D
Claire Hulsebosch	A B C D	A B C D
Patricia Hurn	A B C D	A B C D
Takuji Igarashi	A B C D	A B C D
Larry Jenkins	A B C D	A B C D
Stuart Lipton	A B C D	A B C D
Andrew I. R. Maas	A B C D	A B C D
Geoff Manley	A B C D	A B C D

	CONTENT	PRESENTATION
Tracy McIntosh	A B C D	A B C D
Mary Ellen Michel	A B C D	A B C D
M. Cristina Morganti-Kossmann	A B C D	A B C D
Paul Muizelaar	A B C D	A B C D
Marion Murray	A B C D	A B C D
Martin Oudega	A B C D	A B C D
Jennie Ponsford	A B C D	A B C D
Phillip Popovich	A B C D	A B C D
Mayumi Prins	A B C D	A B C D
Alain Privat	A B C D	A B C D
Paul J. Reier	A B C D	A B C D
Claudia S. Robertson	A B C D	A B C D
Elisabeth Ronne-Engstrom	A B C D	A B C D
Robin L. Roof	A B C D	A B C D
Tim Schallert	A B C D	A B C D
Lisa Schnell	A B C D	A B C D
Michal Schwartz	A B C D	A B C D
Esther Shohami	A B C D	A B C D
Graham Teasdale	A B C D	A B C D
Angelo Vescovi	A B C D	A B C D
Scott R. Whittemore	A B C D	A B C D
Thomas Woolsey	A B C D	A B C D
Jean R. Wrathall	A B C D	A B C D

In your opinion, was information presented by each speaker presented fairly and without commercial or promotional bias?

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Dates of meeting	A B C D	Location (city)	A B C D
Course content	A B C D	Hotel Selection	A B C D
Course as a whole	A B C D	Welcome Reception	A B C D
Poster Abstract presentations	A B C D	Pharmacia Luncheon	A B C D
WINTR Luncheon	A B C D	WINTR Reception	A B C D
General Sessions	A B C D	Breakout Sessions	A B C D
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Please rank the deciding factors in attending: ___Topics ___ Location ___ Faculty ___ Other

What did you find most interesting?

What was the least interesting?

Suggestions for next course?

COURSE OBJECTIVES

Please rate how well N-INTS 2002 met the following educational objectives.	**CONTENT**	**PRESENTATION**
Upon the completion of The Symposium, participants will be able to:		
Discuss the recent, innovative techniques in CNS injury that include transgenic and gene knockout mouse models, advanced MR methods, stem cell biology, biopolymers and developmental molecules in cord lesions, the role of proteases in neuronal injury and nogo and axonal regeneration in the CNS.	A B C D	A B C D
Outline molecular mechanisms of cell death in cerebral ischemia, the role for caspases, cyclooxygenases and excitotoxin in death due to stroke, and describe the significance of late-breaking news in CNS injury research.	A B C D	A B C D
Discuss the characteristics of successful clinical trials in CNS injury and their limitations.	A B C D	A B C D

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We are interested in understanding your needs for continuing medical education. To help us in developing activities that address your needs, please complete the following information.

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1. _____
2. _____
3. _____
4. _____
5. _____
6. _____

Do you have other areas of interest?

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____

Any other suggestions you may have to improve the educational content?

_____

_____

What type of venue would you prefer? *(Please select one from each category)*

Location: ___ Hospital/Institution ___ Downtown Hotel in Major City ___ Resort

Type of Hotel:
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Amenities: *(check all that apply)*
___ Pool ___ Fitness Center ___ Spa ___ Golf
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___ Other : _____

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___ 2003 NNS conference
___ INTS 2004 in Australia ___ 2003 Society for Neuroscience ___ Other: _____

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7th International Neurotrauma Symposium 04

12-16 September 2004
Adelaide Convention Centre
Adelaide, South Australia

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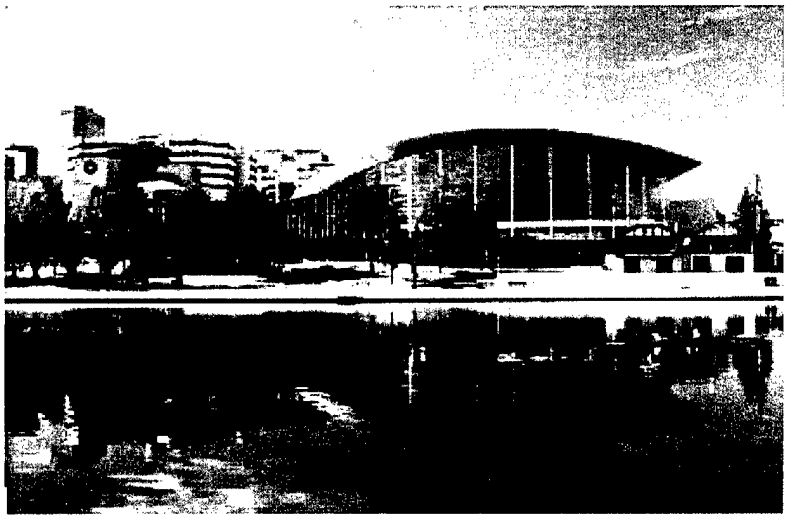

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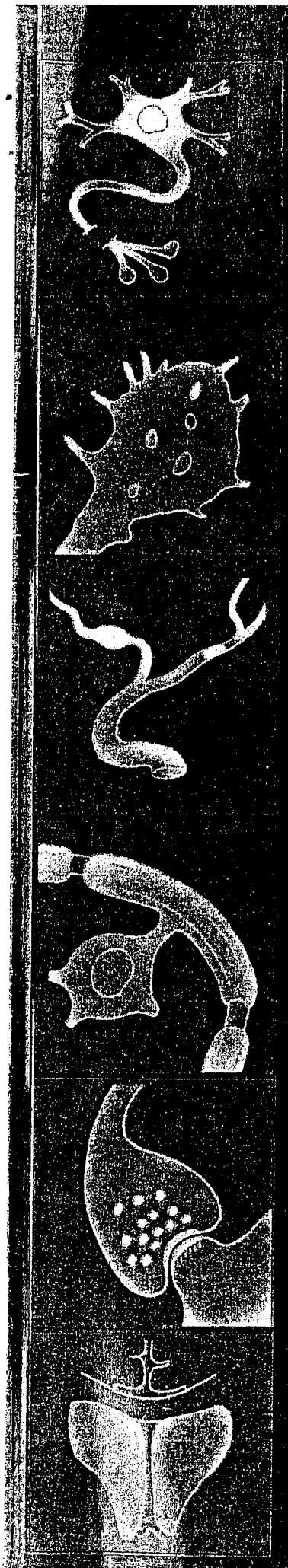
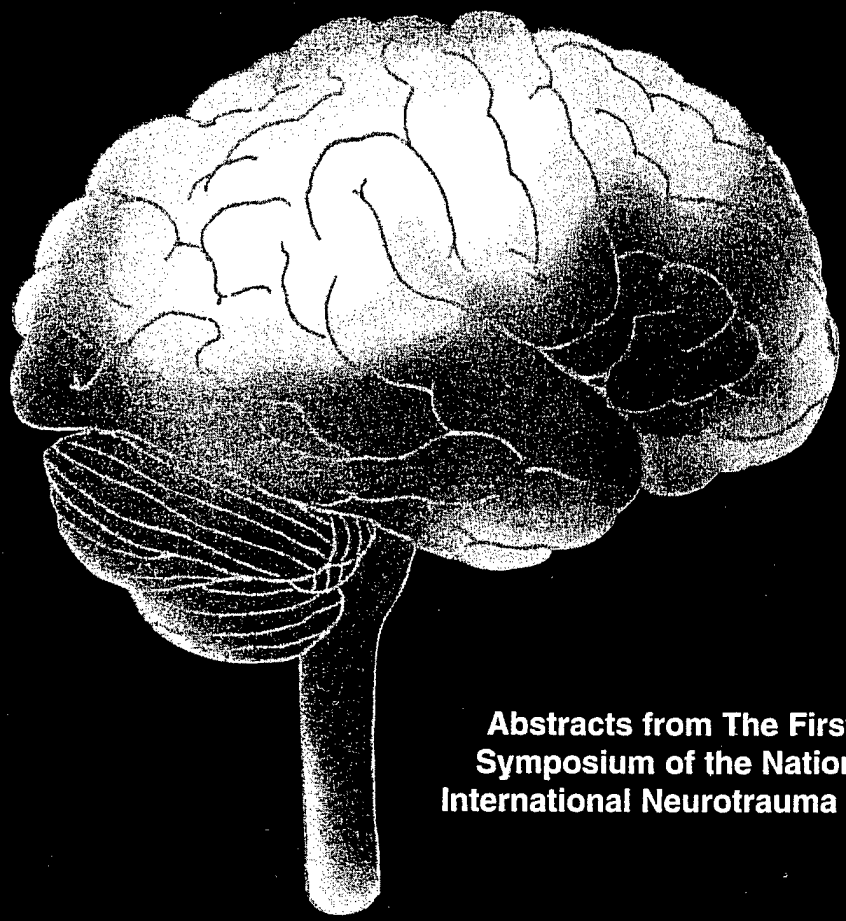
VOLUME 19 NUMBER 10 OCTOBER 2002
ISSN: 0897-7151

Journal of Neurotrauma





Abstracts from The First Joint Symposium of the National and International Neurotrauma Societies

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Abstracts

The First Joint Symposium of the National and International Neurotrauma Societies

The 20th Annual National Neurotrauma Society Symposium

and

The Sixth International Neurotrauma Symposium

October 27–November 1, 2002
Tampa, Florida

P101.

REGIONAL HYPERGLYCOLYSIS IS CHARACTERIZED BY DECREASED GLUCOSE TRANSPORT AND PRESERVED HEXOKINASE ACTIVITY FOLLOWING TRAUMATIC HEAD INJURY.

N. Hattori[1], SC Huang[1], HM. Wu[1], WH Liao[1], TC Glenn[2], PM. Vespa[2], M. Phelps[1], DA Hovda[1,2], M. Bergsneider[2]. ([1]Dept. of Molecular and Medical Pharmacology. [2]UCLA Brain Injury Research Center. David Geffen School of Medicine at UCLA. Los Angeles, CA US).*

Kinetic analysis of dynamic F-18 fluorodeoxyglucose (FDG) positron emission tomography (PET) permits in-vivo assessment of glucose transporter and hexokinase activities regionally in human brain. The purpose of this study was to investigate changes in glucose delivery and phosphorylation regionally in patients with focal head injury with relation to hyperglycolysis. Methods: Twelve patients (4 women, 38 ± 12 years old, range 17 to 58 years) with focal head injury underwent three-dimensional dynamic PET with FDG. Median initial GCS was 11.5 (range: 3–14) and PET scans were performed 3.4 ± 2.7 days after injury. Dynamic tissue activities were obtained from the regions-of-interest (ROIs) on the reconstructed PET images. Activities of glucose transporter (K1) and hexokinase (k3) were estimated using a 2-compartment kinetic model of FDG and a non-linear curve fitting. Results: ROIs of contusion showed minimal FDG uptake, while FDG uptake was relatively preserved in remote cortex (Remote). All patients had at least one PeriContusional area with decreased FDG uptake (PC-Low), while 7/12 also had pericontusional areas showing increased FDG uptake (PC-High) compared to remote cortex. PC-High showed slightly higher metabolic rate of glucose (MRG) than Remote (4.8 ± 1.3 vs. 4.0 ± 0.7, mg/100g/min, $p = 0.05$.). Kinetic analyses showed preserved k3 in PC-High (0.065 ± 0.020 /min) compared to Remote (0.056 ± 0.016 /min, p = n.s.), which was higher than k3 in PC-Low (0.049 ± 0.012/min, $p < 0.05$) and Contusion (0.042 ± 0.018 /min, $p < 0.05$). On the other hand, K1 in PC-High (0.068 ± 0.015 ml/min) was lower than Remote (0.088 ± 0.015 ml/min, $p < 0.05$), similar to PC-Low (0.059 ± 0.026 ml/min, p = n.s.), and higher than Contusion (0.024 ± 0.019 ml/min, $p < 0.001$). Conclusions: Regional FDG uptake generally reflects activity of glucose transporter. However, in the pericontusional region with high FDG uptake, the glucose transporter and hexokinase activity is uncoupled, showing preserved hexokinase activity despite of reduced glucose transporters. (NS30308; UCLA Brain Injury Research Center)

P102.

TOPICAL L-ARGININE, BUT NOT NITRIC OXIDE DONOR, RESTORES CEREBROVASCULAR PRESSURE AUTOREGULATION FOLLOWING TRAUMATIC BRAIN INJURY IN RATS: POSSIBLE ROLE OF ENDOTHELIAL NITRIC OXIDE SYNTHASE.

Candace V. Campos, MD, Fangyi Zhang, MD, Minnette G. Son, MD, Farrokh R. Farrokhi, MD, Shane M. Sprague, Georgina Saravia, Dennis G. Vollmer, MD. (UTHSCSA-Neurosurgery, San Antonio, TX US).*

Cerebral blood flow (CBF) is maintained constant over a range of systemic blood pressure by autoregulation. Impaired pressure autoregulation has been reported in both clinical brain injured patients and animal models of traumatic brain injury (TBI). Nitric oxide (NO) plays a significant role in maintaining pressure autoregulation. We sought to determine 1.) whether NO donor or NO synthase (NOS) substrate can improve the post-traumatic pressure autoregulation, 2.) if so, which isoforms of NOS participate in the autoregulation. Halothane anesthetized rats underwent controlled cortical impact (CCI) injury. Controlled hemorrhage was performed to create a stepwise fall in arterial pressure. Cortical CBF was monitored by laser Doppler flowmetry on an open cranial window. Static CBF pressure autoregulation curve in the hypotensive phase was plotted. Moderate CCI resulted in disruption of autoregulation during hemorrhagic hypotension. The slopes of the autoregulation curves in sham-injured group (n = 7) are significantly lower than that of the CCI group (n = 10) when mean arterial pressure (MAP) dropped from 90mmHg to 70mmHg ($p < 0.05$, t-test). Topical superfusion of S-nitroso-N-acetylpenicillamine (SNAP) does not affect the impaired autoregulation curve in injured rats (n = 10; $p < 0.05$ from sham-injured, ANOVA). In contrast, superfusion of L-arginine fully restores the autoregulation curve during the above MAP range (n = 10; $p > 0.05$ from sham-injured, ANOVA). Further, this restoration effect by L-arginine was not attenuated by intraperitoneal injection of 7-nitroindazole, a selective neuronal NOS inhibitor, (n = 10; $p > 0.05$ from sham-injured, ANOVA). We demonstrated that there is a loss of static CBF pressure autoregulation after moderate CCI. NOS substrate, but not exogenous NO, can restore the autoregulation following TBI. Our data suggests that regulatory NOS activity is required for CBF pressure autoregulation. Furthermore, the fact that 7-nitroindazole does not attenuate the restoring effect rendered by L-arginine indicates that endothelial NOS, but not the neuronal NOS, may be responsible for mediating CBF pressure autoregulation.

P103.

TUMOR NECROSIS FACTOR RECEPTOR FAMILY MEMBERS MEDIATE POSTTRAUMATIC CELL DEATH AFTER CONTROLLED CORTICAL IMPACT IN MICE

Michael J. Whalen, Jianhua Qiu, Deirdra McCarthy, and Michael A. Moskowitz. (Massachusetts General Hospital, Boston, MA US).*

We previously reported upregulation of Fas death inducing signaling complexes (DISC) associated with activation of caspases in brain after experimental and human traumatic brain injury (TBI)(J Neurosci 2002, 22:3504-3511). To test the hypothesis that Fas mediates cell death after TBI, we performed controlled cortical impact (0.9 mm depth, 6 m/s) in Fas knockout vs. wild type mice. Fas knockout mice did not differ from wild type in the number of cortical TUNEL positive cells at 6 or 24 h, number of caspase 3 p20 immunoreactive cells at 48 h, or in contusion volume at 21 d, suggesting that other death receptor(s) might compensate the deletion of Fas. In support of this hypothesis, we detected upregulation of tumor necrosis factor receptor 1 (TNFR1) and TNFR1 DISC assembly in brain homogenates early after CCI using immunoprecipitation and Western blot. In addition, fluorescence immunohistochemistry and laser scanning confocal microscopy demonstrated colocalization of TNFR1 with its adapter proteins TRADD and FADD in neurons and with TUNEL positive neurons early after CCI. However, posttraumatic contusion volume did not differ between TNFR1 knockout and wild type mice. To test the hypothesis that Fas or TNFR1 may compensate antagonism of the other death receptor, we administered blocking anti-TNF antibodies (2 ug i.c.v./2 mg/kg i.p.) to wild type (n = 4) or Fas knockout mice (n = 3) and then performed CCI. Contusion volume in treated Fas knockout mice (Mean + SD; 3.3 + 0.7 mm3) was reduced by over 2.5 fold compared to treated wild type mice (8.5 + 0.9 mm3)($p < 0.001$). The data suggest that both Fas and TNFR1 contribute to cell death after TBI, and that strategies targeting both death receptors simultaneously, or their biochemical convergence points, are required to inhibit posttraumatic cell death. Support: NINDS KO8 NS41969-01 (MJW) and 5 RO1 NS37141-05 (MAM).

P104.

QUANTITATIVE ANALYSIS OF NEUROFILAMENT COMPACTION AND AXONAL TRANSPORT FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY

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Diffuse axonal injury (DAI) is a feature of traumatic brain injury (TBI) involving focal axonal cytoskeletal change that leads to impaired axonal transport, causing axonal swelling and disconnection. To better define the relationship, if any, between intra-axonal cytoskeletal abnormality and impaired axonal transport and/or swelling and their potential spatial/temporal interrelations, a well-characterized animal model of TBI was used together with antibodies targeting either cytoskeletal/neurofilament modification (RMO14) or impaired axonal transport (Amyloid pre-cursor protein, APP). Quantitative assessment was performed on individual immunopositive axons visualized through the same chromagen. The number of focal intra-axonal cytoskeletal changes approximated the number of APP profiles at 30 minutes and 3 hours post-TBI, with the APP population showing a statistically significant increase over time ($p < 0.05$). This correlation for either marker, however, was not absolute. The numbers of combined RM014/APP immuno-positive profiles exceeded those seen with the use of each immunomarker alone, suggesting that not all RM014 positive profiles were linked to impaired axonal transport. By 24 hours, the number of APP positive axons was significantly increased over the 30 min and 3 hour time periods ($p < 0.05$), with striking differences seen in comparison to the reduced numbers of RM014 immunoreactive profiles. No correlation existed between other markers. These results indicate that altered cytoskeletal integrity does not necessarily equate with impaired axonal transport at 30 min and 3 hours post TBI, with no correlation at 24 hours post TBI. Laboratory and forensic studies using both markers must now consider that they potentially label different populations of damaged axons. This work was supported by NIH grants NS-20193 and T32 NS7288.

P105.

EFFECTS OF INJURY SEVERITY ON REGIONAL AND TEMPORAL CASPASE-12 mRNA AND PROTEIN EXPRESSION LEVELS AFTER TRAUMATIC BRAIN INJURY IN RATS

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A novel apoptotic pathway involving the endoplasmic reticulum (ER) and ER stress has emerged with caspase-12's discovery that is independent of the previously described intrinsic and extrinsic pathways. We examined regional and temporal caspase-12 mRNA and protein expression and its potential downstream protein target caspase-3 after traumatic brain injury (TBI). mRNA expression levels of caspase-9 were also examined. The mRNA transcript levels were determined in ipsilateral cortex and hippocampus after cortical impact TBI by quantitative RT-PCR.

Caspase-12 cortical mRNA expression increased significantly to 1,376%, 1,374%, and 3,315% of naïve by 120 hours while hippocampal mRNA levels reached 677%, 571%, and 695% within six hours post-TBI for 1.0mm, 1.2mm, and 1.6mm injury magnitudes, respectively.

In addition, we have shown that caspase-3 RNA levels increased by 400% in the cortex and by 200% in the hippocampus samples compared to naïve. In contrast, cortical and hippocampal mRNA levels for caspase-9 either narrowly or never exceeded naïve.

Western blots showed caspase-12 protein upregulation and activation within 24 hours, peaking within three days, while activated caspase-3 expression peaked later at five (cortex) and seven (hippocampus) days.

The over 1300% increase in cortical caspase-12 mRNA expression and near 600% for the hippocampus plus significant increase in active form of caspase-12 protein suggests that caspase-12 may play an important role in cellular apoptosis following TBI and indicates an ER-mediated pathway to capase-3 activation. The results also suggest that caspase-3 may be the downstream target of caspase-12. (Supported by DAMD 17-99-1-9365 and NIH R01 NS 39091)

P106.

5.6-EPOXYEICOSATRIENOIC ACID - MEDIATED Ca2+ SIGNALING IS ENHANCED IN MICROGLIA ACTIVATED BY EXPOSURE TO SOLUBLE FACTORS FROM TRAUMATICALLY INJURED ASTROCYTES.

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Traumatic brain injury (TBI) induces a state of microglial activation, including upregulation of macrophage characteristics and activation of an inflammatory response. Activated microglia (MG) are reported to have both neuroprotective and neurodegenerative effects, depending on the degree of activation and type of injury. However the microglial signaling pathways leading to activation in response to TBI are not clear. Using an in vitro model for TBI (cell strain or stretch), we have previously shown that MG are not directly activated by strain injury. Indirect activation of MG was induced by exposure of uninjured MG to medium conditioned by traumatically injured astrocytes. We now report that indirect activation of MG increases activity of intracellular store-operated Ca2+ channels (SOC). The arachidonic acid epoxide 5,6-epoxyeicosatrienoic acid (5,6-EET), is a putative "Ca2+ Influx Factor", which activates SOC-mediated Ca2+ influx. Exposure of resting MG to 5,6-EET elicited only a weak influx of extracellular Ca2+. Exposure of indirectly activated MG to 5,6-EET induced a dose-dependent influx of Ca2+, suggesting increased SOC channel activity. The glutamate- and 5,6-EET-stimulated Ca2+ influx observed in activated MG, was blocked by the imidazole antimycotic econazole, the SOC inhibitor SKF96365, and MS-PPOH, a specific inhibitor of the P450 isozyme that produces 5,6-EET. These results suggest that injured astrocytes release soluble factors that upregulate SOC activity in MG, possibly via increased microglial cytochrome P450 activity and production of 5,6-EET. Supported by NS40490.

P107.

LOCAL TREATMENT WITH PHOSPHOCREATINE IMPROVES INJURY-INDUCED METABOLIC AND ELECTROPHYSIOLOGICAL CHANGES AFTER TBI.

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Introduction: Energy failure is a ubiquitous complication after traumatic brain injury (TBI). We have studied whether phosphocreatine (CrP), a high energy substrate, delivered through a microdialysis probe, would ameliorate TBI-induced metabolic and electrophysiological changes.

Material and Methods: Adult Sprague-Dawley rats were submitted to 2.1 ± 0.05 atm fluid percussion injury. A custom probe (4 mm microdialysis membrane with attached recording electrodes) was inserted in the injured hemisphere. After 1 hour of baseline recording, using 0.9% NaCl perfusion, 10 mg/ml CrP was added to the perfusion solution, and measurements continued for 3 hours. Brain biopsies under frozen conditions were obtained for tissue adenosine triphosphate (ATP) measurements.

Results: Local treatment with CrP resulted in 75(±5)% increases in dialysate glucose ($p < 0.05$), 60(±4)% decreases in lactate ($p < 0.001$), and 70(±]6)% decreases in glutamate ($p < 0.001$). Additionally, multiunit neuronal firing rate was increased, as well as brain ATP levels.

Conclusions: Local treatment with CrP seems to ameliorate brain energy metabolism by bolstering intracellular "energy" substrates, and possibly through a stabilizing effect of creatine on mitochondria permeability transition pore.

Supported by NS 12587 and FCT no SFRH/BD/3421/2000.

P108.

HEME OXYGENASE-2 PREVENTS LIPID PEROXIDATION-MEDIATED CELL LOSS AND PROMOTES FUNCTIONAL RECOVERY AFTER TRAUMATIC BRAIN INJURY

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After traumatic brain injury, extracellular heme, derived from both hemorrhage and cell injury causes oxidative stress to neuronal tissue. Oxidative stress, induced by free radical reactions and lipid peroxidation, has been implicated as a key mechanism of secondary traumatic brain injury. Heme oxygenase (HO) has been proposed as a critical mediator of the detoxification of heme, because it metabolizes the pro-oxidant heme to the potent antioxidant, bilirubin. It is currently unclear what role HO-2, the predominant and constitutively expressed isozyme in neurons, plays in traumatic brain injury. We used HO-2 knockout mice to determine the extent and mechanism of damage following controlled cortical impact (CCI) injury. Based on NeuN (neuronal nuclei antibody) immunohistochemical cell counting, regional cell loss was more severe in knockout than in wildtype animals, especially in the peritraumatic cortex and thalamus. In addition, HO-2 knockout mice demonstrated significantly limited recovery on rotarod and inclined beam walking tasks, suggesting compromised motor function and behavior. Brain sonicates of knockout mice revealed significantly less total HO activity than wildtype littermates. Knockout mice also demonstrated decreased ability to reduce oxidative stress, as measured with an Fe2+/ascorbic acid-mediated CO generation assay for lipid peroxidation. Finally, Western blots showed that the low HO-1 expression did not change in injured HO-2 knockout animals. These findings suggest that HO-2 activity protects neurons in the setting of traumatic brain injury by reducing lipid peroxidation, possibly through its catabolism of heme.

Supported by NS 14543 and the UCLA Neurotrauma Initiative.

P109.

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY ASSESSMENT OF F2-ISOPROSTANE LEVELS IN CSF AFTER TRAUMATIC BRAIN INJURY IN RATS

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Traumatic brain injury (TBI) causes 50,000 deaths/year. Although recent studies indicate that oxidant injury may be deleterious, progress in this area has been hampered by lack of adequate biomarkers for oxidant injury. F2-isoprostane is a stable peroxidation product of cell membrane phospholipids that increases dramatically during oxidant injury. The objective of our study was to provide a quantitative and sensitive assessment of F2-isoprostane levels in CSF after TBI.

A cortical impact injury device was used to produce TBI in rodents (n = 30). Sprague-Dawley rats (280–300 g) were anesthetized with isoflurane and mounted in a stereotactic frame. A craniotomy was performed, and TBI was produced by impacting the cortex with a 5 mm diameter impactor tip at a velocity of 3.5 m/s with a 1.6 mm compression and 150 ms dwell time. Sham-injured animals (n = 30) underwent identical surgical procedures without receiving an impact injury.

Methods: CSF was collected at—preset time intervals after sham-injury or TBI. F2-isoprostane levels were measured with a stable isotope dilution assay using capillary gas chromatography / negative ion chemical ionization mass spectrometry. Investigators performing measurements were blinded to the experimental condition the animals. Group means were compared by one-way analysis of variance where $p < 0.05$ was considered statistically significant.

Results: Mean (±SD) F2-isoprostane CSF levels for sham-injured vs. TBI animals were 11 ± 10 vs. 36 ± 18 at 30 min post-injury ($p = 0.003$), 1 ± 2 vs. 91 ± 69 at 2 hours post-injury ($p = 0.03$), and 4 ± 4 vs. 33 ± 13 (6 hours after TBI) ($p = 0.0003$), respectively.

Conclusion: Compared to the sham-injured group, CSF F2-isoprostane levels were significantly higher in the TBI group at 30 minutes, 1 h and 6 h after TBI. These results indicate that oxidant injury occurs rapidly after TBI, and that assessment of F2-isoprostane levels in CSF can provide a quantifiable and sensitive measure of oxidant damage. (Supported by DAMD17-99-1-9565, DAMD17-01-1-0765, NIH R01 NS39091, and NIH R01 NS40182)

P110.

TEMPORAL AND SPATIAL PROFILE OF PHOSPHORYLATED MITOGEN-ACTIVATED PROTEIN KINASE PATHWAYS FOLLOWING LATERAL FLUID PERCUSSION BRAIN INJURY IN RATS

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(Introduction) Mitogen-activated protein kinases (MAPK), which play a crucial role in signal transduction, are activated by phosphorylation in response to a variety of mitogenic signals. The MAPK cascades are composed of extracellular signal-regulated protein kinase (ERK), c- Jun NH(2)-terminal kinase (JNK), and p38 pathways. The aim of this study was to investigate the temporal and topographic expression of the activated MAPK pathways after traumatic brain injury (TBI) in rats. (Material & Methods) Adult male Sprague-Dawley rats (300–400 g) were subjected to lateral fluid percussion injury of moderate severity (3.5–4.0 atm) using the Dragonfly device model (No. HPD-1700). The phosphorylated- or total-MAPKs protein level 5, 15, 30 min, 1, 6, 24, 72 hrs after TBI was quantified using Western blot analysis in each the cortical or hippocampal tissue. Topographic distribution of immunoreactivity for p-MAPKs was examined using immunohistochemistry at the same time course. (Results) TBI significantly increased the p-ERK and p-JNK levels, but not the p-p38 protein levels. The immunoreactivity for p-JNK was uniformly induced regardless of any regional selective vulnerability to TBI. In contrast, the immunoreactivity for p-ERK was confirmed up until 30 min after TBI in the superficial neuronal layers, and was not detected in the CA1 neurons, but was localized in the dentate hilar and the damaged CA3 neurons after 30 min of TBI. Double immunostaining using a glial-specific marker demonstrated that p-ERK was prominent in astrocytes 6 hrs after TBI. (Conclusion) The current results suggest that the ERK and JNK pathways, but not the p38 MAPK pathways are involved in signal transduction after TBI. Strong immunoreactivity for p-ERK was observed in the dentate hilar and the CA3 pyramidal neurons, selective hippocampal vulnerable lesions to TBI. These findings suggest that a distinct MAPKs cascade might therefore participate in the selective vulnerability after TBI.

P111.

TRANSPLANTATION OF NGF-EXPRESSING NT2N NEURONS ATTENUATES A LEARNING DEFICIT FOLLOWING CONTROLLED CORTICAL IMPACT BRAIN INJURY IN MICE

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In this study, we tested the hypothesis that nerve growth factor (NGF)-expressing NT2N neurons transplanted into the basal forebrain of brain-injured mice can attenuate long-term cognitive dysfunction by protecting the NGF-responsive cholinergic neurons of the septo-hippocampal pathway. Undifferentiated NT2 cells were transduced with a lentiviral vector to release NGF (0.2 ng/hr/10(4) cells), differentiated into NT2N neurons by exposure to retinoic acid and transplanted (20,000 cells in 2 ul) into the medial septum of mice 24 hours following controlled cortical impact (CCI) brain injury or sham injury in anesthetized mice. Mice (n = 78) were randomly assigned to one of four groups: 1) sham (operated but uninjured) injected with vehicle; 2) brain-injured injected with vehicle; 3) brain-injured injected with untransduced NT2N neurons; 4) brain-injured injected with transduced NGF-NT2N neurons. Cognitive function (learning) was evaluated with the Morris Water Maze at 4 weeks post-injury/surgery. Sham injured mice performed better than the brain-injured mice ($p < 0.01$). Cognitive function of the brain-injured group engrafted with NGF-NT2N neurons was significantly better than the injured group receiving vehicle ($p < 0.05$) or untransduced NT2N transplants ($p < 0.01$). These data suggest that ex-vivo gene therapy may attenuate cognitive dysfunction following traumatic brain injury. Supported by a Merit Review grant from the Veterans Administration, NIH PO1-NS08803, NIH RO1-NS40978, NIH DK42707 and NS38690

P112.

NEURAL PROGENITOR CELL TRANSPLANTS SHOW LONG-TERM SURVIVAL AND ENHANCE BEHAVIORAL RECOVERY IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY

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The goal of this study was to assess whether neural progenitor cell (NPC) transplants could enhance recovery from behavioral deficits resulting from traumatic brain injury (TBI). NPCs were derived from E14.5 mouse brains containing a transgene for an actin promotor tagged with green fluorescent protein (GFP) and cultured as neurospheres in FGF-containing medium. NPCs were injected into the ipsilateral striatum of adult C57/BL6 mice 1 wk following unilateral cortical impact injury. Motor and spatial learning abilities were assessed on a rotorod task and in a Morris water maze (MWM) task over a 12-month period. Significant improvements in motor abilities were observed in NPC-treated mice as early as one week and were sustained out to 12 months post-transplant. In addition, NPC-transplanted mice showed significant improvement in spatial learning abilities at 3 months, whereas an intermediate treatment effect was detected at 1 and 12 months. Following behavioral testing, animals were perfused and NPC survival, migration, and differentiation were assessed. Initially, NPCs remained near the injection site and subsequently migrated into the penumbra surrounding the injured hippocampus where they were observed at 3 and 12 months post-transplant. Confocal microscopy revealed that transplanted GFP+ NPCs co-label for NG2 but not for neuronal, astrocytic, or microglial markers, suggesting that these cells are NG2+ oligodendrocyte progenitor cells. In conclusion, transplanted NPCs survive in the host brain up to 12 months, enhance motor and cognitive recovery, and may play a role in remyelination following TBI.

Acknowledgments: Support for this project was generously provided by an NSF Fellowship (to DAS) and a Field Neurosciences Institute Research Gift (to DGS).

P113.

ACTIVATED EGFR SIGNALING AND TRANSPLANTED NEURAL STEM CELL MOTILITY

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The epidermal growth factor receptor (EGFR) has been shown to regulate migration in stem cells, through direct and indirect mechanisms. To test the hypothesis that activated EGFR signaling enhances transplanted stem cell motility we transduced neural stem cells with the constitutively active EGFR (EGFRvIII).

The ligand independent, constitutively active EGFRvIII receptor (and empty vector controls) was stably transfected using the lipofectamine reagent into the C17.2 mouse neural stem cell line. The C17.2 EGFRvIII cells (15,000 cells/ml; 2ml per injection; one injection site per animal) and empty vector controls were then transplanted into anesthetized (sodium pentobarbital 60mg/kg), immunosuppressed (Cyclosporin A 10mg/kg i.p injection), uninjured (n = 12/cell type) adult Sprague-Dawley male rats and into rats subjected to lateral fluid percussion (FP) brain injury (n = 12/cell type). All stereotactic injections were done into the corpus callosum (Bregma +1ML, 2.2DV, −4.5 AP) contralateral to the site of injury.

At 2 weeks post transplant, all animals were sacrificed under anesthesia and assayed by X gal immunohistochemistry and by immunoreactivity to rabbit anti-EGFRvIII (kind gift of Albert Wong, M.D., Philadelphia, PA). At 2 weeks post transplant, only C17.2 EGFRvIII-overexpressing cells were found to survive and migrate extensively in all transplanted animals. In the animals subjected to lateral FP injury, transplanted C17.EGFRvIII stem cells were seen crossing the corpus callosum (migrating as far as far as 2 mm from the transplant site) and entering the injury cavity. Many cells were found within the injury cavity as well. In none of the C17.2 empty vector transplants were cells seen to cross the corpus callosum. In naïve (uninjured) animals, the empty vector C17.2 cells were also non-migratory. Membrane EGFRvIII expression was seen to remain robust in the transplants after 2 weeks in vivo.

These studies suggest that activated EGFR signaling may contribute to a motile phenotype in neural progenitor cells in vivo. By enhancing EGFR signaling on stem cells ex vivo, these highly migratory stem cells may represent a better source of cells for neuro-transplantation following CNS injury.

P114.

INHIBITION OF NOGO-A IMPROVES RECOVERY OF NEUROMOTOR AND COGNITIVE FUNCTION FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY IN RATS

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Severe traumatic brain injury (TBI) leads to long-term neuromotor and cognitive deficits, and only limited recovery occurs in human patients. The inability of the central nervous system (CNS) to regenerate appears to be due, in part, to inhibitory molecules associated with myelin. The role of one of these myelin-associated proteins, Nogo-A, has been extensively studied. Nogo-A inhibits neurite outgrowth in vitro, and blockade of this molecule, in vivo, leads to functional recovery as well as regeneration and plasticity of the injured CNS.

We subjected rats to a lateral fluid percussion (FP) brain injury, a well characterized model of TBI, and administered a novel and purified monoclonal antibody against Nogo-A (mAb 11C7C7, kindly provided by Novartis, Basel Switzerl.) for two weeks intracerebroventricularly (ICV). Rats were assessed behaviorally using several neuromotor function tests up to 4 weeks post injury. Brain injured rats receiving mAb 11C7C7 recovered significantly better than animals receiving a control antibody. Interestingly, a test assessing predominantly sensorimotor function (adhesive paper test) was not influenced by the type of antibody used. Furthermore, Nogo-A inhibition significantly improved performance of brain injured rats in a spatial learning task in the Morris water maze at 4 weeks post injury. However, using anterograde tract tracing methods with biotinylated dextran amine (BDA), we were unable to observe an improvement, due to mAb 11C7C7, of sprouting of intact corticospinal and corticopontine fibers into the denervated side of the spinal cord. These findings indicate that neutralization of inhibitory molecules in the CNS may improve recovery from traumatic brain injuries. Supported, in part, by a Veterans Administration Merit Review grant. PML was supported by a NIH training grant on NRSA T32 NS07413-04 and a Research Fellowship by the Swiss National Science Foundation (SNF).

P115.

VOLUNTARY EXERCISE THERAPY AFTER TBI: A CRITICAL WINDOW OF OPPORTUNITY.

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Voluntary exercise leads to an upregulation of brain derived neurotrophic factor (BDNF) in the rat. This activity-induced enhancement of neuroplasticity may be considered for the treatment of TBI. During the first postinjury week, the brain is undergoing dynamic restorative processes and metabolic changes that will strongly influence the outcome of exercise. Therefore, the postinjury timing of exercise is crucial. To address this, rats underwent either sham or fluid-percussion injury (FPI) and were housed with or without access to a running wheel (RW), from postinjury day 0–7 (acute) or 7–14 (delayed). Rats were cognitively assessed in the Morris Water Maze after RW exposure. As reported previously non-injured animals benefited from RW exposure. RW exposure also proved to be beneficial in the delayed FPI-RW animals, as indicated by a 52% improvement in the MWM, compared to the FPI-sedentary rats. However, cognitive performance in the acute FPI-RW rats was impaired. Whereas the sham exercised animals showed an improvement, the acute FPI-RW rats were impaired compared to all the other groups ($p < 0.05$). No difference was observed between the FPI and sham-sedentary rats. Exercise was related to increased levels of hippocampal BDNF in the sham ($p < 0.05$) but not in the acute FPI-RW rats. Increased levels of hippocampal synapsin I ($p < 0.001$) and cyclic AMP element-binding-protein (CREB) ($p < 0.001$) were present in the FPI-sedentary rats at postinjury day 7. In this group, regression analysis indicated a strong relationship between phosphorylated CREB and phosphorylated synapsin (R-squared ipsi: 0.9; contra: 0.8). However, in the acute-RW-FPI rats a decrease in phosphorylated synapsin I ($p < 0.005$) and CREB ($p < 0.05$) was seen. These results are opposed to those in the RW-sham, in which exercise was associated to an increase of synapsin I and CREB. These results suggest that even voluntary exercise can be deleterious when administered to soon after injury. (:NS30308, NS27544, NS38978, BIRC).

P116.

UP-REGULATION OF THE CELL CYCLE/ INHIBITOR OF APOPTOSIS PROTEIN SURVIVIN IN ASTROCYTES AND NEURONS AFTER TBI IN RATS.

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This investigation examined mRNA and protein up-regulation of the cell cycle/ inhibitor of apoptosis protein survivin after traumatic brain injury (TBI) in rats. Additionally, survivin cell subtype expression was determined. Levels of survivin mRNA and protein were significantly elevated in the ipsilateral cortex and hippocampus after injury as compared to sham injured control rats. These levels increased at 1 day, peaked at 5 days and returned to baseline by 14 days post injury in both regions. To determine if survivin up-regulation is correlated with the up-regulation of other cell cycle proteins, western blots of the cell cycle associated proteins survivin and proliferating cell nuclear antigen (PCNA) were compared. PCNA showed significantly elevated protein levels with a similar expression pattern to survivin. With immunohistochemistry (IHC), both neurons and astrocytes showed survivin immunoreactivity in the ipsilateral cortex. Additionally, survivin localized to neurons in the contralateral hippocampus and astrocytes in the ipsilateral hippocampus. Interestingly, although the majority of survivin positive cells were astrocytes that are known to proliferate after injury, a small population of survivin-positive neurons was identified. Future studies will investigate whether these neurons express other cell cycle proteins in an attempt to initiate a cell cycle related program in response to TBI. (Supported by DAMD17-99-1-9565, DAMD17-01-1-0765, NIH R01 NS39091 and NIH R01 NS40182).

P117.

A SUBPOPULATION OF MITOTICALLY-ACTIVE CELLS MIGRATE ECTOPICALLY FROM THE ANTERIOR SUBVENTRICULAR ZONE FOLLOWING EXPERIMENTAL BRAIN INJURY

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Neuronal precursors in the adult rodent anterior subventricular zone (SVZ) proliferate, migrate to the olfactory bulb via a restricted pathway known as the rostral migratory stream (RMS), and differentiate into neurons. A thorough understanding of these processes is necessary to utilize the SVZ as a source of neuronal and glial precursors for genetic manipulation, transplantation, or brain self repair following CNS injury. In the current experiment, we sought to determine whether the normal migratory pattern of SVZ-originating neural precursors was altered following experimental brain injury. Anesthesized mice (n = 30) were subjected to controlled cortical impact (CCI) brain injury (5 m/s, 0.5 mm), injected intraperitoneally (ip) with 50 mg/kg bromodeoxyuridine (BrdU) three times over a six-hour period beginning seven days postinjury, and then sacrificed at 0, 3, 7, 14, or 21 d post BrdU injection. Control mice were anesthesized and surgically prepared and received the identical BrdU administration paradigm without brain injury. Immunohistochemical analyses were performed with antibodies directed to BrdU; nestin (a cytoskeletal protein associated with stem cells); doublecortin (Dcx; a microtubule-associated phosphoprotein expressed in migrating neuroblasts); glial filament acidic protein (GFAP), and class III beta-tubulin (TuJ1; a tubulin isoform expressed by immature and mature postmitotic neurons). In addition to the RMS and olfactory bulb, in brain-injured animals Dcx+, BrdU+ and Nestin+, BrdU+ cells were localized to the anterior periphery of the injury cavity, organized in a chain-like formation originating at the same ventricular location as the RMS, and ensheathed by astrocytes after 7 d post BrdU administration. These data suggest that normal cues responsible for directed migration of SVZ-residing neural precursors may be disturbed following CNS injury.

Supported, in part, by NIH NS40978, NS08803, GM34690, MH17168, and a Veterans Administration Merit Review grant.

P118.

GENDER DIFFERENCES IN COGNITIVE RECOVERY AFTER INTERVENTION WITH ENVIRONMENTAL ENRICHMENT FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY

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Environmental enrichment clinically after traumatic brain injury (TBI), and enrichment of the housing environment has been shown to improve spatial memory after experimental TBI in male rat models, and therefore may have some parallel to receiving rehabilitation. However, the impact of gender on how environmental enrichment affects behavioral performance after experimental TBI has not been studied. Therefore, the purpose of this study was to examine the therapeutic effect of environmental enrichment on post TBI recovery in both male and female rats. Male (n = 32) and normally cycling female (n = 33) Sprague-Dawley rats underwent either controlled cortical impact (2.7 mm, 4.0 m/s) or sham injury and were housed in either standard or enriched environmental conditions, which consisted of novel and social living conditions as well as gustatory, olfactory, tactile and visual stimulation. There were no differences in peri-injury plasma estrogen (n = 15) and progesterone (n = 16) levels for injured females in each of the housing conditions. Motor function was assessed both pre-injury and for the first 5 days after injury. Spatial memory was assessed beginning 14 days after injury using the Morris Water Maze task. Repeated measures ANOVA post-hoc analysis showed that enriched injured males exhibited significantly shorter latencies to find the hidden platform than standard injured males ($p = 0.0165$). Surprisingly, enriched injured females performed worse than enriched injured males on this task ($p = 0.0252$). Enrichment did not improve cognitive recovery in injured females, as they performed no differently than other injured groups in the standard housing environment. Enrichment did not affect motor performance for either males or females. These results suggest that environmental enrichment after TBI beneficially affects cognitive recovery for male, but not female rats. More work is needed to determine the effect of sex hormones and environmental enrichment on cortical plasticity and specific tissue markers of neurotransmission that influence spatial memory. K08HD40833, RO1NS40125, NIDRR#H133P970013-00

P119.

EXTRACELLULAR SIGNAL-RELATED KINASE/MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION IS CRITICAL FOR ASTROCYTE PROCESS EXTENSION AND MIGRATION IN THE SETTING OF BRAIN INJURY

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Extracellular signal-related kinase (ERK/MAPK) is a member of the MAP kinase family involved in diverse cellular functions including apoptosis, motility, and differentiation. We have previously shown that reactive astrocytes in a variety of human neuropathologies exhibit chronic activation of ERK/MAPK. We hypothesized that activation of ERK/MAPK is involved in the induction/maintenance of reactive phenotypes. Accordingly, we characterized activated ERK immunoreactivity (pERK-IR) from 1h-30d after a forebrain stab lesion (FSL) in the adult C57bl/6 mouse. Perilesional neurons demonstrated pERK-IR primarily at the 1h timepoint, whereas staining in astrocytes was found at all timepoints peaking between 3d-7d. pERK-IR in astrocyte processes could be appreciated from 1d-30d. We tested the functional relevance of pERK-IR in cell processes in vitro with the specific MAP kinase kinase (MEK1/2) inhibitor U0126 (20mM). MEK1/2 blockade inhibited process extension after replating of both primary astrocytes and C6 glioma cells. U0126 also attenuated growth factor-stimulated migration in a scratch-wound model. To evaluate the role of ERK/MAPK in astrocyte process extension and migration in vivo we administered a blood-brain-barrier permeant analogue of U0126, SL327 (100mg/kg IP; 2-3h half-life), to mice twice daily for 6d beginning 1d after FSL. Mice were sacrificed at 7d, 12h after the final dose. In vehicle-treated animals, glia arising from the medial wall of the ipsilateral lateral ventricle demonstrated polarized morphology suggestive of migration, pERK-IR in nuclei and leading cell processes, and were found throughout the septal parenchyma. These cells were also immunoreactive for phosphorylated EGF-receptor and ezrin, known mediators of cell motility. Corresponding glia from SL327-treated animals demonstrated nuclear pERK-IR, but were negative for ezrin and phosphorylated EGF-receptor. Further, they exhibited non-polar morphologies, lacked cell processes, and were clustered subependymally. Taken together, we demonstrate a critical role for the ERK/MAPK cascade in process extension and migration of astrocytes after brain injury.

P120.

CO-ACCUMULATION OF AMYLOID-BETA, BETA-SECRETASE, AND PRESENILIN-1 IN CULTURED AXONS FOLLOWING STRETCH INJURY

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We have previously found that amyloid-beta (A-beta) accumulates in damaged axons following brain trauma in humans and animal models. In Alzheimer's disease (AD), A-beta is thought to be primarily produced via transmembrane cleavage of amyloid precursor protein (APP) by beta-secretase (BACE) and presenilin-1 (PS-1). However, the source intraaxonal A-beta following trauma is unknown. Using a cultured axonal injury (CAI) system, we examined potential co-accumulation of A-beta with BACE and PS-1 in damaged axons. CAI was induced by rapid selective stretch of axons bridging a 2 mm gap between two populations of human neurons (N-tera2-N). For each stretch, the rise time 20ms, duration <50ms, and uniaxial strain of 75%. The cultures were then either fixed with 4% paraformaldehyde or frozen with selective protein extraction of the axons in the gap and from the culture media at 0, 3, 6, and 24 h following injury. Double immunohistochemisty using highly specific antibodies was performed to detect co-localization of APP, A-beta, BACE, and PS-1. ELISAs were performed to detect an increase of A-beta1-40 and A-beta1-42 in extracts from the media and axons. We found that CAI induced accumulation of A-beta in axonal swellings remarkably similar in appearance to that observed in humans and animals following brain trauma. In addition, we observed that this A-beta co-localized with APP, PS-1, and BACE in axonal swellings. Moreover, we detected an increase of both A-beta1-40 and A-beta1-42 in the media and in homogenates of axons for at least 24h following injury. These results demonstrate that axonal trauma in vitro can induce the production of A-beta. Like AD, this process may depend on BACE and PS-1 cleavage of APP. Surprisingly, however, this activity appears to occur within the axonal membrane compartment. These data may have important implications for the link between brain trauma and AD. Supported by NIH grants AG21527 and NS38104.

P121.

IDENTIFICATION OF MULTIPLE DISTINCT PATHOLOGIC NEURONAL PHENOTYPES WITHIN DIFFUSELY INJURED BRAIN

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Although traumatic brain injury (TBI) is known to evoke axonal injury (TAI), resulting in delayed axotomy, little is understood regarding the neuronal somatic response to either the diffuse mechanical forces of injury or the retrograde sequelae of TAI. We have recently demonstrated that neuronal somata axotomized by TAI do not progress to cell death within 7 days postinjury, suggesting the potential for recovery. However, in these foci, a distinct population of neurons that did not sustain TAI revealed rapid degenerative changes suggestive of cell death. To better understand these differing primary somatic and delayed/secondary retrograde neuronal responses, we subjected rats to moderate central fluid percussion TBI, followed by perfusion fixation at varying times over 30d postinjury. Antibodies to amyloid precursor protein were used to detect axotomy, antibodies to the 70kD heat shock protein (HSP-70) and phosphorylated eukaryotic translation initiation factor 2 alpha [eIF2alpha(P)] were used to detect potential neuronal perturbation and recovery, and TUNEL as well as antibodies to single stranded DNA were used to detect apoptotic cell death. Fluoro-Jade (FJ) was used as a more generalized marker of cell death. Both single and double labeling immunocytochemical strategies revealed TAI-linked somata that colocalized with eIF2alpha(P), and, infrequently, with HSP-70 in the early postinjury period. After 72h, the increased expression of both HSP-70 and eIF2alpha(P) returned to sham levels, suggesting recovery. Apoptotic labeling was rare, with no apoptotic cells correlating with either axotomized neurons or with HSP-70 or eIF2alpha(P) labeled somata. Some adjacent somata, however, stained positively with FJ within 7d postinjury, suggesting the presence of necrotic cell death. Collectively, these findings emphasize the occurrence of diffuse somatic injury following TBI that involves a spectrum of pathological change, ranging from cell perturbation with the potential for recovery to overt cell death.

P122.

RELATIONSHIP OF 40kD, 10kD, AND 3kD FLUORESCENT INDICATORS OF ALTERED AXOLEMMAL PERMEABILITY TO IMPAIRED AXOPLASMIC TRANSPORT IN TRAUMATIC AXONAL INJURY.

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Traumatic axonal injury (TAI) evolves within minutes to hours following traumatic brain injury (TBI). Previous studies have identified axolemmal disruption and impaired axoplasmic transport (AxT) as key mechanisms in the evolution of TAI. While initially hypothesized that axolemmal disruption led to impaired AxT, recent studies employing antibodies to amyloid precursor protein (APP) to identify impaired AxT and 40kD fluorescently-tagged dextrans to identify axolemmal disruption suggest these processes occur within distinct populations of TAI.

Building on these studies, the current investigation employs smaller molecular weight (MW) dextrans to determine whether more subtle alterations of the axolemma may co-localize with impaired AxT. Specifically, rats were administered an intrathecal mixture of either 40kD+10kD or 40kD+3kD fluorescently-tagged dextrans, with brains subsequently prepared for APP immunofluorescence. APP and all MW dextrans consistently localized to two distinct classes of TAI. The first class demonstrated influx of all MW dextrans across a damaged axolemma, was thin and elongate, sometimes vacuolated, and revealed little progressive change over time. The second class was distinguished by the presence of APP alone within swollen axons at early time-points and APP + all MW species of dextrans within disconnected axonal bulbs at later time-points. Interestingly, there was no co-localization of smaller MW dextrans with APP prior to disconnection.

These studies confirm axolemmal disruption and impaired AxT are distinct events early in TAI. Further, these studies provide evidence that the process of impaired axoplasmic transport and subsequent axonal disconnection leads to delayed axolemmal instability, rather than being a consequence of initial axolemmal failure. This finding underscores the need of multiple approaches to fully assess the axonal response to TBI. Supported by the Commonwealth Neurotrauma Initiative.

P123.

QUANTITATIVE DIFFUSION WEIGHTED IMAGING ANALYIS OF CELL-PERMEANT CALCIUM BUFFER INDUCED NEUROPROTECTION AFTER CORTICAL DEVASCULARIZATION IN RATS

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An excitotoxic cascade resulting in a significant intracellular calcium load is thought to be the primary mechanism leading to neuronal death. One way to protect neurons from injury is through the use of cell-permeant calcium buffers (CPCBs). These molecules have been reported to be neuroprotective via their ability to increase the cell's overall Ca2+ buffering load as well as by attenuating neurotransmitter release. We used the CPCB, 2-aminophenol-N, N, O-triacetic acid acetoxymethyl ester (APTRA-AM), to determine its effectiveness in providing neuroprotection after a cortical devascularization injury. Injured animals were given two intravenous injections of saline, DMSO, or APTRA-AM at 1 and 12 hours after injury. Animals were imaged on a Bruker 4.7 T MRI using a diffusion-weighted imaging sequence prior to injury and at 12, 24, 48 hours, 3 and 7 days after injury. Correlative histological and immunocytochemical studies were performed on euthanized rats 7 days after injury. In saline treated rats, a decrease in the apparent diffusion coefficient (ADC) of the injured area was observed by 12 hours after injury, and remained below pre-injury values throughout the next 7 days. DMSO treated rats also exhibited a decreased ADC within the injured area. In contrast, animals injected with APTRA-AM showed almost no change in the ADC of the injured area. APTRA-AM also significantly reduced infarct volume and inflammatory cell infiltration. The results presented here clearly demonstrate the effectiveness of APTRA-AM in preventing neuronal cell death and the accompanying inflammatory response which further contributes to our understanding of the mechanisms associated with post injury inflammation and infarct development in brain injuries.

P124.

SPINAL CORD OLIGODENDROGLIA EXPRESS ACTIVATED CASPASE-3 FOLLOWING K+ INDUCED DEPOLARIZATION AND NMDA EXPOSURE

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Despite evidence of widespread apoptosis of neurons and glial cells following spinal cord injury (SCI), the extra- and intracellular molecular signals that activate the apoptotic pathway remain poorly understood. Soon after injury, the spinal cord is exposed to numerous secondary insults, including elevated levels of glutamate that contribute to cell dysfunction and death. While glutamate mediated excitotoxicity is typically associated with necrosis, recent studies suggest a role for glutamate in apoptosis. In this present study, we examined the actions of glutamate by performing intrathecal injections of the selective glutamate receptor agonist, N-methyl-D-aspartate (NMDA), into uninjured rat spinal cord. Even though oligodendroglia are believed to contain only AMPA/ kainate receptors, immunohistochemical colocalization studies demonstrated a significant increase in the percentage of oligodendroglia exhibiting activated caspase-3 in comparison to aCSF controls at 4 hours following NMDA injection ($p < 0.05$, $n = 6$). At the later time points examined (24 and 96 hours following injection), there was no evidence of caspase-3 activation in this cell type. However, significant oligodendroglia loss was present at 96 hours following NMDA exposure in comparison to aCSF controls ($p < 0.03$, $n = 6$). In an attempt to better define a mechanism for the action of NMDA in oligodendroglia, we examined the effect of spinal cord depolarization induced by intrathecal injections of high K+ aCSF (50 mM KCl) on caspase-3 activation. Infusions of high K+ aCSF also resulted in caspase-3 activation in oligodendroglia, suggesting one putative and indirect mechanism by which NMDA may lead to caspase-3 activation in oligodendroglia. Supported by: PHS Grant NS40015 and KSCHIRT.

P125.

DIFFERENTIAL GENE EXPRESSION PROFILING IN THE EMBRYONIC AND ADULT-INJURED SPINAL CORDS

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The failure of descending pathways to regenerate after spinal cord injury (SCI) may be due to the failure of the injured spinal cord to express genes that promoted descending axonal growth and targeting during development and/or the expression of genes in the injured spinal cord that create an environment hostile to regeneration. An indication of the differing abilities of the embryonic and adult-injured spinal cords to support regeneration is offered by studies showing that wile the adult spinal cord is inhospitable to the regeneration of brain-spinal cord connections, an embryonic spinal cord grafted into the site of injury promotes this regrowth and regeneration. We hypothesize that these differing regenerative abilities of the adult-injured spinal cord and the embryonic spinal cord will be reflected by differences in their gene expression. The purpose of this study was to identify three classes of genes: 1. Genes expressed only in the embryonic spinal cord (may have an ameliorating effect on SCI recovery), 2. genes expressed solely in the injured cord (may have a maladaptive role in SCI) and 3. genes-common to embryonic and injured but not to a normal tissue (indicative of the reactivation of embryonic genetic programs). Three subtracted cDNA libraries were created to isolate these gene populations. A novel three-way approach to subtractive hybridization was used. To identify genes in each library, the subtracted cDNA populations were fluorescently labelled and used as microarray probes. Differentially expressed genes, as detected by clustering analysis of subtracted cDNA populations, include entire classes of genes (pro necrotic genes were found only in injured tissue) and different genes within a single class (different cell adhesion molecules were found in all three cDNA populations). Putative roles of differentially expressed genes in SCI is also discussed.

P126.

RAPID FUNCTIONAL RECOVERY AFTER THORACIC SPINAL CORD INJURY IN YOUNG RATS

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Glutamate excitotoxicity contributes to secondary injury after trauma in the adult CNS. Glutamate receptor subunit protein expression is elevated during the first 2–3 weeks after birth as compared to that in adults. This relative "overexpression" of glutamate receptors could contribute to differences in both secondary injury processes and plasticity related to functional recovery in young animals. To investigate this hypothesis, we modified an adult model of spinal cord injury (SCI) for use in Sprague-Dawley rats at postnatal day 14–15. A laminectomy was performed at the T8–T9 vertebral level, a 1.5 mm diameter impounder was lowered onto the dura, and a contusion was produced with a 10 g weight dropped from heights of 2.5 or 5 cm. Hind limb function was evaluated 24 hours later and at 1, 2, 3 and 4 weeks with the Combined Behavioral Score to estimate overall hind limb sensory-motor function and the BBB rating scale for open field locomotion. Results showed that rats injured at P14–15 with a 5 cm weight drop (n = 10) exhibited significantly less recovery of function at 4 weeks than those rats injured with a 2.5 cm weight drop (n = 27). The degree of hind limb deficit at 4 weeks was similar to that previously described in adults with comparable SCI. However, animals injured at P14–15 exhibited a significantly faster rate of recovery than adults. Recovery in the young rats was maximal by 1–2 weeks as compared to 3–4 weeks in adults subjected to similar SCI. The rapid recovery in young rats suggests that this model may be useful to study potential mechanisms of recovery after incomplete contusion injury in the spinal cord. (NIH R01 NS 37733)

P127.

BENEFICIAL EFFECT OF AN EARLY ANTI-INFLAMMATORY STRATEGY AFTER ACUTE SPINAL CORD INJURY: COMPARISON TO THE EFFICACY OF METHYLPREDNISOLONE

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We propose that targeted inhibition of early inflammation due to hematogenous infiltrates remains an important first step in neuroprotection after spinal cord injury (SCI). In this study we hypothesized that suppression of leukocyte extravasation early after SCI would lead to improved neurological outcomes. In accordance with our goal of reducing the early inflammatory response, we used a paradigm of treatment [saline or monoclonal antibody (mAb) to the aD subunit of B2 integrin or methylprednisolone (MP) or combined mAb/MP] administration in the first three days after clip-compression SCI at the 4th thoracic segment in rats. The outcome of treatment with this selective mAb was compared with that after treatment with the pleiotrophic MP. We assessed neurological outcomes using BBB open field locomotor scores and evaluation of autonomic dysreflexia. We used histological methods to assess the amount of spared tissue and the pathological changes below the injury site. Mean arterial pressure (MAP) induced by colon distension was used to assess autonomic dysreflexia. Baseline MAP was similar in all groups (~110 mmHg). MAP increased by 37 ± 3 mmHg in untreated rats (n = 14), by only 27 ± 3 mmHg after mAb treatment (n = 11), by 26 ± 2 mmHg after MP (n = 7) and by 28 ± 2 mmHg after mAb/MP treatment (n = 7). mAb treatment improved BBB locomotor scores from 3 ± 1 to 6.7 ± 1 whereas MP or the mAb/MP treatment did not improve them. The amount of white matter was greater in mAb-treated rats throughout the lesion site than in untreated rats. MP alone had little impact on the lesion size. Addition of MP to the mAb treatment paradigm diminished the effect of the mAb on lesion size. In conclusion, the outcome after mAb treatment was clearly superior to that after corticosteroid therapy and demonstrates that early selective intervention in the inflammatory process after SCI can be highly beneficial. Support: Ontario Neurotrauma Foundation and ICOS.

P128.

OLFACTORY ENSHEATHING CELLS PROMOTE ROBUST AXON GROWTH FOLLOWING COMPRESSIVE SPINAL CORD INJURY

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Strategies to promote axonal regeneration and functional recovery after spinal cord injury (SCI) have included application of exogenous neurotrophic factors, neutralizing the inhibitory environment of the CNS, and implantation of various cell types at the site of SCI, such as olfactory ensheathing cells (OECs). We have utilized a clinically relevant model of compressive spinal cord injury in order to characterize the interaction between fetal rat OECs and injured spinal cord tissue. One week following injury using modified aneurysm clips, OECs were implanted into the cystic cavity, which had formed at the site of injury. Three weeks after injection, OECs occupied most of the cystic cavity, and were observed intermingling with reactive astrocytes. Double immunofluorescence for GAP-43 and neurofilament demonstrated numerous axons that had invaded the intraspinal grafts of OECs, and extended from the rostral and caudal portions of the cystic cavity. Ultrastructural examination of the cystic cavity demonstrated that implanted OECs were associated with both myelinated and unmyelinated axons. These observations provide the first direct evidence that a purified population of fetal rat OECs facilitate axon growth in a clinically relevant model of SCI. We hope that data from this investigation will provide novel insight into this type of cell therapy, as a clinically applicable technique that could be used to reduce functional deficits in humans with SCI.

P129.

CYCLIC AMP INDUCES FUNCTIONAL REGENERATION-ASSOCIATED GENES AND REPRESSES GAP-43

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Increasing cyclic AMP (cAMP) allows neurons to grow on myelin, which normally inhibits growth. While dorsal root ganglion (DRG) neurons extend short neurites on myelin, neurons growing in the presence of cAMP extend longer processes. cAMP levels are elevated in DRG neurons after peripheral lesion and during development, times when DRG neurons extend neurites on myelin. Protein kinase A inhibitors block the ability of cAMP to overcome inhibition. By comparing gene expression of neurons grown in the presence or absence of cAMP, we identified several genes expressed when neurons switch from an inhibited state to a growth state. We dissociated P5 rat DRGs and plated the cells on myelin overnight. At 1 hour to 18 hours after addition of dbcAMP or medium alone, the cells were harvested and their RNA was assayed on microarrays containing 5,000 spots of oligonucleotide probes. cAMP markedly induced expression of the pro-inflammatory cytokine IL-6. Real-time PCR showed 15-fold induction of IL-6 mRNA after 18 hours of cAMP treatment. IL-6 protein applied directly to DRG cells growing on myelin showed equivalent growth-promoting effects as cAMP. Although cAMP treatment repressed GAP-43 expression, GAP-43 is expressed at high levels in DRGs after peripheral nerve lesion and during development. Thus, cAMP induces regeneration-associated genes and allows growth on myelin while repressing GAP-43.

Supported by: Bryon Riesch Paralysis Foundation, SCI Project at the W. M. Keck Center, NMSS, NS 37060, RCMI-NIH, SNRP-NIH, NYSDOH.

P130.

NOVEL SYNTHETIC GRAFTS THAT ARE BIOCOMPATIBLE AND PROMOTE AXONAL REGENERATION AFTER SPINAL CORD INJURY

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While synthetic grafts to promote peripheral nerve axonal regeneration have been widely investigated, their use in promoting spinal cord axonal regeneration after injury has been limited. We examined the biocompatibility of a novel synthetic hydrogel tubular device, composed of a rigid or malleable formulation of poly (2-hydroxyethyl methacrylate) (PHEMA), that can be bioengineered to provide improved haptotactic and chemotactic cues for regeneration. Adult Sprague Dawley rats underwent complete spinal cord transection at T8 and repair with PHEMA tubes of two different elastic moduli: rigid (260 kPa; n = 8) or a malleable (178 kPa; n = 8). The cord stumps were inserted into the tube, fibrin glue was applied to the cord-tube interface, and a Preclude® membrane used for duraplasty. Controls (n = 4) underwent cord transection alone. Half the animals underwent axonal tracing with anterograde DiI and retrograde Fluoro-Gold. Survival times were 2, 4, or 8 weeks. Gross and histological examination of the spinal cords showed continuity of the tube and the cord stumps as early as 2 weeks. Continuity of neural tissue was more consistently seen with the rigid tubes, and neurofilament stained axons were visualized within the continuous neural tissue. Supraspinal serotonergic axons were found growing into the graft and were found to enter the caudal spinal cord. Calcium deposits occurred more frequently on the external surface of the rigid tubes, and with both tube types there was minimal scarring at the tube-cord interface, and significantly less scarring at the tube-dura interface compared to the Goretex. For the first time, we have evidence of axonal regeneration in a rat after complete spinal cord transection using synthetic hydrogel tubes without a contained matrix. Present work is examining the effects of contained matrices on axonal regeneration and functional recovery.

P131.

NOGO-66 RECEPTOR ANTAGONIST PEPTIDE PROMOTES AXONAL REGENERATION AND FUNCTIONAL RECOVERY AFTER SPINAL CORD

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After trauma to the adult mammalian CNS. axonal regeneration is minimal. Myelin-derived axon outgrowth inhibitors such as Nogo may account for this lack of CNS repair. The IN-1 antibody recognizes Nogo-A and promotes corticospinal tract (CST) regeneration and locomotor recovery. However. the limited specificity of IN-1 for Nogo and the non-specific anti-myelin effects have prevented a firm conclusion about the role of Nogo-66 or its receptor (NgR). Here. we identify a peptide antagonist (Nogo Extracellular Peptide residues 1-40. NEP1-40) of the Nogo-66 Receptor derived from amino terminal fragments of the Nogo-66 domain. This antagonist binds to the Nogo-66 Receptor competitively with nanomolar potency but does not stimulate axonal growth cone collapse in the cultured dorsal ganglion cells. We delivered this peptide or vehicle intrathecally to adult rats at the site of a mid-thoracic dorsal hemisection injury via an osmotic minipump. The integrity of the descending CST was traced by biotin-dextran-amine injection into the motor cortex. The integrity of serotonergic raphespinal tracts was evaluated with immunostaining for 5-HT fibers. The administration of this NgR antagonist to spinal cord injury rats results in a significant regeneration of both CST and raphespinal axons. and remarkably improves locomotor functional recovery assessed with a standardized BBB score. The sprouting from severed CST tracts following peptide treatment extends at least 1.5 cm caudal to the lesion. These findings reveal the central role of the Nogo-66 Receptor in limiting axonal regeneration after adult mammalian CNS injury. and NEP1-40 provides a potential therapeutic approach to treating traumatic CNS axonal injury.

P132.

TRANSPLANTATION OF RODENT SKIN-DERIVED PRECURSOR CELLS ONTO RAT HIPPOCAMPAL SLICE CULTURE

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One approach to repair damaged central nervous system (CNS) is to transplant new neural cells to restore functional circuitry. As accessible candidate cells for transplantation, we have recently isolated multipotential precursor cells from dermis of the skin. These skin-derived precursor cells (SKPs) form spheres in the presence of mitogens in suspension culture and differentiate into neuronal and glial cells in vitro as determined by the expression of appropriate marker proteins. The objectives of this study are to test the feasibility of SKPs as transplantable cells and to examine if SKPs acquire neuronal phenotypes when transplanted into the CNS environment. SKP spheres grown in proliferation condition (with EGF and FGF) were immediately dissociated, prelabeled with a fluorescent dye, and grafted onto the hippocampal slice. Alternatively, 5–7 days prior to transplantation, spheres were plated down in medium lacking growth factors, but supplemented with FBS. This condition significantly increased the number of neuronal cells in vitro. These cells were then, labeled, and grafted onto the hippocampal slice culture. 7–14 days after transplantation, SKPs prepared from mitogen containing medium proliferated more vigorously than those treated with FBS. Immunostaining revealed a robust growth of nestin-positive fibers that appeared to originate from FBS-treated SKPs, whereas little nestin immunoreactivity was seen in the untreated SKP graft. Furthermore, FBS-treated SKP grafts were immunoreactive for early neuronal markers like bIII-tubulin and HuC/D, and in some cases, for mature neuronal markers like MAP-2 and phosphorylated neurofilaments. Host hippocampal slices showed no adverse reaction to SKP grafts derived from either culture condition. These observations suggest that SKPs survive transplantation procedures and are capable of acquiring a neuronal phenotype in the grafted host environment. N.R.K. is supported by Christopher Reeve Paralysis Foundation.

P133.

PROGNOSTIC VALUE OF SPECT IN PATIENTS WITH POST-TRAUMATIC TRANSTENTORIAL HERNIATION

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Objectives: Cerebral perfusion disturbances are common in patients after the severe head injury, particularly in patients with transtentorial herniation and may influence outcome.

Material and methods: We present a group of 36 head injured patients admitted to neurosurgery with the syndrome of transtentorial herniation (GCS 3-5, homolateral dilated pupil and disturbed vital functions). There were 15 epidural, 19 subdural and two intracerebral hematomas. Mean age 40,8 years. All patients had urgent surgery and then continuous monitoring of ICP, CPP, blood pressure and jugular bulb oxymetry was instituted. Two postoperative CT and SPECT examinations were performed in each patient.

Results: 10 patients had visible ischemia on the first postoperative CT scan, 7 of them died. All patients except 3 had ischemia on SPECT (92%). Ischemia improved on the 2nd SPECT in 17 patients and 16 of them (94%) had a favourable outcome. On the other hand there were 16 patients with no change or even worse ischemia on 2nd SPECT and only 3 (19%) of them had a favourable outcome ($p < 0,05$). 12 out of 17 patients (70%) with improvement of perfusion on 2nd SPECT had a normal CPP during the whole posttraumatic course. On the other hand only 5 out of 16 patients (31%) with no improvement on SPECT had a normal CPP all the time.

GOS (mean follow up 12 month): 19 patients good, 3 moderately disabled, 1 severely disabled, 2 vegetative, 11 died.

Conclusions: SPECT is very sensitive to impaired cerebral perfusion and may be helpful as a serial study. This might be beneficial in patients with reversible ischemia and all the effort has to be made to keep CPP on normal levels. Improvement of perfusion detected by SPECT is related with a good outcome. Moreover SPECT shows the real areas at risk of ischemia which might be chosen for brain oxymetry monitoring.

P134.

LEFT-RIGHT ASYMMETRY OF THE ESTIMATION OF CEREBRAL PERFUSION PRESSURE USING TRANSCRANIAL DOPPLER ULTRASONOGRAPHY IN HEAD INJURY: A PRELIMINARY REPORT.

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Background: The estimation of cerebral perfusion pressure (eCPP) using transcranial Doppler (TCD) has recently been evaluated [1]. We investigated time trends in left-right asymmetry of eCPP and its correlation to CT scan patterns after traumatic brain injury.

Methods: In 25 sedated and paralysed head injured patients, arterial blood pressure (ABP) and intracranial pressure (ICP) were monitored continuously. Left and right middle cerebral arteries were insonated daily (108 measurements) using a purpose built transcranial Doppler monitor (NeuroQ(tm), Deltex Ltd, Chichester UK). NeuroQ's software is capable of on-line bilateral measurement of mean and diastolic flow velocities (FVm, FVd), and calculation of left-right eCPP following: $eCPP = (ABPm * FVd / FVm) + 14$ [2].

Results: The mean absolute value of left-right difference in eCPP (ΔeCPP) was 6 ± 5.8 mmHg. Daily observations showed that ΔeCPP progressively increased with time after head injury (ANOVA; $p < 0.012$).

Similarly, the absolute value of the difference between left and right FVm increased with time (ANOVA; $p < 0.006$). The ΔeCPP was significantly correlated with left-right asymmetry in FVm ($r^2 = 0.61$; $p < 0.0001$), and ICP ($r^2 = 0.18$; $p < 0.04$). Evidence of global brain swelling on CT, was significantly associated with an increase in absolute value of ΔeCPP ($p < 0.05$).

Comparing patients with and without midline displacement on CT ($n = 4$ and $n = 21$ respectively), showed that ΔeCPP was significantly greater ($p < 0.04$) for patients with midline shift, eCPP beeing higher on the side of the expanding brain.

Conclusion:

1. TCD is not only able to measure left and right flow velocities, but also to identify interhemispheric gradients of cerebral haemodynamics.
2. After head injury, left-right differences in estimation of CPP seems to follow a specific pattern.
3. Assessment of left-right asymmetry of cerebral haemodynamics should be of clinical significance.

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P135.

PERFUSION WEIGHTED MAGNETIC RESONANCE IMAGING (MRI) IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY.

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Data from a pilot study to apply Arterial Spin Labeled (ASL) Perfusion MRI to a well-characterized Controlled Cortical Impact (CCI) mouse model of TBI is presented. Our aims were to detect relative changes in Cerebral Blood Flow (CBF) in the injured hemisphere (compared to the contra-lateral hemisphere) and to compare changes in perfusion brought about by three differing injury levels (mild, moderate, and severe).

Animals were anaesthetized with Isoflurane (4% knock down, maintained at 1.5% in air/ nitrogen mixture) during CCI and MRI imaging. Traditional MRI techniques and arterial spin labeled perfusion MRI were used to detect T2, T1 and cerebral blood flow changes in the initial two hours after differing levels of injury. Our data show that CBF at the site of injury is reduced after TBI in this model and that CBF changes scaled with the level of injury. Severe CCI produced the greatest change, leading to a decrease in CBF of 53% in the contusion and 18% in sub-cortical tissue in the injured hemisphere (compared to contra-lateral tissue). Moderate CCI caused a smaller decrease in CBF, 29% in the contusion, 7.5% in sub-cortical tissue, while mild CCI lead to an even smaller change, 8.4% in the contusion and 4.3% in the sub-cortical tissue. This study demonstrates that MRI has utility in characterizing blood flow after injury, that CBF is reduced after injury and that the level of this perfusion deficit is directly related to the level of injury.

P136.

EFFECTS OF EARLY AND LATE INFUSION OF NOREPINEPHRINE ON CEREBRAL BLOOD FLOW, BRAIN TISSUE OXYGENATION, AND BRAIN EDEMA FORMATION IN BRAIN-INJURED RATS

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Reduction of CBF during the early phase after traumatic brain injury (TBI) is followed by a later phase of normal to increased cerebral perfusion. Thus, a pharmacological elevation of mean arterial blood pressure (MABP) with the aim of improving posttraumatic cerebral blood flow (CBF) may exert different time-dependent effects on CBF, tissue oxygenation (ptiO2) and brain edema formation after TBI.

Thirty-seven male Sprague-Dawley rats were subjected to a focal controlled cortical impact injury (CCI). At 4 or 24 hours after CCI, MABP was increased to 120 mmHg for 90 minutes by infusing norepinephrine. In rats receiving physiological saline MABP remained unchanged. In the first series, pericontusional CBF was measured using a laser Doppler flowmetry scanning technique before CCI, before, during, and after the infusion period. In a second series, intracranial (ICP) and cerebral perfusion pressure (CPP) as well as intraparenchymal CBF and ptiO2 measured within the pericontusional cortex were recorded continuously before, during, and after norepinephrine infusion. At the end of each experiment the brain was removed to determine hemispheric swelling and water content.

At 4 and 24 hours after CCI intravenous norepinephrine significantly increased, in parallel to the CCP increase, pericontusional cortical perfusion and ptiO2, whereas pericontusional parenchymal CBF was only significantly increased at 4 hours after trauma. Hemispheric swelling and water content did not significantly differ between the norepinephrine and control animals either with early or late infusion.

Following CCI early and late norepinephrine induced elevation of MABP significantly increased CBF and tissue oxygenation without aggravating or reducing brain edema formation. There was no evidence for a norepinephrine-induced reduction in perfusion due to vasoconstriction in the cortical or subcortical brain tissue at the early or late timepoint after trauma.

P137.

EFFECTS OF HYPERHAES ON POSTTRAUMATIC CEREBRAL PERFUSION AND EDEMA FORMATION AFTER CONTROLLED CORTICAL IMPACT INJURY IN RATS.

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Due to its hyperosmolar and hyperoncotic properties HyperheasTM might influence posttraumatic impaired cerebral perfusion and secondary brain damage. These possible effects following controlled cortical impact (CCI) injury in rats were investigated.

In 19 Sprague Dawley rats a moderate left focal cortical contusion was induced using the CCI. HyperhaesTM (4ml/kg bw) was intravenously administered within 2 min following trauma in 8 animals. In control animals [n = 8] physiological saline was administered. In all animals blood gases were drawn before and following infusion. Temperature and mean arterial blood pressure (MABP) were monitored continuously. 24 hours following trauma brains were removed and posttraumatic edema was quantified gravimetrically. In three additional animals Laser Doppler flowmetry was used to assess pericontusional cortical perfusion before and after trauma, after drug application as well as 4 and 24 hours after trauma.

Temperature and arterial blood gases were determined within physiological limits during the entire study. Following administration of HyperhaesTM no significant changes in MABP could be observed. In HyperhaesTM treated animals posttraumatic swelling (8.2 ± 0.3%) was moderately decreased versus placebo (8.9 ± 0.6%, non significant). Posttraumatic water content was significantly increased in both groups in traumatized versus non traumatized hemispheres. There were no differences in water content in non traumatized hemispheres. In HyperheasTM treated animals water content in traumatized hemispheres were (79.95 ± 0.05%) no significant changes versus placebo (80.1 ± 0.08%) were observed. Pericontusional perfusion was only moderately reduced at 4 hours following trauma in HyperheasTM treated animals in comparison to significant hypoperfusion in placebo group.

HyperhaesTM increases posttraumatic pericontusional perfusion while no significant effect on posttraumatic edema formation was shown. The effect on neuronal damage still needs to be clarified.

P138.

MAPPING FLOW-METABOLISM AND EVOLVING AXONAL INJURY AFTER EXPERIMENTAL BRAIN TRAUMA

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Blood flow-metabolism uncoupling is a well-documented phenomenon after traumatic brain injury but little is known about the direct consequences for the underlying tissue. The aim of this study was to quantitatively assess the topographic inter-relationship between local cerebral blood flow (LCBF) and metabolic rate of glucose uptake (LCMRglu) in the same rat after contusion injury and to determine the degree of correspondence with the evolving axonal injury.

Isofluorane-anaesthetised rats were injured by controlled cortical impact over the left parietal cortex (4m/s velocity, 2mm deformation). Quantitative mmeasurements of regional LCMRglu and LCBF were obtained at 3hr in the same rat from 18F-flurodeoxyglucose (30MBq) and 14C-iodoantipyrine (20μCi) co-registered autoradiographic images, and compared to the density of damaged axonal profiles in adjacent sections using beta-amyloid precursor protein (β-APP) immunohistochemistry. Sham-injured rats were used for comparison of all data together with an additional 24hr injury group that was assessed for β-APP alone (all groups n = 6).

LCBF was significantly reduced over the ipsilateral hemisphere versus sham-controls, for example in the contusion core it was 26 + 2.6 versus 86 + 8.2 ml.100g-1.min-1, respectively and 29 + 3.2 versus 87 + 14.4, ml.100g-1.min-1 in the cingulum ($P < 0.01$). By contrast, LCMRglu was unaffected, apart from foci of elevated LCMRglu in the contusion margin versus sham-controls (86 + 2.5 versus 65 + 6.5 μmol100g-1 min-1, respectively, $P < 0.05$). Flow-metabolism was uncoupled indicated by a significant 2-fold elevation in the metabolism/blood flow ratio within most of the structures analysed in the ipsilateral hemisphere ($P < 0.05$). β-APP staining was evident even by 3hr, with significant increases in injured axon density by 24hr ($P < 0.01$). No staining was present in sham-injured rats. The increase in β-APP axon density was negatively-correlated with LCBF and positively correlated to the metabolism/blood flow ratio ($r = 0.62$ and 0.77, respectively, $P < 0.001$) indicating the critical dependence of axonal outcome on flow-metabolism in the acute injury stage.

P139.

INTERPRETATION OF CEREBRAL LACTATE REDUCTION IN SEVERE HEAD INJURY. A MICRODIALYSIS STUDY.

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Objectives. Since ventilation with pure oxygen seems to reduce high brain lactate levels following head injury (TBI), some authors have proposed hyperoxia for correcting the underlying metabolic disorder. The aim of the study was to investigate the effects of this procedure on the energy metabolism in cerebral tissue.

Materials and methods. We performed 18 tests (3 hours of 100% inspired oxygen concentration) on different days in 8 patients with severe traumatic brain injury (TBI). During the tests brain tissue oxygen tension (PtiO2) was recorded and microdialysis (flow rate 0.3 μl/min) was performed to measure brain levels of lactate, pyruvate and glucose. The ratio of lactate to pyruvate (L/P) and the arterio-jugular difference in oxygen content (AvdO2) were also calculated. Significance refers to repeated measures analysis and paired t-test.

Results. The mean values ± standard deviation at baseline and after 60, 120, 180 minutes of hyperoxia are: PtiO2 (33 ± 18, 119 ± 45, 118 ± 47, 123 ± 45 mmHg, $p < 0.0001$), Lactate (3.21 ± 2.77, 3.23 ± 2.86, 2.99 ± 2.70, 2.90 ± 2.58 mmol/L, $p < 0.01$), Pyruvate (153 ± 56, 158 ± 64, 149 ± 59, 141 ± 56 μmol/L, $p < 0.05$), glucose (2.28 ± 1.35, 2.45 ± 1.46, 2.39 ± 1.34, 2.45 ± 1.63 mmol/L, NS), Lactate/Pyruvate (19 ± 12, 18 ± 11, 18 ± 11, 18 ± 12, NS). AvdO2 drops from a baseline of 4.55 ± 1.22 to 4.15 ± 0.76 ml/100ml after 180 minutes, $p < 0.05$).

Conclusions. Our data confirms that 3 hours of ventilation with pure oxygen rises brain PtiO2 and reduces brain concentration of lactate. The L/P ratio, a marker of cerebral oxidative status, however does not change, underlining that lactate reduction per se does not necessarily indicate an improved aerobic metabolism. In conclusion, this data indicates that prolonged hyperoxia does not affect the cellular redox state, suggesting a reduction in the metabolic rate rather than an improvement in oxidative metabolism. This hypothesis seems to be confirmed by an AvdO2 reduction.

P140.

AMYLOID BETA 1-42 AND TAU IN CEREBROSPINAL FLUID AFTER SEVERE HUMAN TRAUMATIC BRAIN INJURY

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Background: Traumatic brain injury (TBI) is a recognized risk factor for Alzheimers disease (AD). Related to histopathological changes in AD, Amyloid beta 1-42 (Abeta-42) levels are decreased and tau levels increased in cerebrospinal fluid (CSF).

Methods: CSF samples were collected from 29 patients with severe head trauma between 1 and 284 days post trauma. Abeta-42 and tau levels were measured using sandwich ELISA techniques and compared with CSF levels in patients with cognitive disorders and headache.

Results: At all time points, concentrations of Abeta-42 were significantly lower in TBI patients than in control groups. A statistically significant correlation existed for Abeta-42 levels and outcome of patients. Below a cut-off of 230 pg/mL, the sensitivity of Abeta-42 to discriminate between good outcome (GOS 4 and 5) and worse outcome (GOS 1 to 3) was 100% at a specificity of 82%. CSF tau levels were significantly higher in TBI patients compared with control groups. In patients with multiple CSF samples collected at various time points between 1 and 32 days after trauma, tau levels increased early after TBI, peaked 11 days post trauma and slowly decreased thereafter. Independent of outcome, all patients had normal tau levels when CSF was collected more than 43 days post trauma.

Conclusions: Abeta-42 and tau may play a potential role in the pathophysiology of TBI. Furthermore, the results of our study suggest that Abeta-42 might be a supportive early predictor for recovery after severe head injury.

P141.

TEMPORAL AND SPATIAL PROFILE OF BID CLEAVAGE AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

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This study examined the temporal profile and cell subtype distribution of the proapoptotic protein Bid from 6 hours to 7 days following cortical impact injury in the rat. Increased protein levels of the truncated active form of Bid (tBid) were seen in the cortex ipsilateral to the injury site from 6 hours to 3 days after trauma. Immunohistological examinations revealed expression of tBid in neurons, astrocytes and oligodendrocytes from 6 hours to 3 days after TBI and concurrent assessment of DNA damage using TUNEL identified tBid immunopositive cells with apoptotic-like morphology in the traumatized cortex. Moreover, Bid cleavage and activation of caspase-8 and caspase-9 occurred at similar time points and in similar brain regions after impact injury. In contrast, there was no evidence of processing of caspase-8, caspase-9 and Bid cleavage in the ipsilateral hippocampus, contralateral cortex and hippocampus up to 7 days after the injury.

Our results provide evidence of Bid cleavage in the traumatized cortex after experimental TBI in vivo and demonstrate that tBid is expressed in neurons and glia. Further, our findings indicate that cleavage of Bid may be associated with the activation of the initiator caspase-8 and caspase-9. Last, our data support the hypothesis that cleavage of Bid contributes to the apoptotic degeneration of different CNS cells in the injured cortex.

Supported by grants from the FWF (P15308), the NIH (NS36821, R01 NS40182; R01 NS39091), and the U.S. Army (DAMD17-9-1-9565).

P142.

TEMPORAL AND SPATIAL PROFILE OF CASPASE-6 EXPRESSION AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

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This study investigated the temporal expression and cell subtype distribution of the executioner caspase-6 and the cell death receptor p75ntr from 6 hours to 14 days following cortical impact-induced traumatic brain injury in adult rat. Western blotting analysis revealed increased immunoreactivity of caspase-6 and p75ntr in the cortex ipsilateral to the injury site from 6 hours to 3 days after trauma. Immunohistological examinations revealed expression of caspase-6 in neurons and glial cells from 6 hours to 3 days after TBI and concurrent assessment of DNA damage using TUNEL identified caspase-6 immunopositive cells with apoptotic-like morphology in the traumatized cortex. Moreover, double labeling experiments demonstrated expression of both, caspase-6 and the cell death receptor p75ntr in individual CNS cells after impact injury. In contrast, there was no evidence of caspase-6 expression and upregulation of p75ntr in the ipsilateral hippocampus, contralateral cortex and hippocampus at all time points investigated.

Our results provide evidence of caspase-6 expression and upregulation of the cell death receptor p75ntr in the traumatized cortex after experimental TBI in vivo and demonstrate that caspase-6 is expressed in neurons and glia. Further, our data indicate that expression of caspase-6 contributes to the apoptotic degeneration of different CNS cells in the injured cortex. Last, our findings indicate that upregulation of the cell death receptor p75ntr may be associated with the activation of the executioner caspase-6.

Supported by grants from the Austrian Science Fund (P15308) and the National Institutes of Health (NS36821).

P143.

MULTIDIMENSIONAL IMPAIRMENTS OF ATTENTION FOLLOWING PEDIATRIC TRAUMATIC BRAIN INJURY

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Pediatric traumatic brain injury (TBI) is a serious public health issue in the U.S., affecting approximately 200/100,000 children each year. Of the children who survive a TBI, up to 80% may experience persistent impairments in physical, behavioral, cognitive, and self-care functioning. Despite the high incidence of childhood TBI, the nature of associated cognitive impairments has been relatively unstudied. Neuropsychology strives to examine brain-behavior relationships by characterizing the cognitive impairments associated with damage to various brain regions. Frontal and temporal brain regions essential for attentional processing are particularly vulnerable to damage following TBI. Few studies have examined the multidimensional characteristics of attention within a pediatric TBI sample of varying severity levels. The current study examines possible attentional impairments in children/adolescents in the months following head injury (HI). Pilot data are presented for children/adolescents ages 6-16 classified with "mild" or "moderate-severe" HI, with groups comparable on age and gender. Participants were assessed once medically stable. IQ tests and the Test of Everyday Attention for Children (TEA-Ch), a standardized measure of three dimensions of attention (selective, sustained, and attentional control/switching) were generally administered within six weeks of HI. The moderate-severe group obtained a significantly lower Full Scale IQ compared to the mild group. Across the attention measures, the mild group consistently performed better than the moderate-severe group. Further, the moderate-severe group performed statistically worse on tasks of selective and sustained attention. No significant differences were noted on tasks measuring attentional control/switching. Results suggest that moderate-severe acute TBI is associated with specific types of attentional impairments that may have specific effects on the course of cognitive recovery. This study is unique in that it evaluates multiple dimensions of attention within the same test measure in acute HI. Future studies will examine the relationship between attentional impairments and other cognitive abilities within a longitudinal design.

Sponsored By: Florida Brain & Spinal Cord Injury Research Trust Fund

P144.

TEMPORAL PROFILE OF alfa-II-SPECTRIN BREAKDOWN PRODUCTS AFTER TRAUMATIC BRAIN INJURY IN IMMATURE RATS.

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Previous studies have shown that calpains and caspase-3 like proteases play a key role in cellular dysfunction and/or cell death after brain injury. Current work in our laboratory has demonstrated the role of caspase-3 and calpain in protein breakdown with the formation of distinct neuronal breakdown products of alfa-II-spectrin (SBDP) after TBI in the mature brain. Age dependent increase in caspase-3 activity suggests a relatively greater role of programmed cell death in the pathophysiology of traumatic and ischemic brain injury in the developing brain. In the present study, protein analysis was used to immunolabel specific proteolytic fragments of alfa-II-spectrin produced by calpains (145 kDa) and caspase-3 (120 kDa) proteases in the immature rat. Our experiments provide evidence for a role for both calpain and caspase-3 in the production of signature proteolytic fragments after TBI in immature rats. Increases in both the 120 kDa and the 145 kDa fragments of alfa-II-spectrin were detected as early as 15 minutes after injury and persisted for as long as 7 days. These results lay the ground to additional experiments aimed at further defining the relative contribution of each calpain and caspase-3 to the pathophysiology of TBI in the immature brain. (Supported by DAMD17-99-1-9565, DAMD17-01-1-0765, NIH R01 NS39091 and the Howard Hughes Medical Institute Biomedical Research Support Program)

P145.

ZINC CHELATION ALTERS THE MOLECULAR PROFILE OF STRESS SIGNALING PATHWAYS IN TRAUMATIC BRAIN INJURY

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Considerable evidence implicates zinc neurotoxicity in the pathogenesis of brain injury and neurodegenerative diseases such as Alzheimer's disease (AD). In a transgenic mouse model of AD, zinc chelation significantly reduced brain b-amyloid deposition. This evidence, together with the known risk of traumatic brain injury (TBI) for development of AD, suggests that dysregulation of zinc homeostasis could contribute to the pathology of both diseases. We hypothesized that chelating zinc using calcium EDTA would alter the expression of stress response genes following fluid percussion TBI in rats. Zinc chelation had a salutary effect on temporal patterns of gene expression. Most notably, the chelation treatment enhanced the upregulation of neuroprotective genes after TBI. These included the heat-shock proteins HSP70 and HSP27, antioxidant genes such as cellular glutathione peroxidase-1, and heme oxygenase-1, which is activated by inflammatory and oxidant stress and has been shown to have a protective role in vascular wound repair. The expression of genes, such as the intermediate filament protein vimentin, that are involved in regenerative processes was also further upregulated by zinc chelation. After TBI alone, multiple members of the mitogen-activated protein kinase (MAPK) family that regulate many downstream cell signaling pathways, including those involved in the pathophysiology of neurodegenerative diseases, are activated. Treatment with the zinc chelator had either no significant effect on these pathways or increased the expression of some of the MAPK genes, which have a positive role in cell survival. Finally, there was a salutary effect of zinc chelation on the expression of various cell cycle regulatory genes that are dysregulated following TBI.

P146.

EFFECTS OF INJURY SEVERITY ON REGIONAL AND TEMPORAL mRNA EXPRESSION LEVELS OF CALPAINS AND CASPASES AFTER TRAUMATIC BRAIN INJURY IN RATS

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Despite a preponderance of studies demonstrating gene expression and/or enzymatic activation of calpain and caspase proteases after traumatic brain injury (TBI), no studies have examined the effects of injury severity on these important families of cell death effectors after TBI. In addition, relative expression levels of these various gene products for a given injury is unknown. Thus, determination of the effects of injury severity on specific expression profiles will be critical to understanding the various pathways to parenchymal pathology. This investigation tested the hypothesis that different injury magnitudes cause different regional and temporal patterns of mRNA expression of mu- and m-calpain, calpastatin, caspases -3, -8, -9, and bid after 1.0, 1.2, and 1.6 mm lateral cortical impact TBI in rats. Methods: Quantitative RT-PCR was used to compare effects of injury on mRNA levels in ipsilateral (injured) cortex and hippocampus from 6 h to 5 days post-injury compared to sham-injured controls. Results: TBI resulted in significant increases in all genes examined with highest expression in the cortex. Generally, higher injury magnitude caused higher gene expression. The most robustly expressed genes were bid, caspase -3, and -8. Caspase-9 was the lowest expressed gene and levels were undetectable in hippocampus. Interestingly, although calpains are known to be activated within minutes after TBI, mRNA expression of calpains was highest 72 h to 5 days post-TBI. Discussion: This study provides a detailed analysis of regional and temporal expression of calpains and caspases after TBI. Studies provide critical insight into distinct profiles of individual patterns of gene transcription during the evolution of the sub-acute response to TBI between various injury magnitudes. Studies comparing mRNA expression vs. enzymatic activity will be critical for developing gene and protein based therapies for treatment of TBI. (Supported by DAMD17-99-1-9565, DAMD17-01-1-0765, NIH R01 NS39091, NIH R01 NS40182, and USAMRMC)

P147.

THE PREDICTIVE VALUE OF PROCALCITONIN AND S 100 B IN TRAUMATIC BRAIN INJURY

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Background: Procalcitonin (PCT) is a marker of posttraumatic inflammation but has recently also been associated with local hypoperfusion and secondary brain damage after traumatic brain injury. S 100 B is a well-known marker of traumatic brain injury. The object of this study was to compare the predictive value of plasma PCT and S 100 B in patients with traumatic brain injury for the development of septic inflammatory response syndrome (SIRS) and outcome.

Patients and Methods: 102 consecutive patients with traumatic brain injury (ISS > 23, GCS < 8) admitted to either of the two participating level II trauma centers were included in this prospective study. Plasma PCT and S 100 B were measured at admission and daily thereafter for a maximum of 21 days using commercially available immunoluminometric assays (LUMItest PCT, B.R.A.H.M.S.-Diagnostika GmbH, Henningsdorf, Germany and LIA-mat Sangtec 100, Byk-Sangtec Diagnostika, Bromma, Sweden.). The courses of PCT and S 100 B were evaluated with regard to SIRS (patients with and without SIRS according to the Bone criteria) and outcome (survivors versus non-survivors).

Results: A total of 68 survivors (8 with SIRS, 60 without SIRS) and 34 non-survivors (9 with SIRS, 35 without SIRS) were evaluated. Though elevated PCT levels later than 24 hours after trauma were always associated with SIRS, we found no relationship between elevated PCT and outcome. In contrast, the course of S 100 B later than 24 hours after trauma was always associated with outcome. In all non-survivors, regardless of whether they had SIRS or not, S 100 B was either continuously elevated or increased at least 48 hours before death.

Conclusion: PCT may be a sensitive predictive indicator of SIRS but is not associated with outcome following traumatic brain injury. S 100 B, however, appears to be a sensitive predictive indicator of outcome.

P148.

CONTEXTUAL FEAR CONDITIONING TO ASSESS COGNITIVE DYSFUNCTION IN BRAIN INJURED MICE

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The Morris Water Maze (MWM) is commonly used to assess the cognitive effects of fluid percussion injury (FPI) in rodents. Special obstacles unique to mouse species that may complicate the interpretation of MWM experiments have been identified (Carbonell, 1998); for example a significant percentage of individuals may be poor swimmers or non-learners. The present study examined the potential of contextual fear conditioning (CFC) as a technique to circumvent these issues and assess hippocampus-dependent cognitive deficits in FPI-injured mice. Mice were trained in CFC, injured and then assessed for cognitive function 6-8 days post-injury. Injured mice displayed significant deficits with respect to sham-operated control mice in freezing behavior when tested in the training context. Injured mice demonstrated a 38% decrease in freezing compared to sham-operated controls over the 5 min retrieval test (15.2% ± 1% compared to 24.4% ± 2%; $p < .05$; $n = 17, 18$ for FPI and SHAM respectively). In previous experiments, injured mice tested in the spatial version of the MWM displayed significant increases in both latency and search error, a measure of deviation from the optimal swimming path to the goal platform (Carbonell, 1998), with respect to sham-operated controls. Mice in both behavioral assays displayed similar and significant deficits in hippocampus-dependent tasks. As CFC avoids some of the inherent difficulties of the MWM when applied to mice, we propose that CFC is an appropriate and accurate technique to assess hippocampus-dependent cognitive deficits in FPI mice.

P149.

A 4-AXES MODEL OF THE STRESS RESPONSE

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This paper proposes an expanded 4-axes model of the physiological stress axis to help identify cross-cultural risk factors for traumatic brain injury (TBI), and its sequela.

The 4-axes stress response model includes the interrelated fast-acting hypothalamic-pituitary-adrenal (HPA) axis, the intermediately fast-acting pineal-opioid (PO) axis, the slow-acting glucose-glutamine-GABA (GGG) axis, and the hippocampal-cortical (HC) learning/memory axis. An example of the interrelatedness is the high density of cortisol receptors and the co-localization of glutamate and opioid peptides within the hippocampus. Also, plasma glucose levels have now been identified as an early marker to predict survival after TBI, since the brain is continually permissive to glucose.

Two relevant studies address the cognitive impairments seen even in mild TBI: damage to the HC axis. In the first study, tetris game experts, tetris game novices, and tetris trained amnesiacs scored similarly in recall learning when awakened from slow wave sleep. Since the amnesiac group had no recent recall, the results established a dual system for memory and learning, whose path, from hippocampus to prefrontal cortex, is unidirectional. In the second study, it was found that a minimum of 6 hours sleep, preferably 8, was needed for memory consolidation, and that an "all-nighter" severely blighted memory consolidation even post 3rd day.

An indirect value of the expanded 4-axes stress response model is that it is easier to identify multiple points at which risk factors for TBI act, for example, alcohol intoxication. Alcohol disregulates the 4-axes stress response at two points: by potentiating the glutamate receptor and by inhibiting the GABA receptor.

TBI is a towering, unresolved, worldwide public health concern. In an expanded stress response model, ongoing surveillance for the underdiagnosed TBI, for improvement of the consequences of TBI, and for understanding risks for prevention can augment our evolving efforts.

P150.

INDUCTION OF HIGH PURITY OLIGODENDROCYTE CULTURES FROM HUMAN EMBRYONIC STEM CELLS

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The broad developmental potential and replicative capacity of embryonic stem cells promise a virtually unlimited supply of specific cell types for transplantation therapies. One of the current challenges in the field is the development of methods to selectively differentiate multipotential human embryonic stem (huES) cells to cell-type specific precursors. Here we describe the induction of high purity oligodendrocyte cultures from huES cells. huES cell lines H1 and H7 were provided by Geron Corporation and expanded in culture conditions that did not contain mouse fibroblasts. Neural differentiation was initiated in embryoid bodies using retinoic acid and basic fibroblast growth factor (bFGF). Glial-restricted precursor differentiation was initiated by gradually transitioning the cells from huES expansion media to a glial-restriction media containing mitogens. Clusters were enriched by an overnight incubation on adherent substrate, dissociated, then plated a week later on adherent imaging chambers at low density for cell counting and immunostaining. These studies demonstrated greater than 95% pure populations of oligodendrocytes. Thus, huES cells can serve as a plentiful source of oligodendrocyte-lineage restricted cells, which may have use in transplantation regimes aimed at treating demyelinated states of the central nervous system. This study was supported by Geron Corporation and BioSTAR.

P151.

QUANTIFICATION OF DIFFUSION TENSOR IMAGING PREDICTS DIFFUSE AXONAL INJURY FOLLOWING TRAUMATIC BRAIN INJURY IN RATS

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Diffuse axonal injury (DAI), commonly observed following human traumatic brain injury (TBI), is well characterized in animal TBI models. Diffusion tensor imaging (DTI) characterizes molecular displacement in highly oriented tissues and allows calculation of displacement pathways (white matter). Case studies suggest DTI may be altered in injury or disease states. This is the first study to quantitatively compare DTI with a pathological outcome related to TBI—DAI.

Moderate-severe TBI in adult male rats was induced via controlled cortical impact. Fixed brains were excised and DTI of 1H in water measured at 17.6 Tesla using a spin-echo sequence with diffusion gradients applied in 7 directions using 3 weightings. The diffusion tensor of water was calculated and fiber tracts derived from the principal diffusion direction. Fractional anisotropy (FA) and directional coherence (DC) of specific white matter tracts were calculated. Number of injured axons, retraction balls immuno-positive for NF68, within specific tracts were counted and compared with DTI related parameters.

Derived fiber tracts were consistent with corresponding sections in a rat brain atlas. FA and DC values were inversely related to number of injured axons. These results indicate that DTI is a useful tool in assessing diffuse white-matter tract damage. Additionally, they suggest DTI quantification techniques may allow clinicians to better assess damage following TBI. We intend to extend this technology to in vivo. BSCIRTF 2001, NIH P01 NS35702, P41 RR16105, NIH R01 NS39091, R01 NS40182, US Army DAMD17-99-1-9565

P152.

RESPONSE OF NEURONS CULTURED IN TWO- AND THREE-DIMENSIONS TO DYNAMIC SHEAR DEFORMATION

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In vitro models of traumatic brain injury (TBI) have evaluated post-injury alterations in cell biochemistry and viability by utilizing 2-D cell cultures, a contrast from the 3-D architecture of native brain that may lead to a deviation in the injury response. Fundamental differences exist between cells cultured in 2-D and 3-D in terms of cell morphology, cell-cell/cell-matrix interactions, response to biochemical stimuli, and gene expression (1). However, it is unknown whether a differential response to high rate shear deformation—the most prevalent type of deformation in TBI (2)—will exist between these configurations. Primary rat cortical neurons (E17) were plated in 2-D (2.5×10^5 cells/cm^2, below an acellular 3-D extracellular matrix (ECM) gel) or 3-D (6.0×10^6 cells/cm^3, distributed throughout an ECM gel) configurations. Cultures in these two configurations were characterized based on viability, neuronal purity, and neurite extension, and injured via high strain rate simple shear deformation (20s-1 or 30s-1, 0.50 strain). Prior to injury, both 2-D and 3-D cultures formed mature neuronal networks consisting of ~97% neurons with no significant differences in cell viability at one-, two, or three-weeks post-plating. However, neurons plated in 3-D ($n = 3$) had a 1.9-fold increase in the expression of Tau, a cytoskeletal protein associated with neurite outgrowth, versus 2-D cultures ($n = 3$; $p < 0.05$). After high rate shear deformation cells cultured in a 3-D orientation ($n = 17$ at 20s-1, $n = 14$ at 30s-1) experienced a significant decrease in cell viability compared to cells in a 2-D orientation ($n = 19$ at 20s-1, $n = 14$ at 30s-1; $p < 0.05$). These results begin to establish fundamental characteristics of primary cortical neurons in 2-D and 3-D configurations and, furthermore, show differences in the response of these cell cultures to high rate shear deformation, suggesting a possible role for cell orientation and cytoskeletal protein expression in the response to a mechanical insult. Accurate cellular models of TBI are important to develop mechanistically-driven intervention strategies and can therefore serve as valid pre-animal and pre-clinical test beds.

Funding provided by a Pfizer/Parke-Davis ARA Research Grant. (1) Hoffman, R. (1993) Stem Cells 11:105, (2) Holbourn, A. (1943) The Lancet 2:438.

P153.

ADAPTATION OF SENSORIMOTOR AND COGNITIVE TASKS FOR USE WITH MICE: EFFECTS OF CONTROLLED CORTICAL IMPACT INJURY AT VARIED INSULT LOCATIONS

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In an effort to characterize the behavioral deficits seen after traumatic brain injury (TBI), specific sensorimotor tasks, the gridwalk task and spontaneous forelimb use task (SFL), along with the Barnes circular maze spatial task, were adapted for use with the mouse. The SFL and gridwalk tests have not been used extensively in mice, particularly with forebrain insults. Male C57BL/6 mice were anesthetized and given parasagittal controlled cortical impact (CCI) injury or sham procedures, targeting right anterior, middle, or posterior locations relative to bregma (n = 9–10 per group). All injuries were performed using a 3mm impact tip at a velocity of 6.89m/sec, to a depth of 1mm. Animals were tested pre-operatively on the sensorimotor tasks, and post-operatively once per week for four consecutive weeks, and again at five months. There was no overall pre-operative forelimb use asymmetry on the SFL task. However, significant contralateral forelimb deficits were observed in each task for at least one month post injury, depending upon insult location. Barnes maze testing during month two revealed significant TBI-related and insult location dependent deficits in spatial acquisition and on several probe trial measures, relative to shams. Histopathological analysis indicated that all TBI animals displayed cortical and striatal damage, with anterior TBI mice presenting with the most extensive striatal lesions and posterior TBI animals having hippocampal pathology. The present results demonstrate the effectiveness of these tests for use in evaluating behavioral deficits following TBI in the mouse. Supported by NS30291.

P154.

PROGESTERONE IMPROVES BEHAVIORAL AND MORPHOLOGIC OUTCOMES AFTER TRAUMATIC BRAIN INJURY IN MALE C57BL4/6 MICE

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The beneficial effects of progesterone in rat models of traumatic brain injury have been well documented. With the development of progesterone receptor knockout mouse strains, there is increased interest in exploring the effects of progesterone on traumatic brain injury in a mouse model. This study aims to characterize the behavioral and morphologic effects of exogenous progesterone after traumatic brain injury in mice.

Male C57BL4/6 mice were subjected to sham surgery or lateral impact cortical contusion. One hour after surgery, animals were injected intraperitoneally with vehicle (cyclodextrin) or progesterone (8mg/kg, 16mg/kg, or 32mg/kg). Subsequent injections were given subcutaneously at 6-hours post-injury, and daily for 5-days. Behavioral outcomes were assessed using the rotorod and the Morris Water Maze (MWM). Rotorod performance was measured one day before surgery, and at 7 and 14 days post-injury. MWM was assessed daily on days 15–28 post-injury.

No significant injury-induced deficits were observed on the rotorod. MWM testing showed that the 8mg/kg and 16mg/kg doses of progesterone attenuated injury-induced cognitive impairment, whereas the 32mg/kg dose had no beneficial effect.

Collectively, these results suggest that the behavioral benefits of progesterone are conserved in this mouse model of TBI and that 16mg/kg/day of progesterone for 5 days is the optimal dose for facilitating recovery of function. Supported by NIH grants 1R01NS38664, 1R01NS40825, & 5R03HD040295.

P155.

THE NEUROPROTECTIVE EFFECTS OF PROGESTERONE ARE ASSOCIATED WITH MODIFIED GENE EXPRESSION IN RAT CORTICAL IMPACT MODEL

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Although progesterone has been shown to be therapeutic in animal models of traumatic brain injury, the cellular mechanisms behind this therapeutic neurosteroid remain unknown. Goal: characterize and quantify the influence of progesterone on post-injury gene expression related to cellular pathology and/or protection.

Adult male rats received either bilateral prefrontal cortical contusion or sham surgery. Post-injury and sham rats were given 25% Cyclodextrin (vehicle), or 16mg/kg progesterone in vehicle. Treatments were given 1hr, 6hr, and 24hr post-injury. At 48hrs post-injury, the rats were killed, and brains extracted. Both frontal lobes were dissected out; right for edema measures and the left for RNA isolation. The isolated RNA served as a template for cDNA, which was then used as a template for the labeled cRNA that was hybridized to the Affymetrix™ U34A (8800 genes) chip. Chips were scanned and analyzed in Emory's core facility.

Results indicated that animals given vehicle had higher water content than either the sham-operated or post-injury rats given 16mg/kg of progesterone ($p < 0.05$) (previously presented). Rats given progesterone post-injury had modified expression of 768 genes with 96 genes increased, and 405 decreased versus those given vehicle- post-injury. Genes were divided into groups related to: oxidative stress, inflammation, growth factors, neural and metabolic activity, cell death, cytoskeleton, and myelination. Progesterone decreased expression of pro-inflammatory cytokines, markers of metabolic activity, indicators of injury, and genes promoting apoptotic and necrotic cell death. Progesterone increased the expression of cytoskeletal and myelin proteins. The data shows complex gene modulation by progesterone that will be further explored with descriptive and quantitative studies of related protein distribution. Support: NIH/NINDS 1R01NS38664 and General-CologneRe.

P156.

ANESTHESIA AFFECTS GENDER-RELATED FUNCTIONAL OUTCOME FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY IN RAT

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A number of studies have demonstrated that outcome in males and females are different following traumatic brain injury. Some report increased mortality and morbidity in males following trauma while others report that males do significantly better than females under certain conditions (1). These conditions involve type of anesthesia and outcome measure. In the present study we have used three different anesthetic protocols and four different outcome measures to determine how these parameters affect functional outcome following traumatic brain injury in male and female rats. Diffuse traumatic brain injury was induced in adult male and female animals using the impact-acceleration brain injury model (2). Mortality in female animals was no different than males when using halothane anesthesia, slightly less than males when using isoflurane anesthesia, but significantly worse than males under pentobarbital anesthesia. Female animals always performed better than males on rotarod tests of motor outcome, with this effect being unrelated to anesthetic effects. Conversely, in cognitive tests using the Barnes Maze, only isoflurane-anesthetized females performed better than their male counterparts. Similarly, in an open field activity task, females always performed better than males after trauma, with isoflurane-anesthetized females doing significantly better than the halothane-anesthetized female group after injury. Our results suggest that female animals do better than males after diffuse traumatic brain injury, although this observation is dependent upon the type of anesthesia and the functional task employed. Isoflurane is particularly protective in females, pentobarbital is deleterious to female outcome, while halothane anesthesia has the least influence on gender-related outcome.

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(2) Marmarou, A., Foda, M.A.A., Van den Brink, W., Campbell, J., Kita, H. and Demetriadou, K. (1994) J. Neurosurg., 80: 291-300.

P157.

A PARALLEL RANDOMIZED DOUBLE-BLIND MULTICENTRE CLINICAL TRIAL FOR THE EFFICACY AND SAFETY OF NALOXONE IN ACUTE TRAUMATIC BRAIN INJURY

Yuanli Zhao MD,[1] Jiyao Jiang MD,[2] Li Li MD,[1] et al. on behalf of the National Naloxone Study Group. ([1]Beijing Neurosurgical Institute. [2]Shanghai Neurosurgical Institute).

This clinical trial was designed to test the efficacy and safety of naloxone in treatment of traumatic brain injury. It was organised by Chinese neurosurgical society and Chinese Journal of Neurosurgery, which was carried out in 18 major neurosurgical centres in China.

Methods: From 1999 to 2001, a randomised double blind prospective multicentre clinical trial was implemented to compare the difference of naloxone and saline in moderate or severe traumatic brain injured patients. Naloxone or saline placebo was intravenously given for 10 days and follow-up for 3 months. The dosage of Naloxone is 0.3mg/day/kg. Glasgow Coma Scale, Glasgow outcome scale, Karnofsky performance scale, motor function and verbal function were the index of assessment for patients' prognosis.

Results: A total of 530 cases were enrolled in our clinical trial and 511 cases met the need of statistics. The mortality of naloxone group and saline placebo group was 12.5% (32/256) and 17.3% (44/255) respectively ($P < 0.05$). Glasgow coma scale in naloxone group was significantly better than that in saline placebo group starting at 5th days after treatment ($P < 0.05$). Glasgow Outcome Scale, KPS, verbal and motor functions in naloxone group were also statistically better than those in saline placebo group with a mean follow-up period of 3 months ($P < 0.05$). In addition, naloxone did not show any side effects.

Conclusions: The data of our trial has confirmed that early application of naloxone for acute brain injured patients could significantly reduce the mortality and morbidity without side effects.

P158.

DOWNREGULATION OF AMYLOID PRECURSOR PROTEIN (APP) mRNA EXPRESSION FOLLOWING POST-TRAUMATIC CYCLOSPORIN-A ADMINISTRATION

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Amyloid precursor protein (APP) mRNA and antigen are rapidly increased following traumatic brain injury (TBI) and the expression is further increased following administration of the neuroprotectant magnesium sulphate (MgSO4)[1]. The aim of these studies was to assess and quantitate the effects of another neuroprotectant, Cyclosporin-A (CyA), on APP mRNA expression in sheep brains after a controlled focal head impact. Two-year-old merino ewes were injured under isoflurane anesthesia using the humane stunner as previously described in detail elsewhere [1]. Animals were then killed at 2 h or 6 h after injury, and their brains removed, sectioned and snap frozen in liquid nitrogen for PCR analysis. In contrast to the upregulation of APP previously observed with MgSO4, post-traumatic administration of CyA resulted in a rapid reduction in APP mRNA expression. CyA treatment caused a statistically significant 1.3 ± 0.1 fold decrease in APP mRNA in the central grey matter of CyA treated impacted sheep compared to untreated impacted sheep 2 hours post-injury ($p < 0.05$). A more profound reduction in APP mRNA synthesis (1.6 ± 0.2 fold) was evident at 6 hours ($p < 0.001$). These results show that CyA has a downregulatory effect on the increased APP expression caused by TBI. This has potential implications as a means of preventing APP overexpression and possibly the pathophysiological processes underlying AD.

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P159.

THE NEUROPROTECTIVE EFFECTS OF PROGESTERONE AND ALLOPREGNANOLONE AFTER CONTROLLED CORTICAL IMPACT IN RATS

Myriam J. Djebaili, Stuart W. Hoffman, Donald G. Stein. (Emory University, Atlanta, Georgia US).*

We have previously shown that systemic injections of progesterone (16mg/kg) are neuroprotective, leading to improve behavioral outcomes following impact injury (CCI) to the frontal cortex. In order to determine if the neuroprotective effect is specific to this neurosteroid, we compared the effect of progesterone (16mg/kg) injections to different doses (0, 4, 8, and 16mg/kg) of one of its metabolites, allopregnanolone. The vehicle for these substances was cyclodextrin.

Our results show that 24h after CCI, progesterone and allopregnanolone (4mg/kg) decreased both body weight and cortical neuronal loss compared with injured animals injected only with vehicle. The higher doses of allopregnanolone did not affect body weight loss, but surprisingly the number of spared cortical neurons was reduced as compared to those in injured-vehicle animals. This demonstrates that allopregnanolone at doses between 8–16mg/kg are toxic in our injury model. Under light microscopy we found that the number of apoptotic neurons was greatly reduced in rats given progesterone, while number of apoptotic neurons was only slightly reduced in the allopregnanolone-treated rats. Currently, histological assays using Akt and caspase-3 immunocytochemistry are in progress to determine the molecular anti-apoptotic mechanisms being affected by these neurosteroids. Additional behavioral assays will determine if there is a relationship between type of treatment, apoptosis, and behavioral outcome.

This study indicates that progesterone and allopregnanolone both inhibit apoptosis after injury, but progesterone seems to be more efficient in this effect. Supported by NIH grants 1R01NS40825, 1R01NS38664, & 5R03HD040295.

P160.

PREGNENOLONE FACILITATES RECOVERY FOLLOWING TRAUMATIC BRAIN INJURY

Melissa A. Arellano, Robert M. Simkins, IV, Stuart W. Hoffman, Donald G. Stein. (Emory University, Atlanta, Georgia US).*

Past studies have shown that both progesterone and its metabolite, allopregnenolone, have been effective in promoting functional recovery following traumatic brain injury (TBI). Pregnenolone, a progesterone precursor, has been associated with neuronal microtubule formation, implicating its role in neural plasticity. In keeping with this we decided to examine the role of chronic administration of pregnenolone in promoting recovery of function following traumatic brain injury.

Male SD rats received either sham surgery or bilateral cortical impact injury to the medial frontal cortex. On surgery day, animals were injected intraperitoneally 1 hour after injury and subcutaneously (SC) 6 hours after with either pregnenolone (6.33 mg/kg) or vehicle (sesame oil). Injections were continued twice a day for 3 weeks post-injury. A baseline measure of bilateral sensory neglect was established one day prior to surgery and then evaluated post-operatively on days 6 and 20. Spontaneous motor activity was also assessed on post-injury days 2, 5, and 10 after injury. Spatial learning was measured using the Morris water maze (MWM) task for 2 blocks of five days, starting 36 hours after the end of the 3-week injection period. Animals were then perfused and their brains paraffin embedded for histological analyses.

Results of behavioral testing show that pregnenolone facilitates improved sensory performance in animals with bilateral cortical contusions. Although initially, pregnenolone-treated animals showed a deficit in MWM, by day 6 of testing, they showed enhanced performance compared to vehicle treated animals. Supported by NIH grants 1R01NS38664, R01NS40825, & 5R03HD040295 and a gift from GeneralCologneRe.

P161.

STEROIDS IN SEVERE TBI: A META-ANALYSIS

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Objectives: We systematically reviewed and quantitatively evaluated the literature to assess the efficacy of steroids in severe TBI to improve survival and/or neurological outcome, in order to determine whether this therapeutic option was discarded prematurely.

Methods: Pubmed, Embase, and the Cochrane Controlled Trials Registry databases were searched from 1964 to 2002. Randomized control trials (RCT) in English or German were extracted, involving adults and/or children with severe closed TBI, receiving either cortico- or amino-steroids compared to placebo. Risk ratios (RR) of events were used to determine pooled estimates of risk using a random effects model.

Results: We retrieved 749 articles. Eleven articles remained for analyses involving 2611 subjects. Dexamethazone was used in seven studies, methylprednisolone in two, triamcinilone acetonide in one, and tirilizad mesylate in one. The doses ranged from 77 mg to 2300 mg of dexamethazone equivalent. The pooled estimate RR of death in the steroid group compared to the placebo group was 0.93 (95%CI 0.78–1.10, p-value: 0.41, homogeneity statistic 15.24 d.f.: 9, p-value: 0.084) and RR of death and disability was 1.09 (95%CI 0.87–1.35, p-value: 0.45, homogeneity statistic 37.86, d.f.: 10, p-value: 0.000). A regression of the RR of death with the dexamethazone dose showed an increase of RR by 1.1% for every increase in 100 mg of dexamethazone (0.011, 95%CI -0.02- 0.04, p-value: 0.442) and the RR of death in the steroid group was 19% higher for patients receiving a dose above 500 mg compared to a dose below this value (0.19, 95%CI -0.17-0.55, p-value: 0.311).

Discussion: Our analyses of the evidence show that steroids neither present a beneficial or detrimental effect on the risk of death of patients with severe TBI. We suggest that the pre-clinical experimental evidence has not been translated into a beneficial clinical endpoint because questions such as determining the efficacious dose and type of steroid remain unanswered. The information reported did not allow us to evaluate the effect of age, gender, and management strategies as confounders or effect modifiers on the outcome. To quantify their impact, reporting of adherence to therapeutic management strategies and documentation of clinical confounders during acute and rehabilitation phases are essential for ongoing trials.

P162.

DELIBERATE MILD HYPOTHERMIA FOR TREATMENT OF SEVERE BRAIN INJURY

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Thirty patients with severe head injuries with Glasgow Coma Scale (GCS) score of 3–8 were enrolled into the study. The subjects were divided into two groups. The average age in the hypothermic group of 15 patients (10 subdural and 3 epidural hematomas, 2 brain contusions) was 35 years. The average GCS was 4.5 at the site of accident. The average age of the 15 patients (7 subdural and 7 epidural hematomas, 1 brain contusion) in the normothermic control group was 39 years with an average GCS of 4.3. All the patients in the normothermic group and 11 patients in the hypothermic group underwent neurosurgery. The standard treatment was guided according to the European Brain Injury Consortium protocol. Cooling to a core temperature of 34°C in the hypothermic group was achieved by forced air cooling in combination with circulating-water mattress cooling (Blanketrol II, Cincinnati Sub-Zero) and maintained for 72 hours.

The difference in the Glasgow Outcome Scale (GOS) between the hypothermic and normothermic groups of patients after six months was not statistically significant (p value 0.0843). In the hypothermic group, however, good neurological outcome (GOS 4 and 5) was reached in 13 patients (87%), which represents a 40% increase compared with the normothermic control group in which good neurological outcome was reached in 7 patients (47%). Mean normothermia ICP value of 18 ± 2 mmHg was significantly (p value 0,0007) reduced during mild hypothermia therapy to 12 ± 2 mmHg. Mean normothermia CPP value of 72 ± 3 mmHg significantly increased (p value 0,0007) during this time to 80 ± 4 mmHg with unchanged systolic arterial pressure (p value 0,9013).

Our results showed that mild hypothermia could be usefull in improving the outcome and neurological recovery, especially in brain injured patients with surgical lesion on admission.

P163.

REGULATION OF HYDROGEN PEROXIDE PRODUCTION BY BRAIN MITOCHONDRIA BY CALCIUM AND A BH3 DEATH DOMAIN PEPTIDE

Gary Fiskum and Anatoly Starkov. (University of Maryland School of Medicine, Baltimore, MD US).*

Background: Abnormal accumulation of Ca2+ and exposure to pro-apoptotic proteins, e.g., Bax, is believed to stimulate mitochondrial generation of reactive oxygen species (ROS) and contribute to neural cell death during acute ischemic and traumatic brain injury. However the mechanism by which Ca2+ or apoptotic proteins stimulate mitochondrial ROS production is unclear. This study tested the hypothesis that Ca2+ can either stimulate or inhibit mitochondrial ROS generation, depending on the source of electrons donated to the respiratory chain and the effects of Ca2+ on the retention of mitochondrial cytochrome c. We also tested the hypothesis that mitochondrial ROS production is stimulated by cytochrome c release elicited by exposure to Bax and a peptide containing a BH3 cell death domain.

Methods: H2O2 production by isolated rat forebrain mitochondria was monitored fluorometrically using Amplex Red in the presence of ATP and Mg2+ and different respiratory substrates. Mitochondrial membrane potential was monitored with the fluorescent dye Safranine O. Ca2+ transport was measured by monitoring the medium [Ca2+] using the fluorescent dye Calcium Green. Cytochrome c was quantified using ELISA.

Results: Ca2+ uptake suppressed H2O2 generation and reduced the membrane potential of mitochondria oxidizing succinate or glutamate plus malate. In the presence of the respiratory chain inhibitor rotenone, Ca2+ stimulated H2O2 production by mitochondria oxidizing succinate and induced the release of cytochrome c. In the absence of Ca2+, release of cytochrome c induced by BAX protein plus a BH3 cell death domain peptide also stimulated H2O2 production.

Conclusions: In the presence of physiological concentrations of ATP and Mg2+, Ca2+ accumulation suppresses mitochondrial ROS production by decreasing mitochondrial membrane potential. However, Ca2+ as well as Bax plus a BH3 domain peptide stimulate ROS production when conditions favor mitochondrial release of cytochrome c.

Support: NS34152 and ES11838.

P164.

EFFECT OF DEXTROMETHORPHAN—A NON-COMPETITIVE NMDA ANTAGONIST—ON THE SECONDARY GROWTH OF A CORTICAL NECROSIS FROM FOCAL COLD INJURY

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Objective: A cortical lesion induced by cold injury leads to an immediate burst of excitatory amino acids into the traumatic penumbra. Aim of the study was to investigate, whether the secondary growth of a cortical necrosis can be attenuated by the non-competitive NMDA antagonist dextromethorphan.

Material & Methods: Male SD-rats (n = 17) were anesthetized (N20/halothane) and ventilated. The tail artery/jugular vein were cannulated for measurement of mean arterial blood pressure/blood gases and drug administration. Brain temperature was kept constant (37.0°C) by an electrode in the temporal muscle and feedback-controlled heating. Dextromethorphan (solved in NaCl) was given i.v. 20 min before trauma (20 mg/kg) followed by 10 mg/kg/h until sacrifice. The sham group received NaCl (4 ml/kg followed by 2 ml/kg/h). After right parietal trephination, a standardized freezing lesion was induced onto the brain cortex. 24 h later, the animals were sacrificed and brains were removed for histology.

Results: At 24 h after focal cold injury (i.e. maximal necrosis spread in rats) sham treated animals had a cortical lesion with a necrosis volume of 3.82 ± 0.45 mm3. After treatment with dextromethorphan, the animals developed a necrosis of the cortex with a volume of 3.87 ± 0.33 mm3 (n.s.). In relation to the volume of the ipsilateral hemisphere, the cortical necrosis expanded to 5.6 ± 0.1 % in sham treated animals and to 5.9 ± 0.2 % in the dextromethorphan group. Throughout the observation period, mean arterial blood pressure remained constant (sham: 82 ± 4 mmHg, dextro: 81 ± 3 mmHg) without statistically significant differences between the groups.

Conclusion: Administration of dextromethorphan prior to a focal cold injury to the brain does not attenuate the amount of tissue damage 24 h after trauma. Accordingly, it must be surmised that glutamate does not mediate secondary necrosis growth of a traumatic cortical lesion via the NMDA receptor.

Supported by BMBF-Verbund "Neurotrauma" München, FKZ: 01 KO 94026

P165.

NEURAL STEM CELL TRANSPLANTS FOLLOWING BRAIN INJURY IN RATS: CELLULAR SURVIVAL AND BEHVIORAL CONSEQUENCES

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Certain members of the newly discovered family of intrinsic inhibitors of apoptosis (IAP) proteins can directly bind and inhibit caspases. However, IAPs have been shown to undergo cleavage by caspases in response to inducers of apoptosis, but the significance of IAP cleavage has not been established. One IAP family member that is of particular interest in gender studies is the X-linked inhibitor of apoptosis (XIAP) that undergoes cleavage following traumatic brain injury. Since estrogen has been shown to have anti-apoptotic properties, this study examined gender differences and the influence of estrogen on XIAP processing during apoptosis after TBI. Male (TBI-M, n = 6), female (TBI-6, n = 3), ovariectomized female (TBI-OVX, n = 5) and ovariectomized females supplemented with estrogen (TBI-OVX+EST, n = 7) Sprague-Dawley rats were intubated, anesthetized (70%N2O, 0.5% halothane, 30%O2) and subjected to a moderate (1.7–2.2 atm) fluid percussion injury (FPI). Animals were sacrificed 24 hrs after FPI; cortical tissue (ipsilateral and contralateral) was dissected and analyzed for XIAP processing by immunoblot analysis and quantitative densitometry. Significant differences in XIAP cleavage in the ipsilateral cortex were found between groups ($p < 0.03$). Post-hoc analysis showed an increase in XIAP processing in both TBI-F and TBI-OVX+EST compared to TBI-M and TBI-OVX ($p < 0.05$), indicating that more XIAP is cleaved following injury in intact females and estrogen supplemented ovariectomized animals than in TBI-M and TBI-OVX groups. Based on these data, we propose that estrogen may provide neuroprotection by regulating XIAP cleavage after injury. This regulation may be influenced by exogenous estrogen treatment. (NS 30291 & Eli Lilly and Co.)

P166.

COMPARISON OF TWO PROTOCOLS TO DIFFERENTIATE BONE MARROW STROMAL CELLS INTO NEURONS OR GLIA

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Recent reports suggest that adult-derived bone marrow stromal cells (BMSCs) become neuron-like after differentiation, implying that BMSCs could be used for repair after CNS injury. However, in vitro protocols for neuronal differentiation of BMSCs vary substantially and have not been systematically compared. This study replicated and compared the Woodbury et al., 2001 and the Deng et al., 2001 protocols for BMSCs differentiation in sister cell preparations. BMSCs were harvested from adult rat femurs/tibias. passaged at least 6 times. differentiated. and processed for immunocytochemistry for NeuN and GFAP at various time-points. Immunoreactivity was analyzed hourly during the Woodbury differentiation and daily using the Deng method. To determine longevity of differentiated state, cells were then placed in their respective maintenance media or stem cell feed and evaluated by light microscopy for morphology as well as processed for immunoreactivity at 12, 24, and 72 hrs. An adaptation of unbiased sterological technique was used for cell counting. Although highly variable, results show a higher percentage of NeuN and GFAP positive cells in the Woodbury vs. Deng protocol as differentiation time increased. Additionally, cells removed from the maintenance media in both conditions had a significant decrease in expression for either NeuN or GFAP. In conclusion, although results show higher NeuN positivity utilizing the Woodbury protocol. the percentage of differentiated cells is highly variable within each condition in both protocols. This suggests that BMSCs have a large heterogeneity of potential and that maintaining a differentiated state is dependent on specific environmental conditions which may question the benefit of BMSCs in replacement therapy. Supported by UC President's Undergraduate Fellowship and UC Neurotrauma Research Initiative.

P167.

THE EFFECT OF RUTHENIUM RED, AIDA AND MK-801 ON MITOCHONDRIAL MEMBRANE POTENTIAL (MMP) IN STRAIN-INJURED ASTROCYTES

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The purpose of these studies was to determine if astrocytes in neuronal plus glial cultures show a reduction in mitochondrial membrane potential (MMP) in response to strain- (stretch) induced injury, as previously reported in pure astrocyte cultures, and to probe potential mechanisms which reduce MMP in astrocytes of mixed cultures. Rhodamine 123 fluorescence was used as an indicator of MMP. We found that astrocytes in injured mixed cultures displayed an identical drop in MMP to that in pure astrocyte cultures. Previous studies have shown that there is post-traumatic activation of group 1 metabotropic receptors (mGluR1) in astrocytes. However we found no effect of the mGluR1 antagonist AIDA on injured astrocyte MMP.

We have also previously shown that pretreatment with NMDA antagonists partially reduces the loss of MMP seen in neurons of strain-injured neuronal plus glial cultures (J. Neurotrauma 17: 957, 2000). Because neurons and glia signal to each other and NMDA receptors have recently been reported on astrocytes, we examined the effect of MK-801 pretreatment on astrocyte MMP in neuronal plus glial cultures. We found that MK-801 significantly increased uninjured astrocyte MMP by 20%, but did not affect the reduction in MMP found in injured astrocytes.

Following injury, intracellular Ca2+ is thought to be in part buffered by mitochondrial uptake. We used the mitochondrial Ca2+ uniporter inhibitor Ruthenium Red (RR) to determine if blockade of mitochondrial Ca2+ uptake might affect astrocyte MMP. RR pretreatment had no effect on uninjured astrocyte MMP, but reduced the post-traumatic decrease in astrocyte MMP by one-third. These studies suggest that post-traumatic mitochondrial Ca2+ influx may play a role in the reduction of astrocyte MMP, but do not support a role for mGluR1 or NMDA receptors in loss of astrocyte MMP in strain-injured neuronal plus glial cultures. Supported by NS-27214.

P168.

GLIAL NEURONAL SIGNALING IN NEUROTRAUMA, STUDIED IN PRIMARY CULTURES

Elisabeth Hansson and Lars Rönnbäck. (Institute of Clinical Neuroscience, Göteborg, SE).

After a brain damage, nucleosides, nucleotides and glutamate (Glu) are released from the damaged region, and pH drops. These substances and altered conditions induce microglial proliferation, and activation of both microglia and astroglia with a resultant production of an array of molecules, including cytokines. We report on astroglial-microglial interactions in a model system of primary cultures and co-cultures when Glu level is increased and pH decreased. We report on early microglial activation with the formation of vacuoles followed by astroglial and microglial interactions with cytokine production, and we also report on microglial process and network formations. The substances produced are highly neuroactive with effects on other cell types in the nervous system. Interleukin-1beta (Il-1beta) decreases connexin 43 expression, and thereby astroglial gap junction coupling. Tumor necrosis factor alpha (TNF-alpha) reduces astroglial glutamate uptake capacity. Potassium, released from microglial and astroglial cells further impair the astroglial glutamate uptake capacity. Microglia can also produce and release toxic substances such as free radicals, superoxide radicals, and nitric oxide (NO) to participate in cytotoxic reactions. Transforming growth factor-beta (TGF-beta) inhibits the release of TNF-alpha, and vasoactive intestinal peptide (VIP), released from depolarized neurons, and adenosine, released from astrocytes participate in protective mechanisms. VIP induces the synthesis of astroglial derived neurotrophic factor (ADNF), which in turn induces the production of neurotropin-3 (NT-3). Adenosine increases astroglial glutamate uptake capacity and stimulates the production of trophic factors of importance in the rebuilding of the nervous system, such as basic fibroblast growth factor (bFGF), nerve growth factor (NGF) and interleukin-1 (Il-1). In conclusion, close interactions between astroglia and microglia could be important during early phases of a brain injury and during neuroprotection.

P169.

SIGNALING FROM ATP RECEPTORS TO ERK IN AN IN VITRO MODEL OF TRAUMATIC BRAIN INJURY

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Previously we reported that rapid, reversible stretch-induced injury of astrocytes cultured on deformable silastic membranes resulted in activation of extracellular signal regulated protein kinase (ERK), a key regulator of cellular proliferation and differentiation. When ATP released by injury was hydrolyzed by apyrase, ERK activation was significantly reduced, suggesting the involvement of ATP/P2 receptors. To test this hypothesis, we studied the effect of P2 receptor antagonists on injury-induced ERK activation in primary cultures of rat cortical astrocytes. Treatment of astrocytes with suramin, a broad-spectrum P2 receptor antagonist, inhibited injury-induced ERK activation by 65%. Reactive blue 2, an antagonist of P2X2 receptors which are expressed on astrocytes and P2Y6 and P2Y12 which are not, was as effective as suramin. PPADS, an antagonist of several P2X and P2Y receptors subtypes expressed on astrocytes, was partially effective. Brilliant blue G, an antagonist of P2X7 receptors, did not inhibit injury-induced ERK activation. In addition, pre-injury treatment with EGTA almost completely blocked activation of ERK, indicating that an influx of calcium is required for ERK activation. These results, as well as P2 agonist studies of ERK activation in uninjured astrocytes, suggest that ionotropic P2X receptors, perhaps P2X2 receptors, are selectively activated after injury. However, further studies are needed to identify the P2X subtype(s) involved and to evaluate the possible role of P2Y receptors. We conclude that activation of ERK by traumatic injury is mediated in part by ATP/P2 receptors and suggest that this signaling pathway contributes to the development of gliosis after brain trauma. (Supported by the Department of Veterans Affairs.)

P170.

DELAYED TREATMENT WITH DEHYDROEPIANDROSTERONE SULFATE ENHANCES GENE EXPRESSION RELATED TO NEUROPLASTICITY

Stuart W. Hoffman, Edward H. Pettus, Robert M. Simkins, IV, Donald G. Stein. (Emory University, Atlanta, Georgia US).*

Dehydroepiandrosterone sulfate (DHEAS) has been previously shown to be a nootropic/neurotrophic neurosteroid. Our recent results indicate that DHEAS can facilitate recovery of function after a bilateral control cortical impact (CCI) to prefrontal cortex when given after a 7-day delay and then daily prior to behavioral testing. To determine the effects of DHEAS administration on neuroplasticity during this period of recovery, we employed the use of DNA microarray technology.

Starting 7-days after CCI, rats were injected with 10mg/kg of DHEAS (n = 3) or vehicle (n = 3) 1hr before testing on a water maze task. The injections and testing continued for the next 4-days for a total of 5 injections and 5-days of testing. Approximately 2h after the final trial of testing, the rats were killed and the frontal cortices that contained the injury were processed for RNA isolation. The isolated RNA served as a template for cDNA, which was then used as a template for the labeled cRNA that was hybridized to the Affymetrix™ U34A (8800-genes) chip. Chips were scanned and analyzed in Emory's core facility.

The latencies to the platform over 5-days of testing were vehicle 66s ± 14.5 vs DHEAS 44s ± 5.9. The results of the DNA microarray indicated that DHEAS treatments in these animals produced consistent increases in the expression of genes that are linked to neuroplasticity, such as MAP2, GAP43, and CaMKinase when compared to injured-controls. In addition, the expression of genes that counter neuroplasticity (GFAP and S100) were reduced. PCR and Western blot analyses are in progress.

These results indicate that DHEAS promotes behavioral recovery by enhancing mechanisms related to neuroplasticity. Supported by NIH grants 5R03HD040295, 1R01NS40825, & 1R01NS38664.

P171.

TIMING OF PHYSICAL EXERCISE FOLLOWING MILD TRAUMATIC BRAIN INJURY: IMPACT ON STROOP TASK PERFORMANCE

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Functional recovery following traumatic brain injury (TBI) is variable and appears to be dependent on post-injury "critical periods" or windows of opportunity during which the recovery process is exquisitely vulnerable to intervention. While there has been much study of these issues using animal models, less is know about critical periods following human TBI. For example, the role of physical exercise after brain insult is believed to be important, but what remains unclear is the significance of timing, i.e., when exercise is introduced. Thus, the present, retrospective study was designed to delineate the window of opportunity for physical exercise following mild TBI. Cognitive functions (e.g. memory, attention, concentration, selective attention) were assessed using a neuropsychological battery comparing current functional level with initial post-injury performance on the same measures. Independent variables of interest were the timing, type and frequency of post-injury exercise. Results indicate that selective attention measures, such as the Stroop task, are impacted by post-injury exercise. Specifically, the earlier the participant initiated exercise within the first 12 months following TBI, the lower the Stroop interference score. These findings could not be accounted for by time since injury, age at time of injury or the use of certain other rehabilitation interventions. However, exercise type (aerobic vs. resistance training) and frequency appear to be important. Thus, the initiation of exercise within a critical time period after mild TBI can be correlated with improvements in certain aspects of cognitive function.

P172.

THE DELAYED ADMINISTRATION OF DEHYDROEPIANDROSTERONE SULFATE PROMOTES RECOVERY OF FUNCTION AFTER CORTICAL IMPACT INJURY

Sharad Virmani, Robert M. Simkins, IV, Donald G. Stein, Stuart W. Hoffman. (Emory University, Atlanta, Georgia US).*

Traumatic brain injury (TBI) initiates destructive sequelae of secondary events within the brain that lead to permanent cognitive and sensorimotor deficits. Previous research has shown that GABAergic neurosteroids are neuroprotective. The goal of the current study was to show that dehydroepiandrosterone-sulfate (DHEAS), a stimulatory neurosteroid, could facilitate recovery of function in male rats after delayed treatment following TBI. DHEAS has been found to play a major role in brain development by influencing the migration of neurons, arborization of dendrites, and formation of new synapses.

In our study, behavioral assays were conducted concurrently with DHEAS administration (0, 5, 10, or 20 mg/kg in 2-hydroxypropyl-β-cyclodextrin) starting seven days post-injury (PI). Behavioral assays included 10-days of Morris Water Maze testing (MWM; 7d PI), 10-days of Greek-Cross (GC; 21d PI), Tactile Adhesive Removal (TAR; PI days: 6,13,20,27,34), and spontaneous motor behavior (SMB; PI days: 2,4,6,12,19,26,33).

Results showed an improvement in performance in all tasks among injured rats that received 5, 10, or 20 mg/kg DHEAS, yet, the most effective dose was task dependent. The most effective dose of DHEAS in the MWM was 10mg/kg, in the GC it was 20mg/kg, TAR was 5mg/kg, and all doses, except for vehicle, were effective at reducing injury-induced SMB. In no task did DHEAS treated animals perform worse than the injured-controls. In addition, DHEAS had no significant effects on behavioral performance in the sham-operates.

These results demonstrate that after a 7-day delay, the chronic administration of DHEAS to injured rats significantly improves behavioral recovery on both sensorimotor and cognitive tasks. Supported by NIH grants 5R03HD040295, 1R01NS40825, & 1R01NS38664.

P173.

TRAUMATIC PERIMESENCEPHALIC SUBARACHNOID HEMORRHAGE: A SIGN OF BRAINSTEM INJURY

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Objective: To evaluate the frequency, distribution, appearance, and clinical outcome of brainstem injury, as seen on MR, in a prospective study of patients with traumatic perimesencephalic subarachnoid hemorrhage(pSAH) seen on initial CT scan.

Methods: MR images were prospectively obtained in 38 patients with head injury who on initial CT scans showed pSAH. To identify the amount and location of pSAH, the CT scans of all patients were evaluated, and MRI findings were evaluated according to the presence, location and signal intensity of brainstem injury, and other combined intracranial injuries. Initial Glasgow coma scale(GCS) and Glasgow outcome scale(GOS), as noted on clinical records, were reviewed.

Results: Brainstem injury was demonstrated on MR imaging in 30 patients(79%). The majority of these lesions(76.7%) were located in the dorsolateral portion, and nonhemorrhagic lesions were more frequent(70%) than hemorrhagic.

In patients with brainstem injury, as seen on MR imaging, the GOS score was worse, especially in those with combined diffuse axonal injury in the corpus callosum and cerebral white matter.

The location and amount of pSAH seen on CT was not related with brainstem injury or clinical outcome.

Conclusion: The presence of pSAH in patients with acute head trauma, as seen on CT was thought to be an indicator of brainstem injury, and MR imaging was necessary. If such injury was identified on MRI, this was predictive of a worse clinical outcome.

P174.

LOSS IN CORRELATION BETWEEN ADMISSION GCS AND OUTCOME IN PATIENTS WITH MULTIMODAL BEDSIDE MONITORING

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Background: Age and Glasgow Coma Scale (GCS) score on admission are considered important predictors of outcome after traumatic brain injury [1]. More recently data from computerized neuromonitoring systems have been shown to add relevant information for building prognostic models following head injury [2]. We investigated the predictive value of GCS and age in a large group of patients in whom multimodal bedside monitoring data were processed over the last ten years.

Methods: Data from 358 head injured patients collected between 1992 and 2001 were analysed retrospectively. Patients were grouped according to year of admission. GCS and Glasgow Outcome Scores (GOS) at six months were determined. Spearman's correlation coefficients between GCS and GOS were calculated for each year.

Results: On average 34 ($\pm$7 SD) patients were monitored every year. We found a significant correlation between GCS and GOS for the first 5 years (overall 1992–1996 $r = 0.41$; $p < 0.00001$; $n = 183$) and consistent lack of correlations starting from 1997 (overall 1997-2001 $r = 0.091$; $p = 0.226$; $n = 175$). In contrast correlations between age and GOS were in both time periods significant and similar (Age: 1992–1996 vs. 1997-2001: $r = -0.24$ vs. $r = -0.24$; $p < 0.002$).

Conclusions: Admission GCS has lost its predictive value for outcome in this group of patients from 1997 onwards. We can only speculate on which elements of our management have caused this: an inconsistency in obtaining GCS perhaps influenced by more aggressive pre-hospital treatment, as well as progress in clinical management may have influenced the relevance of GCS for outcome. The predictive value of GCS should be carefully reconsidered when building prognostic models incorporating multimodality monitoring after head injury.

References: (1) Bullock et al. J Neurotrauma 2000;17:451–554. (2) Czosnyka et al. Int J Clin Monit Comput 1994;11(4):223–232.

P175.

DIFFUSION WEIGHTED MAGNETIC RESONANCE IMAGING OF EDEMA FOLLOWING TRAUMATIC BRAIN INJURY IN RATS

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Traumatic brain injury (TBI) is often associated with edema that can be examined using diffusion weighted imaging. We examined the development and extent of edema following lateral fluid percussion TBI in rats using diffusion weighted imaging (DWI). Baseline imaging was carried out in uninjured adult, male Sprague Dawley rats on a Bruker 7T magnet with ParaVision software. Animals were anesthetized and mechanically ventilated, with image acquisition gated to respiration cycle. After TBI, DWI was carried out at 1, 2, 24 hr, 1–5, 9 and 10 weeks to examine the development of edema. Apparent diffusion coefficient (ADC) values were calculated for specific regions of interest, including hippocampus, parieto-occipital cortex, temporal cortex, retrosplenial cortex and thalamus, and compared with histopathological findings. ADC values in the hemisphere contralateral to TBI remained unchanged over 10 weeks of imaging. In contrast, a small, localized area of hypotensity (i.e., decreased ADC) in ipsilateral hippocampus developed 1–2 hr after TBI. With later imaging a large area of hyperintensity (i.e., increased ADC) in the hippocampus, parieto-occipital cortex and temporal cortex began 3–4 weeks after TBI. Histopathological evaluations indicated that the late appearing hyperintensity in the ADC map was due to fluid accumulation within enlarged ventricles and a region of cavitation at injury site. The small changes in the DWI and ADC maps immediately after TBI suggest that vasogenic edema is not a major consequence of LFP injury in rodent brain, and that accumulation of extracellular fluid associated with TBI is readily quantified by DWI. (Supported by NIH NS 39090, a UCD NMR Award and the UC Neurotrauma Research Initiative).

P176.

VASCULAR TUNNEL CREATION AND FURTHER SPACE WINNING METHODS IN THE TREATMENT OF AGRESSIVE BRAIN SWELLING.

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Introduction: Decompressive craniectomy with durotomy, is a last resort therapy. Although the method successfully diminishes the ICP, partial or total vascular insufficiency occurs in the herniated part of the brain. The cause of the insufficiency is most likely due to the compression of the cortical veins and arteries, caused by shearing and pressure forces between the dural edge and brain tissue. Furthermore venous congestion may induce edema in the protruding parts of the brain.

Methods: The new surgical technique consists of a stellate type durotomy and the creation of a vascular tunnel, by supporting pillars, on both sides of the main cortical veins and arteries, between the dural edge and brain surface, with the aim that the vessels do not become compressed by the sharp dural or bone edge. The effect of the novel vascular tunnel technique was proven by measuring the blood flow of the protected and non protected veins with doppler UH, intraoperatively. Further space winning surgical methods was applied by scarification and streching the skin or let it open.

Results: Last two years 33 patients were operated on with this method. All were in severe GCS 3 or GCS 4 status, with more than 30 mmHg ICP. In comparison with the traditional treatment, the mortality rate was reduced from 80% to 40% and, recovery (GOS4,5) rate increased significantly in these severe cases.

Conclusions: with this technique the ICP was significantly reduced and further edema and vascular insufficiency was prevented.

References: Csókay et al. Vascular tunnel creation to improve the efficacy of decompressive craniotomy in post-traumatic cerebral edema and ischemic stroke Surgical Neurology 2002-57:2, 126–129. Csókay et al. Vascular tunnel construction in the treatment of severe brain swelling caused by trauma and SAH. (Evidence based on intra-operative blood flow measure). Neurological Research 2002; 24: 157–160

P177.

CONCEPT OF 'TRUE ICP' IN MONITORING AND PROGNOSTICATION IN HEAD TRAUMA

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Objective: Mean ICP is an important prognostic variable in severe head injury. However, absolute ICP would more predictive if we could take into account where on the Pressure-Volume Curve the 'working' pressure point is positioned. ICP above 30 mmHg may not be the danger when compensatory reserve is sufficient, while 20 mmHg, may be life-threatening when compensatory reserve is close to exhaustion. The RAP coefficient helps to monitor this reserve continuously. We propose a new coefficient, which contains information on both the absolute ICP and the pressure-volume compensatory reserve.

Method: ICP was monitored daily in 176 sedated and ventilated patients. The RAP coefficient was calculated as the running (6 minutes) correlation coefficient between slow changes in pulse amplitude and mean ICP. RAP has been demonstrated to have value 0 on the flat part of the Pressure-Volume Curve and +1 on ascending exponential part. Then RAP decreases to zero and then becomes negative when ICP is so high that it affects cerebrovascular pressure-reactivity. Coefficient tICP = ICP*(1-rap) has been called 'true ICP'. It magnifies the critical values of ICP when cerbrovascular pressure reactivity is exhausted and dampens those states where absolute ICP is moderately elevated but vascular reactivity reserve is not affected.

Results: Both Mean ICP and RAP are independently correlated with outcome (ANOVA: ICP-GOS: $F = 5.9$; $p < 0.0007$, RAP-GOS: $F = 3.4$;$p < 0.02$). 'True ICP' has a much stronger association with outcome: $F = 8.8$;$p < 0.00001$.The association between GCS and outcome was weaker: $F = 4.8$;$p < 0.004$ and the association between outcome and CPP was not significant as a majority of patients were managed using CPP-oriented protocol.

Conclusion: The proposed variable is a more powerful predictor of the outcome following head injury than ICP or GCS. It is sensitive to both the rising absolute ICP and the critical change of the pressure-volume compensation.

P178.

ACCUMULATION OF CALPAIN AND CASPASE-3 CLEAVED AII-SPECTRIN BREAKDOWN PRODUCTS IN CSF OF PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY.

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Several lines of evidence in animal models suggest that calpain may play a major role in traumatic brain injury. Moreover the role of caspase-3 protease as key executioner in mammalian apoptosis is well established. Nonerythroid (alpha II-spectrin (alpha-fodrin) is a cytoskeletal protein that is a substrate of both calpain and caspase-3 cysteine proteases. Cleavage of (alpha II-spectrin by calpain and caspase-3 results in accumulation of protease-specific (alpha II-spectrin breakdown products (SBDPs) that can be used to monitor the magnitude and temporal duration of protease activation. We performed a longitudinal western blotting analysis from 3 TBI patients using monoclonal antibodies against (alpha II-spectrin (280 KD), calpain specific (145 KD) and caspase-3 specific (120 KD) SBDPs. Calpain SBDPs were present immediately after injury in all patients and gradually decreased but were still apparent up to 6 days after injury. Caspase-3 SBDPs were also evident immediately after injury in all patients but were not as pronounced as the calpain SBDPs. Caspase-3 SBDPs decreased in intensity and disappeared by the fifth day. Importantly, in one patient ICP increased at 18 h and 3 days and these ICP spikes were associated with concomitant increases in SBDPs (particularly calpain). Thus, (II-spectrin and its calpain and caspase-3 specific SBDPs appears to be sensitive to secondary cerebral injuries. These preliminary data suggest that alpha II-spectrin-SBDPs may be useful biochemical markers of human brain injury, allow for monitoring of specific proteolytic cascades known to play an important role in TBI, and provide potentially sensitive markers for determining severity of brain injury and effects of therapy. (Supported by DAMD17-99-1-9565, DAMD17-01-1-0765, NIH R01 NS39091, NIH R01 and NS40182).

P179.

MODELLING INTRACRANIAL PRESSURE INSULTS IN HEAD-INJURED PATIENTS USING ARTIFICIAL NEURAL NETWORKS

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The objective of this investigation was to model the incidence of intracranial pressure (ICP) insults in head-injured patients with the use of artificial neural networks (ANN).

The BrainIT Multicentre project supplied data from 23 patients admitted to three neurotrauma units between 12/2001 and 4/2002. For each patient mean ICP and compliance were recorded every minute. All patients were aged over 16 and were excluded if they had undergone craniotomy or were brain dead on admission.

ICP insults were defined where within any 5 minute sliding window at least 3 of the measurements were greater than a threshold value (20 and 25 mmHg). Continuous, sub-threshold, ten minute sequences were marked, some of which were "positive sequences" followed by an insult after 5 minutes, and the remainder which were not ("negative sequences"). For each sequence the following were extracted: rates of change in ICP and compliance, standard deviations, maximum ICP, % time ICP > predefined sub-threshold value, minimum compliance, % time compliance < 0.8 ml/mmHg, a flag indicating the sequence class. A dataset balanced between the two classes was created and divided into two parts, 70% for training an ANN, and 30% for testing performance. The process was repeated 20 times for each threshold value.

Where the thresholds were 20 and 25 mmHg the number of positive/negative sequences identified were 426/8043 and 278/10920 respectively. The accuracies of the ANNs in classifying sequences from the test set were 71.6% (CI95: 70.4–72.8) and 68.8% (CI95: 66.6–71.1). The most important predictors were maximum mean ICP, and rates of change in ICP at later time points.

ANNs are a promising methodology for predicting ICP insults and could form the basis of an intensive care early warning system. Improved performance may be achieved by pre-processing the ICP signal, including other physiological indices in the model, and by detailed annotation of clinical interventions.

P180.

THE EFFECT OF MICROGLIA ABLATION FOLLOWING TRAUMATIC BRAIN INJURY IN MICE

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As a whole, whether activated microglia are acting as protectors or attackers to the neurons in response to the certain pathological condition such as trauma? In this study we used microglia-specific immunotoxin, Mac-1-SAPORIN, at the time of injury to temporally eliminate microglia to see the effects following penetrating traumatic brain injury in mice.

At 24 hours after injury, very few arborized microglia were observed around the injury site in microglia ablation group, while arborized microglia were widely distributed around the injury site or throughout the ipsilateral hippocampus and round-shaped microglia/macrophage were sparsely distributed along the needle track in microglia non-ablation group. At 7 days after injury, many arborized microglia were observed around the injury site or throughout the ipsilateral hippocampus even in immunotoxin treated (microglia ablation) group. Gliosis around the injury site was more evident in microglia non-ablation group at 72 hours and 7 days following injury. At 72 hours after injury, the neuronal cell loss became evident in the non-ablation group compared to that in ablation group. However, at 7 days after injury, no statistically significant difference was observed.

The microglia ablation with immunotoxin ameliorated the neuronal cell loss in the dentate gyrus following penetrating traumatic brain injury in the mouse hippocampus. The microglia ablation also inhibited the hypertrophic change and proliferation of astrocytes following traumatic brain injury. We concluded that activated microglia in the acute stage of penetrating brain injury are considered to mainly act on the neurons suffering from injury as attackers through their phagocytic function and/or modification of astrocyte-neuron interaction.

P181.

OVEREXPRESSION OF X-LINKED INHIBITOR OF APOPTOSIS PROTEIN IMPROVES FUNCTIONAL RECOVERY AFTER CONTROLLED CORTICAL IMPACT INJURY IN THE MOUSE.

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Traumatic brain injury (TBI) initiates secondary injury responses that result in apoptotic cell death and neurological dysfunction. Caspases have been implicated in this process. X-linked inhibitor of apoptosis protein (XIAP) is an endogenous inhibitor of intrinsic caspase activation pathways, through its interactions with caspases 3 and 9. Recently, we evaluated whether delivery of XIAP by a non-replicating type 5 adenoviral construct would improve recovery after controlled-cortical injury (CCI) in the C57BL mouse. We injected the left hippocampus and overlying cortex of mice with either Adeno-LacZ, Adeno-XIAP or an equal volume of saline. Seven days later, animals were subjected to moderately-severe, left, lateral CCI. The number of foot-faults on a balance beam test of motor function was significantly reduced at 7, 14, 21 and 28 days after injury in Adeno-XIAP mice, compared to Adeno-LacZ or saline controls. Adeno-XIAP injected mice showed trends toward improved performance compared to controls in a Morris water maze test of cognition administered on days 14-18 after injury. These data indicate that XIAP significantly improves motor outcome after TBI, and lend additional support to the hypothesis that intrinsic apoptotic cascades may play an important role in cell death and neurological impairment after TBI.

Supported by R01 NS36537-01.

P182.

INFORMATION PROCESSING DEFICITS AND THEIR RELATIONSHIP TO NEUROIMAGING FOLLOWING MODERATE AND SEVERE TRAUMATIC BRAIN INJURY

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Neuroradiological and neuropathological studies indicate that diffuse axonal injury (DAI) is a common consequence of traumatic brain injury (TBI) and that the amount of damage increases with injury severity. DAI particularly affects the frontal and temporal lobes, and the corpus callosum (CC). However, the amount of DAI required to cause detectable neuropsychological deficits in TBI patients is not yet known. Moreover, the information processing deficits that are likely to be caused by diffuse damage (measured by reaction time tasks; RT), and the functional integrity of the CC, are not routinely assessed in clinical settings. This study compared the performance of a group of 25 moderate to severe TBI patients with that of 25 matched controls on standard neuropsychological tests as well as visual and tactile RT tasks that required both compatible (intra-hemispheric processing) and incompatible (inter-hemispheric processing) responses. The latter tasks were designed to target the effects of DAI, including DAI to the CC. The neuropsychological data were also analysed in relation to morphometric analysis of the corpus callosum and the results discussed in terms of loss of interhemispheric connectivity.

P183.

"KEY HOLE" APPROACH FOR THE MANAGEMENT OF NEUROTRAUMA

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Minimally invasive surgery is being utilized recently for non-traumatic brain lesions, such as: removal of tumors, clipping of cerebral aneurysms and others.

Recently we enlarged the indication for the utilization of this procedure and we use it also in some trauma cases. Among other trauma cases we used the approach for draining acute epidural hematoma, repair of the anterior skull base and dural tear in cases of acute traumatic CSF leaks and removal of foreign body in penetrating brain injury.

We are reporting herewith 5 such cases with excellent results. A few representative patients will be presented in detail.

P184.

DETRIMENTAL ROLE OF BRADYKININ B2 RECEPTORS FOLLOWING CLOSED HEAD INJURY IN MICE

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The selective non peptide B2 receptor antagonist, LF 16-0687 Ms , and B2 receptor knock-out mice (B2R-/-) were used to investigate the role of bradykinin B2 receptors in traumatic brain injury (TBI).

TBI was produced using a weight-drop device. Neurological deficit (grip test) and brain water content (BWC) were evaluated 4 h post-trauma. Myeloperoxidase (MPO) activity and B1R, B2R, COX-1, COX-2 and iNOS mRNA levels (RT-PCR) were determined 24 h after TBI.

Intact mice had a grip test score of 28.9 ± 0.8 s. It was 12.3 ± 2.9 s ($p < 0.001$), 17.2 ± 3.0 s (NS), 21.1 ± 2.9 s ($p < 0.05$) and 17.4 ± 3.3 (NS) in traumatized mice treated s.c. with vehicle, 1, 3 and 10 mg/kg LF 30 min after TBI, respectively (n = 14-15). Neuroprotection was still observed when LF-treatment was delayed for up to 2 h after TBI. BWC was 79.8 ± 1% and 82.5 ± 0.2% ($p < 0.001$) in intact and injured mice, respectively. LF (3 mg/kg) reduced by 26% TBI-induced increase of BWC ($p < 0.05$). MPO activity increased from 0.017 ± 0.01 to 0.25 ± 0.05 U/g after TBI ($p < 0.01$). LF (3 mg/kg) reduced TBI-induced MPO activity by 50 % ($p < 0.01$). iNOS mRNA which was absent in intact mice was markedly induced after TBI and this was reduced by LF.

The grip test score was 30 s both in intact B2R+/+ and B2R-/- mice (n = 8-10). It was 10.8 ± 5.0 s and 22.6 ± 3 s in injured B2R+/+ and B2R-/- mice ($p < 0.01$), respectively (n = 7-12). However, BWC was unaltered and MPO activity was non significantly reduced by 36 % in injured B2R-/- mice.

Blockade or deletion of B2 receptors produces a neuroprotection associated with a reduction of the acute inflammatory response. Therefore, blockade of B2 receptors might represent a promising pharmacological treatment of severe TBI.

P185.

SECONDARY GROWTH OF A CORTICAL NECROSIS FROM COLD INJURY IN WILD-TYPE AND INOS-DEFICIENT MICE

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Objective: (1) To evaluate the underlying mechanisms of secondary growth of a cortical necrosis from cold injury in animals with selective deletions of specific genes, this model was adapted for mice. (2) To test the hypothesis that the inducible NO-synthase is a mediator of this phenomenon of secondary brain damage.

Material & Methods: (1) C57Bl/6x129 mice (n = 40) were subjected to a right parietal trephination. A lesion was induced to the cortex by focal freezing (−68° C, Æ 1mm). 10 min, 24 h, 72 h, and 3 weeks thereafter, respectively, the lesion was measured histomorphometrically. (2) At the time of maximal lesion spread [(1)], the volume of the cortical necrosis was evaluated in iNOS-/- mice and their wild-type littermates.

Results: (1) Focal freezing produced a cortical lesion with a volume of 0.045 ± 0.014 mm3 10 min after trauma. This lesion was expanding to 0.342 ± 0.026 mm3 at 24 h and to 0.523 ± 0.09 mm3 at 72 h ($p < 0.01$ vs. 10 min). 3 weeks after trauma a glial scar was seen in the area subjected to cold injury. (2) Within 72 h after trauma, the cortical necrosis developed to a volume of 0.33 ± 0.04 mm3 in iNOS-/- mice and to 0.48 ± 0.04 mm3 in their wild-type littermates.

Conclusion: (1) The current cold injury model is highly suitable to investigate the pathophysiology of secondary necrosis growth from trauma in mice, since it produces a sharply demarcated lesion that expands massively after trauma and is limited to the brain cortex. (2) At the time point of maximal lesion spread, the volume of the cortical necrosis in iNOS-/- mice is only 68 % of the necrosis volume in wild-type littermates. This supports the hypothesis that the iNOS-product acts as a mediator of secondary necrosis growth after trauma.

Supported by BONFOR research foundation, Bonn; AZ: O-126.0013

P186.

RECOVERY MECHANISM OF TRAUMATIC CEREBRAL HEMIPLEGIAS

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A cerebral hemiplegia comes from the cerebral ischemia of the ascending frontoparietal artery, which has 2 types: the tree-branch type (57.5%) and the candlestick type (42.5%). In the treatment of hemiplegias, the most useful arteries for a single anastomosis are:

*P–P arteries, parietal branch of the superficial temporal artery-posterior branch of the candlestick artery.

The arteries for a double anastomosis are:

* F–A arteries, frontal branch of the superficial temporal artery—anterior branch of the candlestick artery;

*P–P arteries, parietal branch of the superficial temporal artery—posterior branch of the candlestick artery.

The single anastomosis except for a slow recovery is the same to the double anastomosis. The pressure–flow in the superficial temporal artery was 600 mm H2O and 20 cc Per minute; in the ascending frontoparietal artery, 300 mm H2O and 10 cc Per minute. The high pressure–flow, the amount of which following anastomosis enters the ascending frontoparietal artery for a single anastomosis, was 10 cc Per minute, 600 cc Per hour, 14,400 cc Per day; for a double anastomosis, 28,800 cc Per day. The highly pressure–flow, in the ascending fronto parietal artery, could dilate the closed cortical arteries and subcortical pressure sphincters of distributing arteries to improve the microcirculation, This is the recovery mechanism. The cerebral hemiplegia is an neuronal pseudo–death like a facial nerve paralysis, and is a neuronal true-death like an infantile paralysis.

Now the 5 hemiplegic patients have completely recovered. They may raise their paralysed hands and lift their paralysed feet to walk towards man's world.

P187.

POSTTRAUMATIC LOCAL INFLAMMATORY CELLULAR INTERPLAY DETERMINES NEURONAL FATE

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Inflammation following central nervous system (CNS) injury is still a subject of controversy, regarded by many as a detrimental process. Our group, however, have provided substantial evidence for immune involvement in neuronal protection. In this study we found that, the ability to resist the consequences of CNS axonal injury was characterized by the early onset of site-specific phagocytic activity and MHC class II expression. Our data suggest that post-traumatic CNS inflammation comprise a highly complex cascade of events, in which only a suitably timed and properly balanced innate immune dialog will lead to neuronal survival. Such a dialog was demonstrated in vitro, using rat primary cultures of microglia and astrocytes. In this experiment, both types of cells showed a specific ability to take up 14C-glutamate in a sodium-dependent manner, and their glutamate-scavenging abilities were dramatically enhanced after their pre-incubation with activated autoreactive T cells. These results shed some light on the role of inflammation after CNS injury, and compel us to modify our therapeutic strategy in favor of regulating the inflammatory reaction rather than suppressing it.

P188.

EXPRESSION OF EPHA7 SUGGEST ROLES IN SPINAL CORD INJURY PATHOPHYSIOLOGY

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The molecular mechanisms underlying processes involved in the growth cone response to spinal cord injury (SCI) are incompletely understood. In several SCI animal models, injured neurons have shown the intrinsic capacity to regenerate, but the microenvironment surrounding the lesion site have shown inhibitory and prevents axonal growth. During the past decade, the Eph receptor protein tyrosine kinase family and its cognate ligands, the ephrins, have emerged as key repulsive cues known to be involved in neurite outgrowth, synapse formation, and axonal pathfinding. More recently, it has become clear that in certain situations they can mediate different effects on cells, including adhesion. Developmental analysis of EphA7 regulation nurtures the idea that the truncated receptors, which lack the kinase activity, act as endogenous, dominant-negative suppressors of the full-length EphA7 signaling. Therefore, in the absence of signaling these receptors could promote cellular adhesion. We hypothesize that alternative usage of different splice forms of Eph A7 tyrosine kinase receptor can mediate cellular adhesion or repulsion after neural trauma. We have examined the relative expression of EphA7 full-length and truncated isoforms in adult rats after injury using the NYU compression model and semi-quantitative real time PCR analysis. Standardized real-time RT-PCR analysis showed increase in the full-length EphA7 expression 7, 14, and 28 days post-injury and these results were corroborated by immunohistochemistry. Immunoreactivity was observed in GFAP positive cells located in the ventral region of the white matter. Owing to the limited regenerative capabilities of the central nervous system, these results suggested that EphA7 full length might be involved in the establishment of the restrictive environment for axonal regeneration after SCI. This study is supported by NIH/NINDS (NS 39405), NSF-EPSCOR (EPS-9874782), KSCHIRT (8-29), Norton Health Care, MBRS-SCORE (2 SO6 6M8224).

P189.

TRANSPORT OF POLIOVIRUS RNA FROM THE PERPHERY VIA SCIATIC NERVE AXONS RESULTS IN GENE EXPRESSION IN THE CENTRAL NERVOUS SYSTEM.

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Poliovirus replicons are a unique RNA based gene vector capable of spatially and temporally localized effects on the injured spinal cord. Poliovirus replicons spatially localize to spinal cord motorneurons. Replicons induce a transient burst of foreign gene expression, initiating at 6 hours and peaking at 72 hrs after injection. In mice transgenic for the poliovirus receptor (pvr mice), intramuscular injection of poliovirus replicons encoding gfp reveal expression in the ventral horn motorneurons. Expression is also found within the injected muscle, sciatic nerve, dorsal root ganglia (drg) and within fibers of the dorsal horn. No inflammation in the muscle or spinal cord and functional changes were noted.

Injection of naked (unencapsidated) poliovirus replicon RNA encoding gfp into the muscles of pvr mice or rats also result in gfp expression within the spinal cord. The pattern of gfp expression within the muscle, sciatic nerve, drg and spinal cord was similar to that seen after intramuscular injection of encapsidated replicons. Transection of the sciatic nerve eliminated gfp expression in the sciatic nerve and spinal cord. Thus, replicon RNA is transported via sciatic nerve axons back to the spinal cord, where RNA translation occurs primarily within the motorneuron cytoplasm. The ability of replicons to be transported from the periphery to the central nervous system, coupled with their unique gene expression profile, further enhance the potential uses of replicons for gene delivery of therapeutic proteins to the central nervous system. Supported by AI 25005 to CDM.

P190.

INFLAMMATORY CELLULAR RESPONSE AND CYTOKINES IL-1BETA, IL-6 AND TNF-ALPHA

Liqun Yang, Corinna Van Den Heuvel, Peter Blumbergs, Nigel Jones, Jim Manavis. (University of Adelaide, Adelaide, Australia).*

The inflammatory response following spinal cord trauma plays an important role in the secondary spinal cord injury. We hypothesized that the pro-inflammatory cytokines IL-1beta, IL-6 and TNF-alpha produced by intrinsic cells in the spinal cord may act as messengers to coordinate the inflammatory cascade and the influx of neutrophils and monocytes to the site of damage and that the cytokine response should be greater in severe than in mild injury.

Neutrophils were not detected at 1 and 3 hrs after spinal cord injury, dramatically increased at 6 hrs postinjury primarily around blood vessels in the central gray matter and peaked at 1 day.

Macrophages were noted at 6 hrs and then progressively increased for the first 3 days postinjury. Activated microglia were found as early as 1 h after contusion, increased dramatically at 1 d postinjury and frequently wrapped around axonal swellings and healthy neurons. RT-PCR showed an early and robust up-regulation of IL-1beta, IL-6, TNF-alpha mRNAs in spinal cord after severe contusion injury, maximal at 6 h postinjury with return to control levels by 24 h postinjury, the changes being quantitatively less in mild injury.

RT-PCR analyses together with histological observations suggest that intrinsic CNS cells, not peripheral inflammatory cells, are the main source for cytokine mRNAs because the peripheral inflammatory cells do not invade the injured spinal cord until 6 h postinjury, a time when cytokine mRNA levels have peaked and started to decline. Furthermore, our comparative RT-PCR analyses, showing significantly increased expression of pro-inflammatory cytokine mRNAs in severe injury in contrast to mild injury, support the hypothesis that cytokine up-regulation is an important factor in the generation of the severity of the inflammatory response and thus a suitable target for pharmacological intervention to attenuate this response.

P191.

HEMATOGENOUS MACROPHAGES EXPRESS CD8 AND DISTRIBUTE TO REGIONS OF LESION CAVITATION AFTER SPINAL CORD INJURY

Phillip G. Popovich, Nico van Rooijen, William F. Hickey, Geoff Preidis, Violeta McGaughy. (Ohio State University, Columbus, Ohio US).*

Historically, CD4 and CD8 antigens have been used to designate functionally distinct T-lymphocyte subsets. However, these antigens also have been described on macrophages in the normal and pathologic CNS. Signaling through CD4 or CD8 may impart unique functional attributes in macrophage subsets expressing these antigens. In the current study, the distribution of CD4 or CD8+ cells was evaluated within rat spinal cord following contusion injury. Survival intervals ranged from 6 hours to 6 weeks post-injury (n = 4–6/group). The data reveal divergent patterns of CD4 and CD8 expression on unique macrophage populations. Specifically, in addition to infiltrating lymphocytes, we observed sustained elevations of CD4 expression on microglia and macrophages throughout the lesion site and spared white matter up to 6 weeks post-injury. In contrast, CD8 was predominantly associated with hematogenous macrophages that are recruited from the blood during the first week post-injury. These cells were restricted to zones of necrosis and lesion cavitation. The hematogenous nature of the CD8+ macrophage was confirmed by immunohistochemical analysis in radiation bone-marrow chimeric rats and after intravenous injection of liposome encapsulated clodronate (a method for selectively depleting blood monocytes and hematogenous macrophages). Indeed, macrophage depletion caused a 20-40 fold reduction in CD8+ macrophage infiltration at the injury site. The restricted expression of CD8 on blood-derived macrophages and the limited temporal appearance of this molecule after SCI suggest that CD8 is actively regulated and could play a role in triggering the acute neurotoxic properties of recruited macrophages after SCI. This work supported by NS37846 (PGP).

P192.

HP184 INCREASES CONDUCTION VELOCITY IN THE DYSMYELINATED CNS OF THE LONG EVANS SHAKER (LES) RAT

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Demyelination of injured but surviving axons following trauma results in axonal conduction deficits and altered ion channel distribution. Conduction block in demyelinated fibers is believed to be at least partly due to the appearance of aminopyridine-sensitive potassium channels in areas of myelin loss. Potassium channel blockers, such as 4-AP, increase action potential duration and amplitude in demyelinated fibers and improve conduction of action potentials. In this study, HP184 was administered orally to adult mutant dysmyelinated Long Evans Shaker (LES) and normal Long Evans rats. Conduction velocity (CV) of evoked compound action potentials (CAP) was measured in vivo 2 hours after ingestion. While CV in the untreated LES rats (24.75 m/s) was 3.27 times slower than CV in normally myelinated untreated controls (80.88 m/s) HP184 had a positive effect on CV in treated LES rats. HP184 caused a 56.1% (38.63 m/s) increase in CV at 3 mg/kg body weight and 168.6% (66.48 m/s) increase at 10 mg/kg. HP184 improves conduction velocity of unmyelinated central axons and is therefore proposed as a treatment for symptoms of demyelination resulting from spinal cord injury. The new in vivo model of measurement of CV of evoked CAP developed for this study proved reliable and can be used in chronic studies requiring multiple electrophysiological measurements performed in the same animal.

P193.

BEHAVIORAL OUTCOME FOLLOWING GRADED UNILATERAL CERVICAL SPINAL CORD CONTUSION INJURY IN RATS

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The majority of human spinal cord injuries occur in the cervical cord, however, few animal models have examined the effects of contusion at this level. This study explores behavioral and histological outcomes of unilateral contusions at C5 using the MASCIS device. One advantage of this model is the ability to assess functional effects of gray and white matter injury. Unilateral injury also provides a within subject control, and sparing of bladder and respiratory function. Several well-characterized tests for forelimb and hindlimb function can be applied; including paw preference test (Liu et al., 1999), grooming test (Bertelli and Mira, 1993), Catwalk quantitative gait analysis system (Hamers et al., 2001), horizontal ladder test (Metz and Whishaw, 2002), and open field locomotor test (Basso et al., 1995).

Experiment 1: 6.25mm (n = 5) and 12.5mm (n = 5) unilateral contusion injuries were made at C5-6. Mild and moderate functional deficits were observed. In the open field, 6.25mm subjects recovered some ipsilateral forelimb plantar stepping over 6 weeks whereas 12.5mm subjects did not. For the cylinder test, 12.5 mm subjects rarely used their ipsilateral limb while the 6.25 mm subjects used their ipsilateral limb less than normal but more than the 12.5 mm subjects. The grooming test revealed serious impairment after 12.5 mm injuries that partially recovered over 6 weeks. Minimal impairment was observed in 6.25 mm subjects, with complete recovery observed by 3 weeks. Histological analysis showed ipsilateral sparing of 70% and 43.7% of the cord area after 6.25mm and 12.5 mm injuries respectively. Strong correlations between spared tissue and contralateral paw preference ($r2 = 0.92$) and grooming performance ($r2 = .74$) were observed.

Experiment 2 is underway to evaluate the model for consistency of histologic and behavioral outcomes (n = 10 per group); and to test sensitivity to potentially neuroprotective agents, methylprednisolone and minocycline.

(Supported by NIH NS 31193 and the ISRT)

P194.

HEAT SHOCK REDUCES THE DEGREE OF LIPID PEROXIDATION FOLLOWING ACUTE SPINAL CORD INJURY IN RODENTS

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Following acute spinal cord injury, a period of secondary metabolic injury ensues. One of the hallmarks of these changes is the ongoing oxidative stress that leads to lipid peroxidation. Reduction of lipid peroxidation is felt to preserve the remaining spinal cord ultrastructure and minimize the effects of the secondary injury. Heat shock has been shown in various models of injury to minimize the effects of oxidative stress. The objective of this project was to assess the effect of heat shock on lipid peroxidation in a rat spinal cord injury model.

Fifty female Wistar rats were used in this study. Twenty-five rats were heat shocked, temperature 40oC for 15 minutes, prior to lesion. Five animals from each the heat shocked and normal animals were sacrificed prior to lesion. The remaining animals received a mid-thoracic complete spinal cord injury via clip compression. The animals were randomly assigned to a sacrifice time, either 4, 6, 12, or 24 hours post lesion. Spinal cord tissue was either processed immunohistochemically for hsp27 or assayed colorimetrically for malondialdehyde (Oxford Biomedical Research; Oxford, MI) as a marker for lipid peroxidation.

Immunohistochemical staining revealed increased expression of hsp27 in the neurons of both the heat-shock control and lesion-only animals at all time points. However, the animals that received a lesion after heat-shock displayed increased expression in both neurons and glia. Malondialdehyde levels were reduced by 35 and 70% in heat-shocked animals at 4 and 6 hours, respectively, when compared to lesion-only animals at the same time points.

In summary, we have shown that heat-shock prior to spinal cord injury induces hsp27 expression in both neurons and glia and leads to a reduction in the degree of secondary injury as measured by lipid peroxidation.

P195.

THE EFFECT OF FK506 ON NITRIC OXIDE SYNTHASE ACTIVITY IN THE LACERATION MODEL OF SPINAL CORD INJURY

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The aim of this study was to investigate the alterations in spinal cord nitric oxide synthase (NOS) activity following an experimental spinal cord injury and to assess the effects of FK506 on this NOS activity. A blind study involving seven animal groups was initially performed (Set 1). Four groups underwent a T9 laminectomy and laceration spinal cord injury. with 2 groups receiving subcutaneous (s.c.) vehicle and the other 2 groups receiving s.c. FK506 (2.0mg/kg). Two uninjured groups received s.c. FK506. The 2 vehicle groups differed in survival times (6 and 16 hours post injection). as did the injured and uninjured FK506 groups. A control group consisted of uninjured and untreated animals. A citrulline assay was used to assess the cNOS and iNOS activities in each animal. The results did not reveal any significant changes in iNOS activity in any of the groups. The cNOS in all injured animals was increased at 6 hours and remained similarly elevated at 16 hours. but there was no significant difference between the vehicle and FK506 treated groups. The cNOS activity in the uninjured groups that received FK506 was unaltered at 6 and 16 hours compared to the controls. A further blind citrulline assay study (Set 2) was performed in vitro to observe the effect of three different forms of FK506 (base. oral and intravenous) at different doses (10μM. 100μM. 1UμM and 10μM) on cNOS activity in cerebellar and spinal cord tissue. A negative control group (untreated) and positive control group (treated with the NOS inhibitor. L-NAME) were utilised. The results did not reveal any significant differences between the FK506 groups and negative controls. Both Sets 1 and 2 results indicate that the neuroprotective mechanism of action of FK506 is probably not via an alteration in NOS activity.

P196.

INCREASING DOSAGES OF FIBROBLAST GROWTH FACTOR-2 (FGF-2) DELIVERED NEAR THE SITE OF SPINAL CORD INJURY IMPAIR FUNCTIONAL RECOVERY AND TISSUE SPARING IN RATS

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We conducted a dose-response study of FGF-2 to characterize its efficacy for functional recovery and tissue sparing following contusion spinal cord injury using the Infinite Horizon Impactor®. Immediately after T10 injury a catheter connected to an osmotic pump was inserted intrathecally at T13/L1 and advanced to T11 for continuous delivery of FGF-2 at 3μg, 15μg or 30μg per day versus control vehicle for 1 week (n = 6/group). Additionally, to test the in vivo influence of heparan sulfate (HS) on FGF-2 activity, others received the same dosages plus 10μg/ml HS (n = 6/group) versus HS alone (n = 12). Animals were tested for 8 weeks using the BBB locomotor rating scale and histologically assessed for volumetric tissue sparing of gray and white matter. All injured rats demonstrated an acute loss of hindlimb function followed by a recovery phase that peaked by 2–3 weeks. HS alone did not significantly affect recovery or tissue sparing, but animals that received 30μg FGF-2, with or without HS, demonstrated significant impairment in both acute and long-term hindlimb locomotor function compared to vehicle or HS. The remaining FGF-2 dosages also rendered somewhat lower BBB scores versus controls, except for a marginal improvement with 3μg FGF-2+HS. Accordingly, this group had the greatest amount of spared gray and white matter while the 30μg FGF-2 groups had the least. Since there were no significant differences among all groups in percent tissue sparing at the lesion epicenters, ongoing stereological studies are examining putative cellular alterations that may account for these observations.

Supported by KSCHIRT #9-17 and SCRF 2085-02.

P197.

DEVELOPING NOVEL CALPAIN INHIBITORS: TAT-CALPASTATIN

Tomoko Sengoku, Shu-Xin Zhang, Vimala Bondada. (James W. Geddes Spinal Cord and Brain Injury Research Center, Sanders Brown Center on Aging, and Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY US).*

Following CNS injury, including spinal cord injury and ischemia, excessive calcium influx has been implicated in the ensuing secondary neuron death. Calpains are calcium activated neutral proteases whose substrates include a wide variety of signaling, cytoskeletal, and life/death proteins. Following spinal cord injury, maximal calpain activation occurs within 1-2h and remains elevated for at least 24h. Inhibiting calpain activity is a rational therapeutic target. However, synthetic calpain inhibitors are problematic due to limited cell permeability, short half lives, and inhibition of other proteases. Calpastatin, an endogenous calpain inhibitor is very potent and specific but does not cross cell membranes. By linking the 11 amino acid protein transduction domain of the HIV Tat protein to green fluorescent protein (GFP) we developed a fusion protein that could be easily visualized in order to determine transduction capabilities. At low concentrations Tat-GFP did transduce primary rat hippocampal neurons but appeared to be localized within endosomes. By linking Tat and calpastatin we developed a novel calpain inhibitor that was functionally active and able to transduce neurons of primary cultures at low concentrations. At higher concentrations the Tat-calpastatin appeared to aggregate on the external surface of the cell surface. Our work with Tat-GFP and Tat-calpastatin reveals both the possible problems of fusion proteins as well as the potential of this novel calpain inhibitor.

P198.

POST-INJURY TREATMENT WITH Mn (III) TETRAKIS (4-BENZOIC ACID) PORPHYRIN IMPROVES FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD INJURY.

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Manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP) is a cell permeable superoxide dismutase mimetic and a broad spectrum scavenger of reactive species. The present study compares the effect of MnTBAP and methylprednisolone—the only drug approved for clinic treatment of spinal cord injury (SCI) on neurological recovery after SCI. The rat spinal cord was injured at T10 vertebra by dropping 10 g rod 1.25 cm down to the cord with a NYU device. The force of injury was digitally recorded on a PC equipped with data acquisition board. At 4 and 6 h post-SCI, rats were treated with MnTBAP (10 and 5 mg/kg, i.p., respectively), methylprednisolone sodium succinate (MPSS, 30 and 15 mg/kg, i.p., respectively), or saline as control. Functional recovery was evaluated by the standard Basso, Beattie and Bresnahan (BBB) test and inclined plane test performed on day 3 and every week until 9 week following SCI. The pre-trained rats were individually placed in open field and recorded BBB score based on hind limb movement, support, fore- and hind- limb co-ordination, paw and tail position. The inclined plane test is to test the rat's ability to maintain itself for 5 seconds on the maximum angle of a plane. MnTBAP treatment significantly increased the scores of BBB test ($p < 0.001$) and inclined plane test ($p = 0.01 - 0.007$) compared to saline treatment. MPSS treatment did not significantly improve the scores by both tests, although the scores were better compared to controls. Therefore, MnTBAP is superior to MPSS in enhancing neurological recovery following SCI, indicating that MnTBAP is a potential therapeutic agent for reducing secondary SCI. Supported by NIH grants to DLiu (NS 34048 and NS 35119).

P199.

CHARACTERIZATION OF INTRASPINAL BONE MARROW STROMAL CELL TRANSPLANTS IN THE RAT SPINAL CORD INJURY MODEL

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Previously, we reported that transplanted marrow stromal cells support lesion site axonal regrowth and stimulate hindlimb airstepping—a locomotor-like behavior in the injured rat spinal cord,. Separately, we demonstrated that brain derived neurotrophic factor (BDNF) also provokes airstepping following spinal contusion or transection injuries, suggesting that BDNF activates the locomotor central pattern generators to induce the behavior. Therefore, we performed in vitro ELISA studies to explore the possibility that MSCs induce airstepping by effecting the release of BDNF either directly or from local neurons. Specifically, we tested the supernatants from MSCs that were cultured alone and on a carpet of mouse spinal cord neurons. Because MSC transplants provoke airstepping by 4 days after transplantation, we predicted that the cells produce BDNF prior to transplantation. However, we did not detect BDNF production by either MSCs alone or MSC/SC co-cultures, suggesting either that MSCs are not stimulated to induce BDNF release in artificial culture settings, or that the cells provoke airstepping by another mechanism. Additionally, we transferred supernatants from cultured MSCs to trkB-expressing PC12 cells (trkB/PC12). Although neurite outgrowth was not as robust as when BDNF standard was applied, MSC supernatant treated trkB/PC12 cells displayed short processes, while those treated with unconditioned media displayed none. These results raise the possibility that MSCs produce NT-4/5 or another factor, rather than BDNF. Additionally, we further examined MSC grafts in vivo, with the aim of better characterizing the morphology and composition of the grafts. We observed that grafts contained both laminin and fibronectin and appeared to direct regrowing axons to extend in the rostral-to-caudal orientation, rather than transversely across the cord. The grafts also contained significant amounts of P0-labeled Schwann cell myelin, but were largely devoid of MBP-positive central myelin. Collectively, these results support further examination of MSC transplants for SCI. Supported by NS37321.

P200.

INFLUENCES OF ACUTELY TRANSPLANTED GLIAL RESTRICTED PRECURSOR CELLS ON THE CHRONIC LESION ENVIRONMENT FOLLOWING CONTUSIVE SPINAL CORD INJURY

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Glial restricted precursor (GRP) cells are multipotent cells that can differentiate along oligodendrocyte and astrocyte lineages both in vitro and in vivo. We previously reported that GRP cells transplanted acutely into spinal cord contusion injuries and assessed after 8 days, were able to survive and migrate (based on nuclear labeling with Hoechst) and that GRP cells appeared to reduce astrocytic scaring and the expression of inhibitory chondroitin sulphate proteoglycans (CSPGs). Here we examine whether these alterations persist chronically and whether transplantation of GRP cells alters axonal responses. Cells isolated from transgenic rats that ubiquitously express the human placental alkaline phosphatase (PLAP) gene were isolated and transplanted into the contusion site immediately following injury. Similar to our previous results, 6 weeks after injury GRP cells are able to survive and migrate when transplanted immediately after injury. PLAP expressing GRP cells were observed within the lesion site. The majority of cells were confined to the lesion area, which was partially filled with cells, and some cells were observed in the white matter up to 5 mm rostral or caudal to the lesion center. We also examined the glial and molecular scar and the extent of axonal growth from CST and 5HT fibers 6 weeks after injury and transplantation to determine if the alterations in scaring persisted in the longer term and whether these changes towards a more permissive environment and the presence of an immature astrocyte substrate could result in increased sprouting of descending axons 6 weeks after injury. (Support: NS 38079)

P201.

ADENOVIRAL VECTOR-MEDIATED GENE TRANSFER OF BRAIN DERIVED NEUROTROPHIC FACTOR PROMOTES FUNCTIONAL RECOVERY AND AXONAL REGENERATION AFTER COMPLETE TRANSECTION OF ADULT RAT SPINAL CORD

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MATERIALS AND METHODS: We prepared adenoviral vectors encoding either beta-galactosidase (AxCALacZ) or brain-derived neurotrophic factor (AxCABDNF). The titers of the vectors were adjusted to 5.0 × 1010 plaque-forming units/ml. COS cells were infected with the vectors and western blotting was performed to detect BDNF. Tissue samples were obtained from 8-week-old male Wistar rats. The spinal cord was completely transected at the T8 level. Immediately after the transection, 5ml of the vectors was injected in both stumps. AxCAlacZ-treated rats were perfused transcardially and cryosections of the brain and spinal cord were made. X-gal histochemistry and immunohistochemistry were performed to evaluate transgenic expression. Locomotor activity was evaluated using the BBB locomotor scale. In AxCABDNF group, retransection of spinal cord was performed 6 weeks after injury. For retrograde tracing, fluorogold (FG) was injected into the lumbar enlargement 6 weeks after the transection, and FG-labeled neurons in the brainstem nuclei were evaluated.

RESULTS and DISCUSSION: Western blot analysis revealed that the conditioned medium from AxCABDNF infected COS cells contains BDNF. X-gal histochemistry revealed transgenic expression in the injected site and in the brainstem nuclei. Immunohistochemistry revealed transgenic expression in neurons and glial cells near the injected site, and in neurons of the brainstem nuclei. BBB locomotor scale of AxCABDNF group showed significant recovery and the average score 6 weeks after injury was 6.0, although no recovery was observed in the AxCALacZ group. The average score 6 weeks after injury in AxCALacZ group was 0.4. Retransection caused complete loss of the recovered hindlimb function.

Retrograde tracing revealed FG-labeled neurons in the red nucleus of AxCABDNF group although no FG-labeled cells were found in AxCALacZ group. Thus, we conclude that adenoviral vector-mediated gene transfer of BDNF promotes axonal regeneration, resulting in functional recovery after complete transection of the spinal cord in adult rats.

P202.

TRANSPLANTED HEMATOPOIETIC STEM CELLS@FROM BONE MARROW DIFFERENTIATE INTO NEURAL LINEAGE CELLS AND PROMOTE FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY IN MICE

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Introduction: Recent evidence demonstrates that hematopoietic stem cell (HSC) fraction of bone marrow can differentiate into cells bearing neural markers in brain. In the present study, we employed a mouse model of spinal cord injury and transplanted HSCs from bone marrow into the injured spinal cords. Based on the results, we discuss a possible role of the transplantation on their functional recovery.

Materials and Methods: To purify HSCs, we collected total bone marrow cells from femurs of male Rosa26 mice. The mice were pretreated and overexpressed beta-galactosidase ubiquitously. The cells were analyzed by FACS Vantage (Becton Dickinson), and c-kit+ Sca-1+ Lin- cells were sorted, yielding primitive HSCs. Female C57Bl/6J mice were subjected to a contusion injury of spinal cord using Farooque's technique. HSCs in phosphate buffered saline or buffer alone (control) were injected into the spinal cord 1 week after injury. We evaluated their functional outcome using hindlimb motor function score (Farooque, 2000) every week for total 5 weeks after the transplantation. Fluorescent in situ hybridization for Y chromosome was performed to detect cells derived from HSCs. To visualize the cellular co-localization of beta-galactosidase and cell-type specific markers, we employed double immunofluorescent staining.

Results: Significant recovery of hindlimb motor function score was detected in mice transplanted with HSCs compared with control. Histological analysis showed that the transplanted cells survived and differentiated into cells expressing neural markers.

Discussion: HSC fraction of bone marrow offers several advantages for the clinical use of cell transplantation. Clinical uses of fetal embryonic and neural stem cells are limited both from immunological and ethical standpoints. In contrast, transplantation of patient's own bone marrow cells could circumvent the problems of host immunity and graft-versus-host disease. Our data suggests that transplantation of HSCs from bone marrow may represent an effective strategy for the treatment of spinal cord injury.

P203.

NG2, p75 AND NEUROFILAMENT EXPRESSION AFTER SPINAL CORD INJURY IN RATS: DISTRIBUTION, CO-LOCALIZATION AND QUANTIFICATION

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Oligodendrocyte progenitors, found throughout the adult brain and spinal cord, are thought to be the source of NG2 chondroitin sulfate in the nervous system. We previously determined that NG2+ cells display protracted proliferation after spinal cord injury (SCI). Since NG2 is considered a potent inhibitor of axon growth, this increased level of NG2 may have important implications for regeneration. Currently, we sought to further evaluate NG2 expression after SCI by identifying the cellular source and determining the relationship of its distribution to that of axons within the injury site. Surprisingly, a considerable number of cells were double-labeled for NG2 and p75. These cells likely are premyelinating Schwann cells that infiltrate the spinal cord early after SCI. Indeed, quantification of p75 immunoreactivity revealed increased expression at 3 days post-injury, which remained elevated for 4 months. While p75+ profiles may include oligodendrocytes or axons, the majority had a bipolar phenotype and displayed immunoreactivity for S100b, suggesting they are premyelinating Schwann cells. Acutely, NG2+ p75 cells were distributed in the dorsal funiculus, while chronically they were found throughout the tissue and often were seen lining cavities and forming bands along septae within the cavities. A minority of NG2+ cells displayed fibronectin immunoreactivity, suggesting they were fibroblasts from the meninges or peripheral nerves. NG2 also was detected on macrophages and on myelinating Schwann cells within the spared white matter. Thus, many cells in addition to oligodendrocyte progenitors express NG2 after SCI. An unexpected finding was considerable overlap in the distribution of NG2 and neurofilament immunoreactivity along the septae on which axons grow after injury. This suggests that NG2 is involved in axon growth after SCI, acting either as either a permissive or regulatory substrate. Furthermore, the p75+/NG2+ cells along the septae may play a role in this process. Supported by NS37321.

P204.

PRECLINICAL TRIAL OF INTRATHECAL GABAmide IN THE TREATMENT OF SPASTICITY.

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The most effective current clinical treatment for severe spasticity is baclofen delivered intrathecally. Tolerance development and various side-effects emphasize the need for the development of a new drug that lacks these problems. GABAmide is an active metabolite of Progabide that has been tested extensively in animal studies and in clinical trials and found to be more effective in treating spasticity than baclofen. Its superior effectiveness was probably due to its action at both the GABA-A and GABA-B receptors while baclofen only influences the GABA-A receptor. However, systemic delivery of Progabide resulted in liver dysfunction in some patients. GABAmide can be delivered intrathecally at much lower doses than required for systemic delivery, thus avoiding the problem of liver dysfunction. Rats with chronic spinal cord injury produced using a weight-drop device were evaluated for spasticity every other day using a new test developed by our lab that was based on the Ashworth scoring system. Alzet or ESOX pumps connected to 1 French catheters were used to deliver the drug or vehicle intrathecally. Our drug cross-over studies [GABAmide (5 mg/day), baclofen (15 mg/day), saline] in rats confirm that intrathecal GABAmide and baclofen significantly reduce spasticity. In spastic rats treated with GABAmide intrathecally for 1 month, there was no evidence of the development of tolerance. Intrathecal delivery of GABAmide in normal animals for 3 months did not produce any changes in sensorimotor function based on BBB locomotor scores and CBS testing. Our studies support the conclusion that GABAmide is a better alternative to baclofen in the treatment of spasticity.

Supported by STTR (NIH) to Neurorecovery Inc.

P205.

CELLULAR REACTIONS REMOTE FROM THE LESION SITE AFTER HUMAN SPINAL CORD INJURY

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Little is known about the cellular responses in the human spinal cord after traumatic injury. The growth associated protein GAP-43 and the transcription factor c-jun are believed to play a major role in the process of regeneration and they have been reported to be induced in axotomised neurons in a number of experimental models. In this study, we investigated the possible up-regulation of GAP-43 and c-jun in neurons of Clarke`s nucleus (CN) in human post mortem material of patients who died after severe spinal cord trauma. By non-radioactive in situ-hybridization, a strong induction of GAP-43 and c-jun mRNAs could be observed bilaterally within CN neurons below the lesion site after short, but not after long survival times. Immunohistochemistry demonstrated the enhanced expression of 200KDa neurofilament protein in CN neurons below the lesion. These results confirm experimental data that even non-regenerating CNS neurons can up-regulate regeneration-associated genes such as GAP-43 and c-jun, which might reflect a transient regenerative capacity.

Furthermore, we have investigated the dynamics of Wallerian degeneration within white matter tracts. Neurofilament staining demonstrated a specific spatio-temporal pattern of axonal loss within degenerating fibre tracts which could be detected as early as 12 days close to the lesion. After late survival times the affected tracts were almost devoid of any NF staining. Between 5 weeks to 4 months, activated microglia were abundant within the corticospinal tract. These activated microglia were MHC class II positive and co-localized with the macrophage marker CD68 reflecting the phagocytic role. GFAP-staining revealed a late astrocytic reaction throughout the area of the degenerating tracts leading to a long term deposition of a dense astrocytic scar.

Such studies on post mortem tissues are required to obtain a better understanding of human pathology after SCI and will elucidate the clinical relevance of experimental data.

P206.

EARLY DECOMPRESSION AND ITS RELATION TO THE PHARMACOLOGICAL TREATMENT OF ACUTE CERVICAL SPINAL CORD INJURIES

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PURPOSE: The aim of the research was to examine the impact of timing on the effectiveness of decompression in traumatic spinal cord injury, especially in interaction with pharmacological treatment.

MATERIALS AND METHOD: 216 cervical fracture patients admitted to our tertiary SCI unit were studied retrospectively: 118 treated with immediate decompression, 84 with delayed surgery, and 14 not operated due to their clinical condition. Data was also collected on the administration of methylprednisolone. The timing of surgery and implementation of pharmacological treatment were determined by the time required for the patient to arrive in our unit after injury. Neurological condition was measured using the ASIA scale.

RESULTS: Patients who underwent late surgery had more severe spinal cord injuries initially than those operated. However, no differences in neurological improvement at 1-year follow-up were found between those who underwent early and late surgery. No correlation was found between pharmacological and surgical treatment.

CONCLUSION: The direct clinical benefit of early decompression and stabilization to improve the physiological environment include decreased hospitalization and faster neurological improvement in cases of cervical spine trauma with proven compression of the spinal cord, as well as earlier rehabilitation and mobilization. Over the long term, however, the neurological outcome in late surgery is comparable. The reported positive effects of methylprednisolone treatment are not influenced by surgical outcome.

P207.

CONTUSION SPINAL CORD INJURY (SCI) INDUCED CHANGES IN CATHEPSIN B GENE AND PROTEIN EXPRESSION

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An increase in protease activity is a hallmark event of the secondary injury cascade following contusion SCI. Elevated levels of protease activity result in the degradation of cytoskeletal and myelin proteins essential for cellular function and survival. We have provided the first data that at least one member (CatB) of the cathepsin protease family is upregulated by SCI. The excessive release and activity of cathepsin B, a ubiquitous lysosomal cysteine protease, has been implicated in several pathologies including tumor metastasis, arthritis and Alzheimer's disease. Our goal was to characterize the SCI-induced changes in cathepsin B expression. Following a T12 laminectomy and a moderate contusion (NYU device), the gene and protein profiles of cathepsin B in rats were examined using real-time PCR and immunoblots, respectively. Both the contusion-injured and the matched sham-injured animals exhibited elevated proenzyme (37 kDa) protein levels at the lesion (LX) site, with significant differences between the two groups at 48, 72, and 168 hr. post-SCI. Furthermore, there was an increase in the active species of the protein with significant differences at 72 and 168 hr. post-SCI for the 30 kDa form and at 48 and 168 hr. for the 25 kDa form. Cathepsin B protein levels were also affected in areas rostral (RLX) and caudal (CLX) to the injury epicenter. These levels differed significantly between groups at various post-SCI time points (24 to 168 hr.). Real-time PCR revealed increases in cathepsin B mRNA levels following contusion SCI as early as 6 hr. post-SCI. These data indicate that SCI causes an upregulation of cathepsin gene expression and protein levels, which provides the basis for future studies to determine if these proteases are involved in the secondary injury cascade.

P208.

DESTRUCTIVE CNS AUTOIMMUNE REACTIONS TRIGGERED BY SPINAL CORD INJURY ARE ASSOCIATED WITH THE PRODUCTION OF INFLAMMATORY CHEMOKINES AND ENHANCED RECRUITMENT OF CD4+ T-LYMPHOCYTES

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T-lymphocytes are one of several immune cells that infiltrate the injured spinal cord. Previously, we demonstrated the injurious potential of myelin-reactive lymphocytes isolated from rats following spinal cord injury (SCI). We later confirmed this potential by showing that axonal injury and demyelination are exacerbated and neurological function is impaired after SCI in transgenic (Tg) mice enriched in myelin-reactive T-cells. Together, these studies indicate endogenous T-cells are activated by SCI and contribute to secondary injury. Still, other studies suggest myelin-reactive T-cells are neuroprotective. To further understand the role of CNS-reactive T-lymphocytes after SCI, we completed a time course analysis of the molecular cues necessary for lymphocyte entry and activation within the CNS. Specifically, mRNA levels of co-stimulatory molecules (CD80, CD86) and chemokines (IP-10, RANTES, MCP-1 and MIP-1a) were compared between Tg and nTg mice using quantitative real-time PCR. At 7 and 21 days post-injury, mRNA for co-stimulatory molecules (CD80 and CD86) and inflammatory chemokines (IP-10, RANTES, MCP-1) was dramatically increased in Tg and non-Tg mice. However, expression was significantly higher in Tg mice. For example, relative to uninjured control mice, RANTES mRNA was elevated 180-fold in Tg mice compared to a 33-fold increase in nTg mice. Increased molecular signaling for T-cell recruitment during the first 3 weeks post-injury was accompanied by robust intraspinal accumulation of CD4+ T-cells. In SCI nTg mice, T-cells were localized to the injury site with few cells present in the rostral/caudal spinal cord. However, in SCI Tg mice, significantly larger numbers of CD4+ T-cells were found throughout the rostro-caudal extent of the injury with large infiltrates localized to regions of axon loss and demyelination. These data suggest that if unregulated, the chemokine and co-stimulation profile induced by SCI can initiate feed-forward amplification capable of evoking chronic and destructive CNS inflammation. This work was supported by NS37846 (PGP).

P209.

EFFECTS OF TARGET CONTROL INFUSION OF PROPOFOL ON BURST SUPPRESSION AND BISPECTRAL INDEX IN HEAD INJURED PATIENTS

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The Bispectral Index (BIS; Aspect Medical, Inc) monitor quantifies hypnosis in the form of a BIS score, and cerebral metabolism as a burst-suppression (BS) percentage. The device is widely used to monitor anaesthetic depth and sedation, and could be useful in monitoring sedation and metabolic suppression following head injury. However, acute brain injury has effects on BIS scores [1], and proprietary methodology used in the device has not been evaluated against more conventional measures in this population. We have investigated the relationship between propofol concentration, BIS and BS figures derived from the BIS monitor and conventional electroencephalography (EEG).

Methods: We studied 5 severely head-injured patients, aged 36 years (range 20–53) who required intensive care. Plasma propofol levels were standardized using a Target Controlled Infusion (TCI), commenced at least four hours before the start of the study. Sixteen lead EEG and BIS:A2000 monitors were sited to monitor BIS levels, BIS burst suppression ratio (BISBSR) and EEG burst suppression ratios (EEGBSR) at two target propofol concentrations.

Results: A change in mean (SEM) estimated target concentrations of propofol from 2.3 ± 0.2 to 4.3 ± 0.2 mcg/ml resulted in BIS values of 43.5 ± 2.9 and 37.7 ± 10.2, BISBSR values of 2.8 ± 1.5 and 30.9 ± 8.2, and EEGBSR of 0 and 49.7 ± 4.2, respectively. Propofol levels were unrelated to BIS scores. There was a significant correlation between Propofol levels and BISBSR (r2: 0.615) and between Propofol level and EEGBSR (r2: 0.766). While BISBSR and EEGBSR were significantly related (r2 0.418; $p < 0.05$), there was inter-individual variability in the level of BS assessed by the two techniques.

Conclusion: Current implementations of the BIS score do not correlate with targeted sedative infusions. While the BIS monitor does provide a measure of cerebral metabolic suppression, further work is required to relate this to more conventional measures.

References: [1] J Neurosurg Anesthesiol 1996; 8: 349.

P210.

PREVENTING FLOW-METABOLISM UNCOUPLING ACUTELY REDUCES EVOLVING AXONAL INJURY AFTER TRAUMATIC BRAIN INJURY

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We have previously presented evidence that the development of secondary traumatic axonal injury is related to the degree of local cerebral blood flow (LCBF) and flow-metabolism uncoupling. We have tested the hypothesis that augmenting LCBF in the acute stages after brain injury prevents further axonal injury.

Isofluorane-anaesthetised rats were injured by controlled cortical impact over the left parietal cortex (4m/s velocity, 2mm deformation). Quantitative measurements of regional local cerebral metabolic rate of glucose (LCMRglu) and LCBF were obtained in the same rat from 18F-flurodeoxyglucose (30MBq) and 14C-iodoantipyrine (20μCi)-co-registered autoradiographic images respectively, and the density of injured axons from adjacent beta-amyloid precursor protein (β-APP)-immunostained sections. Data were acquired at 3hr post-injury with and without acetazolamide-induced CBF augmentation immediately following injury (150mg/kg,i.p.). Axonal outcome was assessed at 24hr in two further groups with and without CBF augmentation immediately following injury. Sham-injured rats were used for comparison of all data (all groups n = 6).

In CBF-augmented-injured rats, LCBF was significantly elevated above untreated-injured rats at 3hr, by ~2-fold in ipsilateral and contralateral regions ($P < 0.05$). LCMRglu was globally unaffected by acetazolamide compared to untreated-injured rats although it was no longer significantly increased from sham-control in the contusion margin. Ipsilateral LCMRglu/LCBF ratios were normalised by CBF-augmentation compared to untreated-injured rats from values 2-fold greater than in sham-controls. The density of β-APP-stained axons at 24hr was significantly reduced by CBF augmentation in most regions compared to the untreated-injured group at 24hr ($P < 0.01$). Furthermore, there was generally no significant increase when compared to the 3hr untreated-injured group, indicating that further axonal injury was prevented

These data suggest that increasing post-injury CBF prevents axonal injury by diminishing the pronounced metabolism >blood flow dissociation that occurs in the acute stage of injury. This underlines the importance of maintaining flow-metabolism coupling immediately after injury in order to prevent further axonal injury.

P211.

PHYSIOLOGICAL HETEROGENEITY MASKS HYPERVENTILATION-INDUCED REDUCTIONS IN CEREBRAL OXYGEN METABOLISM IN HEAD INJURY

Jonathan P. Coles, Tim D. Fryer, Pawan S. Minhas, Doris A. Chatfield, Arun K. Gupta, Peter Smielewski, Julian C Matthews, Kenneth Rice, Tim Donovan, Franklin I. Aigbirihio, Guy B. Williams, John C. Clark, John D. Pickard, David K. Menon. (Department of Anaesthesia and Wolfson Brain Imaging Centre, University of Cambridge, Cambridge, Cambrigeshire UK).*

We have previously used positron emission tomography (PET) in patients with head injury to show that hyperventilation-induced reductions in cerebral blood flow (CBF) are associated with increases in regional oxygen extraction fraction (OEF). However, conclusive evidence of critical ischaemia requires the demonstration of hyperventilation-induced reductions in regional cerebral oxygen metabolism (CMRO2). We have used 15O PET to address this issue.

Methods: PET was undertaken in 18 head-injured subjects, 2-5 days post injury, at baseline and following hyperventilation. Maps of CBF, CMRO2 and OEF were calculated, coregistered to X-ray CT, and normalised to Talairach space. 15 Regions of interest (ROIs) covering the whole brain were defined on these metabolic images. Baseline data were collected in two frames to quantify the reproducibility of the technique, and estimate 95% confidence intervals (CI) to test whether changes in CMRO2 (dCMRO2) were real, or were the consequence of intra-subject variability.

Results: Reduction of PaCO2 from 36 ± 0.7 to 29 ± 0.6 mmHg led to significant and consistent reduction in CBF and increase in OEF in all subjects ($p < 0.001$). Despite an overall increase in CMRO2 with hyperventilation ($p < 0.001$), individual responses were highly variable. CMRO2 measurement was highly reproducible, and test-retest analysis showed that a dCMRO2 > 2.4 micromol/100g/min could be defined as significant (>95% CI). 43% of ROIs showed significant increases in CMRO2, but 20% of ROIs showed significant reductions in CMRO2, with significant reductions in one or more ROIs in 10 of the 18 patients studied (56%).

Conclusions: CMRO2 increases with hyperventilation may arise from increased neuronal excitability [1], but these increases may be attenuated or reversed if CBF reductions are critical. Summary statistics may mask this heterogeneity and miss critical regional ischaemia leading to CMRO2 reductions in many patients.

Reference: [1] J Cereb Blood Flow Metab 2001; 21: S169.

P212.

EFFECTS OF CEREBRAL PERFUSION PRESSURE AUGMENTATION ON CAPILLARY-TISSUE OXYGEN GRADIENTS AFTER ACUTE BRAIN INJURY

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We have previously shown [1] that increased capillary to tissue oxygen diffusion gradients exist after traumatic brain injury (TBI). We have used positron emission tomography (PET) and invasive tissue oxygen monitoring to characterise these gradients and determine their response to cerebral perfusion pressure augmentation.

Methods: We have studied 5 patients, within 5 days of a severe closed head injury, using 15O-PET to image cerebral blood flow (CBF), cerebral oxygen metabolism (CMRO2) and oxygen extraction fraction (OEF). Cerebral tissue PbO2 was measured using a multiparameter sensor (NeurotrendTM, Codman), and cerebral venous PO2 (PvO2) was calculated from the OEF in a region of interest around the sensor. Capillary-tissue oxygen gradients (PvO2-PbO2) were calculated for each patient at baseline cerebral perfusion pressure (CPP) and after increasing the CPP by at least 20% using a norepinephrine infusion.

Results: Both OEF and PbO2 varied between patients, but OEF did not predict PbO2, which was determined, in large part, by the gradient between end-capillary and tissue pO2 levels. An increase in CPP from 67.4 ± 3.1 mmHg (mean ± SD) to 88.7 ± 2.7 mmHg ($p < 0.001$) resulted in an increase in PbO2 (19.8 ± 9.9 to 27.4 ± 7.6 mmHg; $p < 0.05$) despite no significant change in OEF or PvO2. This resulted in a variable reduction in the PvO2-PbO2 gradient (12.2 ± 10.6 to 7.6 ± 6.2 mmHg; $p = 0.10$). The magnitude of change in the PvO2-PbO2 gradient, varied inversely with the baseline PbO2 ($r2 = 0.88$; $p < 0.02$).

Conclusions: Significant pO2 gradients exist between the vascular and tissue ECF compartments in head injury. Early results suggest that CPP augmentation may partially overcome these oxygen diffusion barriers, and that such improvements may be more prominent in tissue where the baseline PbO2 is low. Further work is needed to understand the incidence, mechanisms and therapy of these novel pathophysiological processes in head injury.

[1] Coles JP et al. J Neurotrauma 2001; 18 (10): 1186

P213.

EARLY BRAIN SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY IN PATIENTS FOLLOWING CRANIO-CEREBRAL TRAUMA

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Central nervous system (CNS) injuries are among most frequent sequelae of trauma. Patients with CNS injuries often require at least hospital observation, due to the diversity of clinical symptoms and poor prognosis in case of delayed or missed diagnosis combined with shortcomings and deficiencies in management. The aim of the study was to evaluate regional cerebral blood flow (rCBF) after minor cranio-cerebral trauma (Glasgow Coma Scale 13–15 points) by means of single photon emission computed tomography (SPECT). Three types of rCBF changes have been found: local perfusion deficits at the site of trauma (beneath the wound or cranial fracture), multifocal perfusion deficits and diffuse perfusion deficits. Among diffuse perfusion deficits the most common was a bilateral hypoperfusion of frontal lobes. Most of focal perfusion deficits were not linked to the site of trauma but 38,89% were localized at the site of trauma or contralaterally. The most frequent localization was found in frontal and temporal lobes.

The correlations between the rCBF deficits and neurological symptoms have been found. Brain SPECT demonstrates posttraumatic brain lesions earlier, with a greater sensitivity and defines more precisely their extent than by means of computed tomography. Brain SPECT may be helpful in distinguishing patients simulating head trauma symptoms in an attempt to avoid legal problems or army conscription.

P214.

CEREBRAL BLOOD FLOW AND BLOOD VOLUME RESPONSES TO CARBON DIOXIDE AFTER HEAD INJURY

David K. Menon, Jonathan P. Coles, Tim D. Fryer, Pawan S. Minhas, Doris A. Chatfield, Arun K. Gupta, Peter Smielewski, Julian C. Matthews, Kenneth Rice, Tim Donovan, Franklin I. Aigbirihio, Guy B. Williams, John C. Clark, John D. Pickard.. (University of Cambridge, Cambridge, Cambridge, UK).*

Hyperventilation is commonly used to produce reductions in intracranial pressure (ICP), which are thought to be due to reductions in cerebral blood volume (CBV). There is concern that associated changes in cerebral blood flow (CBF) may result in cerebral ischaemia. The relative CBV and CBF responses to PaCO2 reductions have been poorly studied in patients with head injury. We have used O-15 PET to measure CBF, CBV and CO2 reactivity after head injury.

Methods: PET was undertaken in 18 head-injured subjects, 2-5 days post injury, at baseline and following hyperventilation. Maps of CBF and CBV were calculated, coregistered to X-ray CT, and normalized to Talairach space. Fifteen regions of interest (ROIs) covering the whole brain were defined on these maps. Regional CBF and CBV reactivity to changes in PaCO2 were calculated. Data are expressed as median (interquartile range).

Results: Regional CBF and CBV were variable and showed a positive correlation (r: 0.246; $p < 0.001$). CBF reactivity to CO2 in patients (2.8 (1.6 to 3.9) %/mmHg) was similar to published normal values, but CBV reactivity (0.1 (0.1 to −1.1) %/mmHg) was lower than published normal values [1]. ICP reductions with hyperventilation (4.5 (0.8–5.2) mmHg) were unrelated to CBF or CBV changes, but were proportional to baseline ICP (r: 0.498; $p < 0.0001$). Regardless of baseline ICP (range: 5–30 mmHg) none of the regions showed CBF increases with hyperventilation.

Conclusions: The positive correlation between CBF and CBV supports a microcirculatory cause for ischaemia in head injury. CBF and CBV reactivity to PaCO2 show discordant changes in this population. The effect of CBV reductions on ICP depends on intracranial compliance, but regional CBF reductions may be the dominant effect of hyperventilation at this interval post-injury, even in patients with relatively high baseline ICP values.

Reference: [1] J Trauma 1995; 39: 463.

P215.

TRANS SODIUM CROCETINATE INCREASES OXYGEN DELIVERY TO BRAIN PARENCHYMA IN RATS ON OXYGEN SUPPLEMENTATION

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In spinal cord injury (SCI) and traumatic brain injury (TBI), the primary injury largely determines a patient's neurological grade upon admission and thereby is the strongest prognostic indicator. However, secondary mechanisms of injury can exacerbate damage and limit restorative processes, and hence, contribute to overall morbidity and mortality. Hypotension and hypoxia leading to local ischemia following SCI and TBI are associated with worsened neurologic outcomes. Trans sodium crocetinate (TSC) is a carotenoid that has been shown to improve oxygen delivery to ischemic heart and liver tissue and in lower-flow states such as shock, but has never been studied in brain or neurologic disorders. We investigated the effect of TSC on brain oxygen delivery in order to determine indication for study in TBI and SCI with secondary hypoxic insult.

To this end, male rats were ventilated with either 21% or 100% FiO2. Femoral artery and vein cannulation was achieved and baseline arterial blood gas measures taken. Next, a small burr hole was drilled into the right parietal bone. A calibrated Licox rat brain PO2 probe was inserted into the cortical parenchyma and PO2 in brain tissue (PbtO2) plugged into the brain tissue oxygenation monitoring system. PbtO2 was recorded over the course of an intravenous infusion of TSC or saline. Serial ABGs were also monitored.

TSC significantly increases PbtO2 to brain in rats ventilated with 100% FiO2. A similar effect on oxygen delivery is not achieved in unstressed rats ventilated with 21%FiO2. O2 supplementation is the standard of care for hypoxia after neurologic insult. Because TSC increases oxygen delivery to brain in the presence of O2 supplementation, an investigation of the impact of TSC on oxygen delivery in TBI and SCI with secondary hypoxic insult is warranted. Supported by AO North America.

P216.

REGIONAL EFFECTS OF CEREBRAL PERFUSION PRESSURE AUGMENTATION IN HEAD INJURED PATIENTS

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Cerebrovascular autoregulation is often impaired after head injury. However, there is substantial pathophysiological heterogeneity in the injured brain, and global measurements may not predict the response of regional cerebral blood flow (rCBF) to augmentation of cerebral perfusion pressure (CPP). We have used positron emission tomography (PET) to investigate rCBF responses to a change in CPP.

Methods: Fourteen sedated and ventilated head injured patients were studied within 4 days of injury. CBF and cerebral blood volume (CBV) were measured using PET at a CPP of ~70 and ~90 mmHg. CPP was controlled using norepinephrine. Changes in CBF and CBV and the static rate of autoregulation (SROR) were calculated globally (gSROR) and regionally (rSROR) for 15 normalized regions of interest (ROI). ROIs were classified as lesioned or non-lesioned based on CT images.

Results: gSROR ranged from 37–101%; rSROR in 210 ROIs from 14 patients ranged from 20–109%. There was substantial spatial variation in autoregulation within patients, and rSROR was < 50% in 19% of non-lesioned ROIs. Regional dysautoregulation was commoner when global autoregulation was impaired: no patient with gSROR > 85% showed regions with rSROR < 85%, and rSROR < 50% was only found in patients with gSROR < 50%. Assessment of CBV responses to hypertension allowed definition of a subset of ROIs (23% of ROIs with rSROR > 85%) that showed an increase in CBV > 10%, suggesting false autoregulation, possibly due to distal vascular compression leading to an increase in CBV but not CBF.

Conclusions: CPP augmentation leads to unpredictable changes in rCBF due to heterogeneity of vascular reactivity, but global autoregulation is a useful indicator of regional autoregulation. CT scans underestimate the extent of cerebrovascular dysfunction after head injury. Assessment of autoregulation by CBF responses only may be confounded by false autoregulation.

P217.

INHIBITION OF NA+/CA++ EXCHANGE WITH KB-R7943 ATTENUATES EARLY ASTROCYTE LOSS IN HIPPOCAMPUS FOLLOWING FLUID PERCUSSION BRAIN INJURY.

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Astrocytes play a significant role in normal brain function including active neuronal-glial signaling and maintenance of homeostasis in the extracellular microenvironment. Early impairment of astrocyte function after traumatic brain injury (TBI) may compromise critical neuronal-glia interactions and thus, may play a significant role in outcome after injury. Recent studies in ischemia (Mol Brain Res 68:29–41, 1999) as well as in TBI (J Neurotrauma 18:1165, 2001) indicate astrocyte loss in selectively vulnerable brain regions. Overload of intracellular calcium is thought to be a major cause of cellular damage following TBI. We examined the effects of KB-R7943, a novel inhibitor of the reversed Na+/Ca++ exchanger, on early astrocyte loss in hippocampus.

KB-R7943 (0, 0.2, 2, 15, or 40 nmoles) was infused into the lateral ventricle of males Sprague-Dawley rats for 1 hr prior to lateral fluid percussion TBI. At 4 hr after TBI, rats were euthanized and glial fibrillary acidic protein (GFAP) immunohistochemistry was performed. Counts of astrocytes were made in the dorsal hippocampus CA2-3 sectors from coronal sections between Bregma -2.12 through -4.80mm using stereological procedures. Astrocyte counts in the contralateral hemisphere were not significantly different between groups (65,135 ± 2103). Ipsilateral astrocyte counts were significantly higher in the 15nmol (58,442 ± 372) and 40nmol (53,684 ± 1254) KB-R7943 groups compared to the vehicle-treated group (34,170 ± 3735).

Inhibition of the Na+/Ca++ exchanger reduced astrocyte loss in the hippocampus after TBI. These data suggest that TBI may cause reversal of the Na+/Ca++ exchanger, which could be detrimental to astrocyte survival in selectively vulnerable brain regions after TBI. Supported by NIH NS29995 & UC Neurotrauma Research Initiative.

P218.

CSF ACCUMULATION OF CALPAIN-SPECIFIC aII-SPECTRIN BREAKDOWN PRODUCTS ARE ASSOCIATED WITH INJURY MAGNITUDE AND LESION VOLUME AFTER TRAUMATIC BRAIN INJURY IN RATS

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There currently exists no definitive diagnostic tests of traumatic brain injury (TBI) to help physicians determine the seriousness of injury, the extent of cellular pathology, or to guide appropriate therapeutic administration. Although we recently reported that calpain-specific aII-spectrin breakdown products (SBDPs) accumulate in CSF after TBI (Pike et al., 2001, J. Neurochem. 78:1297–1306), correlation of SBDP levels with injury magnitude and outcome is not known. The purpose of this study was to examine SBDP accumulation in brain and CSF at two levels of lateral controlled cortical impact TBI (1.0 mm and 1.6 mm) in rats at 2, 6, and 24 hours after injury. In addition, SBDP levels at each injury magnitude were correlated with rotarod performance on days 1–5 post-TBI, and with lesion volume at 28 days post-TBI (by T2-weighted MRI). Results: Accumulation of SBDPs in brain and CSF was highest after 1.6 mm injury at all time points. Both 1.0 mm and 1.6 mm groups had significantly greater CSF levels of SBDPs than the control group ($p < 0.05$, $p < 0.01$ respectively). In addition, SBDP levels were associated with both rotarod performance and with lesion volume where each was greater after 1.6 mm injury than 1.0 mm injury. Conclusions: This study indicates that CSF levels of calpain-specific SBDPs are sensitive to injury severity and that acute levels of SBDPs in CSF (hours) are associated with more delayed measures of behavioral outcome (days 1–5) and lesion volume (28 days). These findings support the use of SBDPs as biochemical markers of cellular pathology, injury severity, and outcome after TBI. (Supported by DAMD17-99-1-9565, DAMD17-01-1-0765, and NIH R01 NS39091, and NIH R01 40182).

P219.

TISSUE-TYPE TRANSGLUTAMINASE DISTRIBUTION AND EXPRESSION AFTER TRAUMATIC BRAIN INJURY

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Tissue-type transglutaminase (tTG) has been implicated in various diseases including neurodegenerative disease. Tissue transglutaminase (tTG) is a calcium-regulated enzyme and intracellular Ca+2 overload is triggered following traumatic Brain injury (TBI). Therefore, we analyzed the expression of tTG after TBI in a rat cortical impact model. Western blot analysis has demonstrated an increase in tTG protein expression after TBI. In ipsilateral cortex, peak induction of tTG protein (561% ± 16%) was observed five days after injury, with expression remaining elevated after two weeks. Lesser tTG protein induction was observed in hippocampus (194 ± 9%) five days after injury. The tTG protein induction was supported by northern blot and semi-quantitative PCR transcript analysis that demonstrated peak induction three days after injury in ipsilateral cortex with a small induction in hippocampus. Semi-quantitative PCR analysis of tTG mRNA demonstrated a peak induction three days after injury in ipsilateral cortex (414% ± 21% of control, $n = 3$), while in hippocampus, maximal induction was observed one day after injury, 196% ± 14% of control. Further, to elucidate the cell subtype distribution of tTG immunofluorescence was performed. Studies have demonstrated increased expression of tTG in both neuron and astrocyte cell population after injury, however, the expression was stronger in astrocytes than in neuronal cells. Future studies are in progress to determine (the role of tTG in injured cells) whether tTG positive cells are involved in apoptosis or proliferation. These findings will lay the groundwork for elucidating the functional role of tTG in TBI or any other CNS related injury and may result in the development of effective treatment strategies. (Supported by DAMD 17-99-1-9565 and NIH RO1 NS 39091)

P220.

RECOVERY OF SPEECH-SOUND PRODUCTION SKILLS IN YOUNG CHILDREN AFTER SEVERE TRAUMATIC BRAIN INJURY

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The current literature provides little information on the recovery of speech skills in children following severe traumatic brain injury. No data exist on the relationship between age at time of injury and speech recovery in these children.

In this investigation, we examined the rate and level of consonant mastery in 30 children who sustained severe traumatic brain injury (TBI) between 15 months and 10 years of age. Percentage of Consonants Correct was calculated and plotted over twelve monthly speech samples beginning when the child produced at least 10 intelligible words, and compared to a normal Percentage of Consonants Correct growth curve.

Results showed that children who were relatively older (i.e., >49 months) at the time of injury tended to display Percentage of Consonant Correct values that approached the normal performance curve in a shorter period of time than the children injured at younger ages (<48 months). In addition, older children were generally less variable across the 12 sampling sessions than younger children. Despite improvements in consonant production for the majority of these subjects over the 12 testing sessions, prosodic and voice aspects of speech production remained compromised in 83% of the subjects at the final session, suggesting underlying motor deficits.

These findings do not support the traditional view that earlier onset of neurological injury results in greater recovery. The need for predictive models of speech outcome that include age at injury, extent of neurological injury, and severity of oral-motor dysfunction will be discussed. This research was funded by a grant from the National Institute on Deafness and Other Communication Disorders awarded to the first author (R01-DCO 3608).

P221.

EXPERIENCE-DEPENDENT LOSS OF PLASTICITY IS RESTORED AFTER DELAYED EXPOSURE TO AN ENRICHED ENVIRONMENT.

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It has previously been shown that developing rats exposed to an enriched environment (EE) immediately following brain injury fail to show experience-dependent plasticity (Fineman et al, 2000). In this study, entry into an EE was delayed to 14 days after lateral fluid-percussion injury (FPI) to determine the time window during which developmental plasticity is lost. Rats were reared in EE for 30 days. After EE exposure rats were trained in the Morris Water Maze task (MWM) for five days. Both the sham-enriched rats and the FPI-enriched rats showed a significant improvement in the rate of learning compared to the standard-housed rats ($p < 0.05$). A probe test performed one week after MWM training showed that both the FPI-enriched and FPI-standard-housed rats spent significantly less time swimming in the target area compared to the sham animals ($p < 0.01$). In addition, the FPI-enriched rats took significantly more time to initially reach the target area compared to all the other groups ($p < 0.05$). Exposure to EE did not have any influence on delayed probe performance in the sham animals. These behavioral results indicate that plasticity is restored by PND 14, as indicated by the rate of learning. However, injury-induced memory impairments persist after exposure to an EE. Supported by: University of CA BIRC, NS30308, NS27544, NS38978.

P222.

GLUTAMATE AND GLUTAMINE IN EVOLUTION AND DEVELOPMENT

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The shape of the brain is preserved during growth, independent of the health of the child. This is an ontological process that includes growth (size change), development (shape change), and maturation (sexual age). Hominid evolution, a switch from an ape ontogeny to a human ontogeny, is marked by a significant increase in relative brain size (encephalization), from 450cc three million years ago to 1350cc 0.1 million years ago, and linked to energetic requirements to maintain the brain. This paper explores the role of glutamate and glutamine in that evolution and development.

Glutamine synthase (GS) was refined during the preprokaryotic period 3.8 billion years ago to provide glutamate and glutamine for neurotransmitters and metabolic pathways, respectively. In the evolution of biochemical signals, the amino acid, glutamate, also from this early preprokaryotic earth condition, became significant in the evolution of highly excitable signals of communication networks, both for homologous cells and complex organs. The glutamate signal meaning designated a memory in the excitation response, where the phenomenon of behavior could be created.

Glutamine provides nitrogen to be disseminated into metabolic pathways for amino acids, nucleotides, and enzyme cofactors. Metabolism and brain size have been correlated as follows. EQ (encephalization quotient) is a residual value, positive or negative, for brain mass, and has a statistical relationship with dietary strategies: folivory (negative), $p < 0.01$; frugivory (positive), $p < 0.05$; and faunivory (positive), $p < 0.02$. Two million years ago, hominid dietary strategies shifted to include meat and fat; just a 10–20% change in intake of these nutrients may be sufficient to provide the energy to fuel larger brains and for evolutionary consequences in encephalization.

The roles of glutamate and glutamine have been pivotal in the selection for that independent preservation of brain growth, from an ape ontogeny to a human ontogeny, and has led to this appreciable evolutionary change.

P223.

INFLUENCE OF APOE GENOTYPE ON SECONDARY INSULTS AFTER TRAUMATIC BRAIN INJURY

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Objectives: The aim of the study was to determine the influence of APOE genotype on secondary insults after Traumatic Brain Injury (TBI).

Methods: Sixty-two of 262 genotyped patients admitted to a neurointensive care unit with TBI over a 4 year period had monitoring data on intracranial pressure (ICP), blood pressure (bp), oxygen saturation and cerebral perfusion pressure (CPP). Demographic, management and outcome data including admission Glasgow Coma Scale (GCS) and 6 month Glasgow Outcome Score (GOS) were recorded using a standardised proforma for each patient. APOE genotype was determined using polymerase chain reaction. Monitored data was validated manually. Insults were identified using established definitions and total insult duration as a fraction of valid monitoring time and frequency of insults were recorded.

Results: Eighteen patients had one or more APOE e4 alleles. Hypotensive and CPP insults were more frequent in the APOE e4 group ($c2 = 6.236$; d.f.2; $p = 0.04$ and $c2 = 48.9$; d.f.2; $p = 0.00$ respectively). The duration of hypotensive and CPP insults was also longer in the APOE e4 group although this failed to reach statistical significance. There were no significant differences between the groups for insults related to ICP or hypoxia.

Conclusions: Hypotensive and CPP insults were more frequent amongst patients with an APOE e4 allele. Differences in the acute response to TBI may be at least partially responsible for the worse outcome that had been observed in individuals with an APOE e4 allele.

P224.

HIGH-DENSITY HIGH-THROUGHPUT TISSUE MICROARRAY PROFILING OF MOLECULAR ALTERATIONS IN EXPERIMENTAL TRAUMATIC BRAIN INJURY

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Head injury sets into motion a complex neurochemical cascade that may worsen the initial injury and render the damaged tissue more vulnerable to secondary damage from ischemia and hypoxia. These neurochemical alterations may be subject to pharmacological intervention. Study of these processes has been hampered by the lack of high-throughput techniques that allow rapid analysis of large numbers of tissue samples from experimentally produced brain injury. Very recent advances in high-density, high-throughput molecular profiling techniques developed for tumor analysis may be directly transferable to the study of non-neoplastic disorders such as head injury.

One of the most promising of these techniques is the tissue microarray (TMA), in which small tissue cores from hundreds of individual tissue samples are composited into a single paraffin block from which thin microtome sections can be cut and subjected to a wide range of analytical procedures, including routine tinctorial staining, immunohistochemistry, and a wide range of chromogen-based and fluorescent in-situ hybridization and PCR techniques. Existing technology permits the manual or automated compositing of individual tissue cores ranging from 0.6-2.0 mm diameter with 0.1 mm spacing between array elements. TMAs permit the simultaneous staining/hybridization and analysis of up to 1000 different tissue samples on a single 45 × 20 millimeter glass slide.

We constructed a TMA of cerebral cortex cores taken ipsilateral and contralateral to a controlled cortical impact site from 80 rats using an automated tissue arrayer (ATA-27, Beecher Instruments, Silver Spring, MD). The donor blocks comprised archival material from previous experiments in which the animals were sacrificed 14 days after injury. Duplicate punch samples were obtained from each site to yield a total of 320 tissue cores arrayed in a single recipient (TMA) block. From this TMA block, 40 unstained sections were cut. The first and last slides were stained with hematoxylin & eosin and the TMA block was refrigerated for future use. Using two of the unstained sections, we performed glial fibrillary acid protein (GFAP) and HAM56 (a marker for macrophage activation) immunostains. Strong GFAP immunoreactivity was seen in approximately 50% of the cores, and in 90% of these GFAP-positive cores the immunoreactivity localized to the side of the cortical impact. No significant HAM56 immunoreactivity was present in any core. The remaining unstained slides and the TMA block are now archived for additional studies. In addition, the ATA-27 punch order program has also been saved, permitting easy automated construction of additional TMAs from the archived donor block study set as needed.

This proof-of-principle study demonstrates the technical feasibility of using tissue microarrays for protein expression profiling in traumatic brain injury. Tissue microarrays facilitate the preservation of valuable tissue resources by virtue of their "tissue expansion" property and permit an increase in the number of experiments that can be performed on limited and sometimes irreplaceable tissue samples by at least two orders of magnitude. High-density high-throughput TMAs provide a powerful new tool for the dissection of molecular and cellular events occurring in traumatic brain injury.

(The support of NIH PO1-NS38660 is gratefully acknowledged.)

P225.

GENE EXPRESSION FOLLOWING HUMAN TRAUMATIC BRAIN INJURY BY MICROARRAY ASSAY

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Human data accumulated over the past decade has validated animal studies showing gene expression after traumatic brain injury (TBI), including up regulation of c-fos, Jun B and HSP 70. We tested the hypothesis that mRNA expression following human TBI characterized by microarray assay (ma) would be consistent with previous work and uncover novel gene expression.

Methods: This study was conducted with IRB approval. Pericontusional tissue from TBI patients was analyzed against normal tissue removed during surgery for non-traumatic indications using ma. Experimental and control cDNA probes were hybridized against >5000 gene segments on GF200 human GeneFiltersä and quantified with Pathways 4Ô software.

Results: To date we have analyzed samples from five TBI patients against control samples. We identified upregulation of c-fos (4 of 5), Jun B (4 of 5) and HSP 70 (3 of 5) achieving statistical significance in several cases. In other comparisons glyceraldehyde-3-P, dynactin-4, B crystallin, and myelin basic protein were up-regulated, while GFAP was down-regulated, in TBI compared to control patients.

Discussion: These results further address the validity of current animal TBI models by using ma to demonstrate specific, differential gene expression in human TBI. Further study will provide new insight into the pathophysiology and treatment of TBI. Support by the L.M. Thomas, M.D Fund to D.B.M., by NIH R15-DC05179 to L.N.I., and an NIH-NCRR (RCMI) grant to the Univ. of Texas at El Paso.

P226.

THE EXPERIMENTAL STUDY ON EXPRESSION AND ACTIVATION OF CASPASE 3 AFTER ACUTE BRAIN TRAUMA

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To analyze the role of Caspase 3 on delayed neuronal death. Experiments were based on rat diffuse brain injury model. The neuronal DNA injury in cortex and hippocampal was observed by TUNEL stain. The mRNA and protein expression and enzyme activation of Caspase 3 were observed by northern blot, in situ hybridization, immunohistochemistry stain and western blot. Special Caspase 3 enzyme inhibitor were given to observe the therapeutic effect.

After impact, neurons with positive TUNEL stain appeared 2 hours after severe injury, most peaked at 24 hours, last till 7 days. Northern blot shows that the Caspase 3 mRNA expression increased and peaked on 24 hours, 3 times higher than the controls. In the area of cortex and hippocampal, positive mRNA stain neurons appeared most distinct on 24 hour. With the antibody for Caspase 3 P20 subunit, the active Caspase 3 expression peaked on 1–3 days. The electrophoresis band of PARP degradation would be seen by western blot. Caspase 3 enzyme inhibitor decreased apoptotic neuronal death, but had no effect on Caspase 3 P20 subunit expression.

After brain trauma, there were increases on Caspase 3 mRNA and protein expression and enzyme activation, accounting for neuronal DNA injury or apoptosis. Using special Caspase 3 enzyme inhibitor can apparently decrease the delayed neuronal death.

P227.

CXC CHEMOKINES MAY CONTRIBUTE TO INFLAMMATION IN SUBARACHNOID HEMORRHAGE AND ITS CONSEQUENCES

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Activated neutrophils are thought to be involved in neuronal injury following subarachnoid hemorrhage (SAH). CXC chemokines, interleukin (IL)-8 and epithelial neutrophil activating peptide (ENA)-78, are responsible for activation of neutrophils and for neutrophil chemotaxis to site of injury. To understand the importance of these chemokines in the pathogenesis after SAH, we have examined the production of these chemokines in cerebrospinal fluid (CSF) from 19 patients with SAH and in 70 control patients without central nervous system lesions. We also investigated the expression of mRNAs of IL-8, CXC R1 and R2 by RT-PCR and immunocytochemically the expression of IL-8 protein in CSF leukocytes from patients with SAH.

Significantly increased levels of IL-8 ($p < 0.001$) and ENA-78 ($p < 0.001$) were detected in the CSF of patients after SAH compared with levels in the CSF of control patients, and the levels of both chemokines correlated with neurologic severity at admission assessed by Hunt & Kosnik grading and neurologic outcome by Glasgow outcome scale. The elevated leukocyte count in CSF collected by ventricular drainage correlated with the levels of IL-8 ($p < 0.01$) and ENA-78 ($p < 0.05$). We followed time-related changes in concentrations of the CXC chemokines in 7 patients with SAH for up to 30 days, and found peak concentrations of them within 10 days after the onset of injury. Furthermore we detected mRNAs of IL-8 and CXC R1 and R2 in CSF leukocytes and IL-8 protein in macrophages and in polymorphonuclear leukocytes in CSF.

These findings suggest that IL-8 and ENA-78 may contribute to inflammation in SAH and its consequences through leukocyte activation and chemotaxis.

P228.

SYSTEMIC ANTI-INFLAMMATORY REACTION AFTER BRAIN INJURY

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Local release of pro-inflammatory cytokines as well as an increased ICP after brain injury may acitvate the hypothalamic-pituitary-adrenal (HPA)-axis and the sympathetic nervous system (SNS) and induces a systemic anti-inflammatory response syndrome. In order to analyze the mechanisms of a systemic immunodepression resulting from cerebral inflammation and an increased ICP we established different animal models using intra-cerebroventricular (icv) or intra-hypothalamic (ih) infusion of the pro-inflammatory cytokines TNF-α and IL-1β and increasing continuously the ICP using an subdurally placed catheter. Interestingly, icv and ih infusion of IL-1β but not TNF-α produced distinct signs of central nervous system (CNS) inflammation. Correspondingly, icv and ih infusion of IL-1β generated an increase of neutrophils and a decrease of lymphocytes in blood. This could be reduced by hypophysectomy (HPX) and completely blocked by administration of the β2-adrenoreceptor antagonist propranolol. Furthermore, icv and ih infusion of IL-1β produced a diminished TNF-α secretion capacity. This could be reversed by HPX. Finally, icv infusion of IL-1β caused a temporal elevation of the endotoxin-induced IL-10 secretion. This effect was antagonised by propranolol suggesting an involvement of the SNS. Moreover, increased ICP and bolus IL-1β into the brain are able to induce systemic IL-10 release. This effects could be likewise blocked by application of the β2-receptor-antagonist propranolol suggestig that symapthetic activation mediated also the systemic IL-10 release. Interestingly, brain injured patients showed also high IL-10 concentration in plasma and the IL-10 levels were associated with the severity of the injury. Finally high IL-10 levels correlated with systemic immuno-depression and increased risk of infectious complications. So we conclude, that increased ICP and cytokines in the brain can produce vegetative disturbances with sympathetic activation. Catecholamines are able to induce an IL-10 release from white blood cells. This may lead to systemic immuno-depression and infectious complications in brain-injured patients.

P229.

TRAUMATIC BRAIN INJURY (TBI)-INDUCED SPASTICITY: MONOAMINE CHANGES AND POSSIBLE MECHANISMS.

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Little is known regarding the fundamental neurobiology of TBI-induced spasticity that could guide the development of successful treatment strategies. These studies were conducted in Sprague Dawley rats with moderate controlled contusion cortical injuries (CCI), that had just completed three-months of spasticity and behavioral studies (see poster: Thompson et al, 2002). The purpose of the present studies was to investigate two monoamine neural substrate systems known to influence spinal reflex excitability. Fluorescent immunocytochemistry (ICC) was applied to ponto-medullary and lumbar spinal cord tissue to investigate TBI-related changes in the expression of immunoreactivity (IR-) in noradrenergic (NE) cell clusters in the locus cereluous (LC) and fibers in L4-5 spinal segments, as well as in the serotonergic (5-HT) cell clusters in the raphe and fibers in the spinal L4-5 segments. A significant reduction in LC NE cell number was detected in the TBI specimens in conjunction with significantly decreased number of IR-NE positive fibers in the ventral horns compared to that of control specimens. At the same time, a robust hyperinnervation in the dorsal raphe serotonergic positive cells was detected in the TBI specimens, compared with time-matched sham control specimens. Similarly, hyperinnervated IR-5HT positive fibers were also detected in the dorsal, ventral, and intermediolateral column of L4-5 spinal cord of TBI specimens. We hypothesize that this marked asymmetry between noradrenergic and serotonergic expression both in the brain and spinal cord following TBI could substantially contribute to the development of the robust tonic spasticity observed in these animals (see companion poster). Supported by the Brain and Spinal Cord Injury Rehabilitation Trust Fund.

P230.

SIMPLE MORPHOMETRY OF AXONAL SWELLINGS CANNOT BE USED IN ISOLATION FOR DATING LESIONS AFTER TRAUMATIC AXONAL INJURY

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Objectives: Disruption of fast axonal transport due to traumatic brain injury results in the accumulation of b-amyloid precursor protein (APP) in axonal swellings. Using image analysis we have tested the hypothesis that the size of axonal swellings correlates with survival time after injury.

Materials and methods: Paraffin sections of the corpus callosum from 63 cases of fatal head injury were stained for APP and counterstained with haematoxylin. Three different measurements were made of the APP-immunoreactive axonal swellings i) minimum and ii) maximum Feret diameters, iii) area.

Results: Linear regression revealed a significant correlation between survival time and the minimum Feret diameter ($p < 0.0001$) and the area ($p < 0.001$) of axonal swellings.

Conclusions: The findings are in agreement with a previous study showing a significant correlation between axonal swelling size and survival time. However, it is suggested that the large variability in swelling size within individual cases and the heterogeneity of the original trauma seriously compromises the utility of such information in the timing of lesions.

P231.

COGNITIVE IMPAIRMENT WITH MENTAL FATIGUE DURING RECOVERY FROM NEUROTRAUMA. CELLULAR MECHANISMS FOCUSING ON ASTROGLIAL DYSFUNCTION IN GLUTAMATERGIC NEUROTRANSMISSION

Lars Rönnbäck and Elisabeth Hansson. (Institute of Clinical Neuroscience, Göteborg, SE).

During rehabilitation after brain injury, patients often suffer from mental fatigue, specifically having difficulty with attention, concentration, and learning. Glutamate, the most extensively studied excitatory neurotransmitter in the nervous system, is indispensable for information intake and processing within the brain. After glutamate has elicited its effects on the postsynaptic and adjacent glial membrane receptors, the astroglial cells, one of the supporting cells in the brain, remove excess glutamate from the extracellular space. The extracellular concentration of glutamate must be low in order for glutamatergic neurotransmission to be effective. According to our hypothesis, one underlying mechanism at the cellular level for this mental fatigue could be a reduced capacity of the astrocytes to clear the extracellular space of glutamate. We have developed cell cultivation conditions that make us able to study in primary culture, and co-cultivation, of different cell types, effects of slightly increased glutamate levels when astroglial glutamate uptake is impaired. We present results on a slight microglial activation and production of substances, tumor necrosis factor alpha (TNF-a) and interleukin-1b (Il-1b) or altered conditions (slight acidification) after glutamate levels in culture medium has been increased over time.

P232.

DIFFERENTIAL PEPTIDE DISPLAY AND ANALYSIS IN CSF AND PLASMA FOLLWING TRAUMATIC BRAIN INJURY.

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CSF peptides reflect cerebral protein metabolism, blood-brain- and blood-CSF-barrier function, and have regulatory functions. The applied Peptidomics™ technique involves a) high performance liquid chromatography to separate samples in 96 fractions, b) MALDI-TOF mass spectrometry for peptide detection and c) unique software solutions for image generation and statistics. Quantifiable peptide maps displaying the full range of peptide structures were created.

We studied the peptide pattern of rat CSF and plasma in general and the changes of peptide composition following controlled cortical impact injury (CCI). 24 CSF and plasma samples, respectively, were analyzed from normal animals, from sham operated and trauma rats at 1h, 4h, 24h, and 7d after CCI. Maps displayed ~3600 and ~5000 peptide signals in CSF and plasma, respectively. A large increase in selected CSF peptide signals was seen at 1h and 4h reflecting initial disturbance of barrier function. Statistical time course analysis furthermore revealed 9 peptides in CSF and plasma each, which were significantly changed over the whole 7d and each showed a different pattern of intensity changes over time.

This indicates in CSF, besides a remarkable overall stability of the CNS milieu after trauma, barrier-related as well as and metabolic changes, and in plasma, a systemic response to isolated brain injury. Sequence analysis for identification of these isolated peptides is currently underway. This novel technique opens a new window for insights in posttraumatic peptide metabolism and function of CNS barriers.

P233.

TRAUMATIC BRAIN INJURY INDUCED SPASTICITY: NATURE, MAGNITUDE, AND TIME-COURSE

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Traumatic brain injury (TBI) produces major health problems impacting the lives of 1.5 to 2 million people in the United States each year. Spasticity is one of the most significant challenges associated with rehabilitation following moderate/severe TBI. These problems are further exacerbated by the lack of understanding of many essential aspects of this condition. As a first step, our studies have developed a model that incorporates controlled contusion brain injuries (CCI) in adult Sprague-Dawley rats followed by unequivocal measures that quantitate cognitive, spasticity, and vestibulomotor function. Cognitive deficits in these animals were consistent with those previously reported for moderate CCI. Velocity dependent ankle torques and triceps surae EMGs were measured in awake animals over a broad range of rotation velocities (49 0/sec-612 0/sec) before and at weekly intervals following injury. A significant increase in velocity dependent ankle torque and associated EMGs were observed at all the rotation velocities from week 1 through 10 weeks following injury (repeated measures ANOVA). Mean increases of 108% in the peak torque and 177% in peak EMG magnitude at 612 0/sec were observed at week-10 postinjury. Spasticity magnitude was only 50% predictive of the magnitude of anterograde memory deficit. The TBI-induced spastic animals also showed significant vestibulomotor deficit tested on rotorod. These TBI-spasticity patterns, including changes in ankle extensor electrophysiology, differed from those observed in previous studies of spasticity following experimental thoracic spinal cord contusion. These studies represent the first quantitative investigation of the nature, magnitude, and time course of the development of spastic hypertonia following TBI in an animal model. Supported by the Brain and Spinal Cord Injury Rehabilitation Trust Fund.

P234.

ESTROGEN REGULATION OF XIAP PROCESSING FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT

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Certain members of the newly discovered family of intrinsic inhibitors of apoptosis (IAP) proteins can directly bind and inhibit caspases. However, IAPs have been shown to undergo cleavage by caspases in response to inducers of apoptosis, but the significance of IAP cleavage has not been established. One IAP family member that is of particular interest in gender studies is the X-linked inhibitor of apoptosis (XIAP) that undergoes cleavage following traumatic brain injury. Since estrogen has been shown to have anti-apoptotic properties, this study examined gender differences and the influence of estrogen on XIAP processing during apoptosis after TBI. Male (TBI-M, n = 6), female (TBI-6, n = 3), ovariectomized female (TBI-OVX, n = 5) and ovariectomized females supplemented with estrogen (TBI-OVX+EST, n = 7) Sprague-Dawley rats were intubated, anesthetized (70%N2O, 0.5% halothane, 30%O2) and subjected to a moderate (1.7–2.2 atm) fluid percussion injury (FPI). Animals were sacrificed 24 hrs after FPI; cortical tissue (ipsilateral and contralateral) was dissected and analyzed for XIAP processing by immunoblot analysis and quantitative densitometry. Significant differences in XIAP cleavage in the ipsilateral cortex were found between groups ($p < 0.03$). Post-hoc analysis showed an increase in XIAP processing in both TBI-F and TBI-OVX+EST compared to TBI-M and TBI-OVX ($p < 0.05$), indicating that more XIAP is cleaved following injury in intact females and estrogen supplemented ovariectomized animals than in TBI-M and TBI-OVX groups. Based on these data, we propose that estrogen may provide neuroprotection by regulating XIAP cleavage after injury. This regulation may be influenced by exogenous estrogen treatment. (NS 30291 & Eli Lilly and Co.)

P235.

NEURON-GLIA COMMUNICATION: METALLOTHIONEIN EXPRESSION IS RAPIDLY INCREASED BY ASTROCYTES IN RESPONSE TO NEURONAL INJURY

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Metallothioneins (MTs) are stress-related proteins, which respond to numerous stimuli including cytokines, metals and various chemical agents. We examined MT-I/-II expression following scratch wound injury in primary rat embryonic neuron/astrocyte co-cultures and pure astrocyte cultures. Following injury in mature neuron/astrocyte co-cultures (21 days in vitro), MT-I/-II was rapidly induced in astrocytes aligned along the injury site and staining was observed in both cell bodies and processes. At later time points, almost all astrocytes were MT-I/-II immunoreactive, suggesting that a chemical or physical signal spreads from the injury site. In un-injured controls, MT-I/-II staining was either absent or localised solely to the nucleus of a small number of astrocytes. MT was not found in neurons at any time point investigated. Intriguingly, an equivalent scratch wound injury in pure astrocyte cultures resulted in no change in MT-I/-II expression, indicating that the upregulation of MT is specifically induced by neuronal injury and subsequent neuron-glia communication. Further, focal cortical brain injury in anesthetised adult rats also resulted in a similar pattern of MT-I/-II induction, suggesting that our co-culture model will be valuable in discovering the mechanism by which astrocyte gene expression is modulated by neuronal injury. We are currently investigating the involvement of several neuron-derived factors in this response.

P236.

THE EFFECT OF CYCLOSPORIN A UPON MITOCHONDRIAL DYSFUNCTION AND ENERGETIC METABOLISM FOLLOWING DIFFUSE TRAUMATIC BRAIN INJURY

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Introduction: Pre- and post-injury Cyclosporin A (CsA) administration has shown neuroprotective properties by ameliorating mitochondrial damage. The aim of this study was to assess the effect of CsA upon N-acetylaspartate (NAA) reduction and ATP loss, two sensitive markers of mitochondrial dysfunction and bioenergetic impairment.

Methods: Adult male Sprague-Dawley rats were exposed to Impact Acceleration TBI (2m/450g) and randomized into the following experimental groups: Intrathecal (I.T.) CsA/Vehicle treated (n = 12), Intravenous (I.V.) CsA/vehicle treated (n = 24) and Sham (n = 8). I.T. treatment consisted of post-injury (30 min) cisternal bolus of CsA or Vehicle (0.15 ml, 10 mg/kg). I.V. treatment consisted of post-injury infusion of 20 and 35 mg/kg CsA or Vehicle. HPLC analysis of whole brain samples was performed 6 hours post-injury for levels of NAA and ATP.

Results: I.T. CsA delivery demonstrated significant neuroprotection blunting a 32% NAA reduction ($p < 0.0001$) and restoring 30% of ATP loss ($p < 0.005$). The 20 mg/kg I.V. dose failed to ameliorate the biochemical damages. The 35mg/kg I.V. infusion showed 36% NAA recovery and 40% ATP restoration ($p < 0.001$).

Conclusion: CsA is capable of blunting NAA reduction and restoring ATP. Intravenous infusion of 35 mg/kg appears to be the most effective therapeutic strategy. These findings contribute to the notion that CsA achieves neuroprotection preserving mitochondrial integrity and provide a rationale for the assessment of CsA in the clinical setting where MR Spectroscopy can monitor NAA and ATP in brain injured patients.

Research supported in part by NIH Grant 2R01 NS 19235 and in part by Research Funds from Catania and Rome "Tor Vergata" Universities.

P237.

GENE DELIVERY OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) PRIOR TO TRAUMATIC BRAIN INJURY: DIFFERENTIAL EFFECTS ON ANATOMY AND BEHAVIOR

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Traumatic brain injury (TBI) results in significant long-term disabilities in millions of patients, yet no treatment exists. This study is the first to test neurotrophic factor gene delivery to the cortex in an animal model of TBI—the controlled cortical impact (CCI). GDNF protein is neuroprotective in experimental models of stroke, Parkinson's disease and in hippocampal cells following CCI. Here, we investigate whether administering a GDNF gene via an adenovirus(AdGDNF) to the penumbra of the CCI one-week prior to the injury can be neuroprotective and ameliorate behavioral deficits. Adult male rats received two injections of an adenoviral vector harboring GDNF (5×10^8 particles in 4 μl total) into the cortex medial and lateral to the site of injury. One week later, a CCI was administered over the forelimb sensorimotor cortex. Controls received CCI only or a control vector and CCI. Behavioral testing (foot fault and limb-use) was performed on day 0, 2, 4, 7, 10 and 13 post-injury. Rats were sacrificed on day 14. Serial sections through the contusion area were analyzed with NIH image to quantify contusion volume. AdGDNF treatment resulted in significantly smaller contusions ($p < 0.05$) suggesting that AdGDNF is neuroprotective. However, this neuroprotection did not result in a significant decrease in behavioral deficits. AdGDNF slightly decreased deficits on the foot fault at all time points, and on limb use on day 2. However, there were no significant decreases in limb use at all other time points. Although AdGDNF significantly decreased the size of the contusion it did not significantly decrease behavioral deficits suggesting that although the neuronal perikarya in the contusion survived these were not fully functional. Future work will focus on elucidating these differential effects. Supported by: NIH-NS31957 (M.B.), Shaw Fdn (M.B.), Carver Fdn (Univ. Iowa Vectore Core), NIH-NS4258301 (D.K.) DePaul University Research Council and College of Liberal Arts & Sciences (D.K.)

P238.

EXAMINATION OF THE ROLE OF N- AND P/Q-TYPE VOLTAGE SENSITIVE CALCIUM CHANNEL BLOCKERS IN TRAUMATIC BRAIN INJURY PRODUCED BY LATERAL FLUID PERCUSSION

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Voltage sensitive calcium channels (VSCCs) are major sources of cellular calcium (Ca2+) entry. Ca2+ regulates several processes critical for normal cell function such as cellular excitability, neurotransmitter release, and gene expression. Intracellular Ca2+ overload has also been implicated in the pathogenesis of neuronal loss after traumatic brain injury (TBI). Past studies have shown neuroprotection by both N- and P/Q-type VSCC blockers in rodent models of ischemia. Few, if any, studies have been carried out using TBI models. We examined the role of N- and P/Q-type VSCCs in the pathophysiology of TBI using the lateral fluid percussion (LFP) injury model in rats. Immediately after injury, twenty microliters containing 50, 100, or 200 pmol of SNX-185, a N-type VSCC blocker, 10, 25, 50 or 100 pmol of AgaIVA, a P/Q-type VSCC blocker, or ACSF-vehicle was injected into the CA3 subregion of the hippocampus. Histological assessment of neuronal degeneration was visualized in brain sections using cresyl violet and Fluoro-Jade staining. Behavioral assessments were carried out using beam walk, inclined plane, radial arm maze, and Morris Water Maze. Compared to control, rats treated with 100 pmol of SNX-185 or 10 pmol of AgaIVA showed both a significant decrease in neuronal degeneration and improved behavioral outcome. Doses above 50 pmol AgaIVA showed toxicity. Our data indicate that both SNX-185 and AgaIVA may be neuroprotective, implicating both N- and P/Q-type VSCCs' in the pathophysiology of TBI. Blockage of VSCCs after TBI may have important therapeutic potential. (Supported by NIH NS39090 and the UC Neurotrauma Research Initiative)

P239.

ASSESSING THE GLOBAL BURDEN OF PATHOLOGY IN HEAD INJURY USING 2-D PQ HISTOGRAMS AND DIFFUSION TENSOR IMAGING

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Diffuse axonal injury (DAI) results in changes in the mean apparent diffusivity and diffusion anisotropy of tissue water as seen on magnetic resonance (MR) diffusion tensor imaging (DTI). DAI can be widespread and subtle, extending beyond image abnormalities seen on conventional CT or MR. We describe novel graphical tools for visualising and quantifying the global burden of these pathologies across the entire brain, making no assumptions regarding their location.

Methods: Acute DTI was undertaken in five volunteers and five patients with acute head injury on a 3 Tesla MR system, using a pulsed gradient spin echo, echo planar imaging technique. The diffusion tensor was computed on a voxel-by-voxel basis, and deconvolved to its isotropic (p) and anisotropic (q) components. These variables were used to provide a 2-D graphical representation of DTI abnormalities across the brain. Summary statistics for p and q included the peak (mode) location, mean values (p*,q*), and the full width half maximum ranges of distribution (dp,dq).

Results: Volunteers showed highly consistent pq plots, with coefficients of variations for the above parameters ranging from 1.4 to 12.9%. Acute head injury resulted in marked inter-individual variations in these parameters, with increases in isotropic diffusion (probably representing ischemic vasogenic edema), and/or a significant reduction in the number of voxels with high q values, suggesting loss of white matter anisotropy due to DAI. Follow up imaging showed partial resolution of these abnormalities in some patients.

Conclusions: PQ histograms provide a easily comprehensible graphical depiction of the global burden of ischemia and axonal injury, and can be used to obtain parametric measures of these pathologies. Further work is required to clearly define the pathological correlates of these imaging abnormalities, and select individual parameters that best quantify the variable pathology in this patient population.

P240.

REDUCTION IN THE FORMATION OF CEREBRAL EDEMA FOLLOWING FLUID PERCUSSION INJURY FROM FREE RADICAL SCAVANGER AND NSAID COMBINATION THERAPY

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Combination therapy consisting of the free radical scavengers vitamins C and E and the non-specific cyclooxygenase (COX) inhibitor ibuprofen administered 30 minutes post fluid percussion injury (FPI) have been shown to reduce neurological and motor deficits following experimental brain injury. This treatment is hypothesized to reduce the availability of arachidonic acid for conversion to vasoactive prostaglandins by COX via the protective action of the free radical scavengers vitamins C and E. Vitamin C in addition to acting as a general free radical scavenger also converts the radical form of vitamin E back to an active radical scavenger. Further benefit may be derived from protection of cellular structures by the scavengers. Ibuprofen reduces the conversion of arachidonic acid by the inhibition of COX resulting in less vasoactive prostaglandins, and a reduced inflammatory response.

The aim of this study was to determine the effect of this treatment on the formation of cerebral edema 24 hours post injury. Long Evans rats were subjected to severe FPI (3.1 atm. mean duration of unconsciousness 175seconds) centered over the left hemisphere midway between bregma and lambda. Thirty minutes post FPI rats received either 10mg/kg vitamin C, 45mg/kg vitamin E, and 10mg kg ibuprofen or vehicle treatment. Twenty four hours post-FPI the rats were rated on forelimb flexion. Treated subjects demonstrated significantly less deficits compared to the vehicle treated group (<0.05). Following neurological assessment, the subjects were sacrificed and brains removed and dissected into defined regions. The sections were freeze dried for 72 hours and edema levels determined using the wet/dry method. The brains of the untreated subjects had significant levels of edema in the ipsilateral cortex, hippocampus and thalamus. Rats receiving the combination treatment displayed significantly less edema in the ipsilateral cortex (<0.01) and ipsilateral hippocampus (<0.05). No effect was observed in the thalamus.

P241.

SODIUM AND CALCIUM EXCHANGE FOLLOWING IN VITRO MECHANICAL AND/OR ISCHEMIC INJURY IN ASTROCYTES

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Mechanical brain injury is clinically often followed by ischemia but the mechanisms of each insult are difficult to separate. The effect of mechanical injury alone or followed by ischemia on astrocyte intracellular Na+ and Ca2+ concentrations ([Na+]i; [Ca2+]i) was examined using fluorescent imaging of Fura-2-AM or SBFI-AM in cultured astrocytes. Following loading, astrocytes were perfused with standard solution (NORM), stretch-injured, and imaged for 30 min. If applicable, cells were then exposed to hypoxic, acidic, ion-shifted Ringer's (HAIR, Bondarenko and Chesler, 2001) for 5 min., and reperfused with NORM buffer for 30 min. while imaged. Cell viability was evaluated by propidium iodide uptake (PrI). Mild, moderate and severe stretch injury increased [Na+]i by 2, 3 and 5-fold, respectively. When mild injury was followed by mild HAIR, [Na+]i increased by nearly 5-fold, [Ca2+]i increased by 4-fold, and PrI uptake increased 5-fold suggesting that the combination injury is more damaging than either insult alone. Application of KB-R7943, an inhibitor of reversed Na+/Ca2+ exchange, significantly reduced injury-induced increases in [Ca2+]i and PrI uptake after moderate stretch injury, but not after mild or severe stretch, or combined mild stretch/HAIR. This suggests that the Na+ load following mild stretch injury may not be sufficient to reverse the Na+/Ca2+ exchanger, but that Na+ level following moderate stretch injury may drive the exchanger in the reverse direction. Additionally, we hypothesize that both the severe stretch injury and the combination of mild stretch/HAIR may involve additional mechanisms of ionic imbalance beyond reversal of Na+/Ca2+ exchange. Supported by UC Neurotrauma Research Initiative, NIH NS 29995.

P242.

DELAYED TREATMENT OF HEMOGLOBIN NEUROTOXICITY

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Hemoglobin is an oxidative neurotoxin that may contribute to cell injury after CNS hemorrhage. Prior studies have demonstrated that concomitant treatment with iron-chelating antioxidants prevents its neurotoxicity. However, the efficacy of these agents when applied hours after hemoglobin has not been determined, and is the subject of the present investigation. Consistent with prior observations, an increase in reactive oxygen species generation, as detected by 2′,7′-dichlorofluorescin oxidation, was observed in cultures exposed to hemoglobin alone. However, this oxidative stress developed slowly. A significant increase in the dichlorofluorescein signal compared with control, untreated cultures was not observed until four hours after addition of hemoglobin, and was followed by loss of membrane integrity and propidium iodide staining. Treating cultures with the 21-aminosteroid U74500A or the ferric iron chelator deferoxamine four hours after initiating Hb treatment markedly attenuated reactive oxygen species production within 2 hours. Continuous exposure to 5 uM hemoglobin for 24 hours resulted in death of about three-quarters of neurons, without injuring astrocytes. Most neuronal loss was prevented by concomitant treatment with U74500A; its effect was not significantly attenuated if treatment was delayed for 2–4 hours, and it still prevented over half of neuronal death if treatment was delayed for 8 hours. Similar neuroprotection was produced by delayed treatment with deferoxamine or the lipid-soluble iron chelator phenanthroline. None of these agents had any effect on neuronal death when added to cultures 12 hours after hemoglobin. In contrast, delaying treatment of glutamate-induced neuronal injury for two or more hours resulted in complete loss of the protective effects of U74500A and MK-801. These results suggest that hemoglobin is a potent but slowly-acting neurotoxin. The delayed onset of hemoglobin neurotoxicity may make it an attractive target for therapeutic intervention.

P243.

ENHANCED NEURONAL DIFFERENTIATION OF TRANSPLANTED NEURAL STEM CELLS INDUCED BY IN SITU ADMINISTRATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR RESTORES NEUROMOTOR FUNCTION FOLLOWING TRAUMATIC BRAIN INJURY IN RATS

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We have demonstrated that neural stem cell (NSC) transplantation together with in situ administration of brain derived neurotrophic factor (BDNF) could enhance differentiation to neuronal phenotype in rat cortical ablation model. The present study was undertaken to examine whether the similar neuronal differentiation of transplanted NSCs was induced by BDNF administration following traumatic brain injury (TBI) and restored neuromotor functions. Adult male wistar rats were deeply anesthetized and cortical contusion was induced in the unilateral sensorimotor cortex by controlled cortical impact device. Seven days following injury, BrdU-labeled human fetus-derived NSCs (approximately 10 × 104 cells/ animals) were stereotactically transplanted into pericontusional areas together with insertion of BDNF-soaked gel foams into the contusion cavities. Controls animals were given saline-soaked gel foam with NSCs transplantation. Two weeks after the transplantation, neuromotor function was evaluated by rotarod test and sacrificed. The survival and differentiation of transplanted cells were examined immunohistochemically by NeuN, MAP2, GFAP, vimentin, BrdU. The BrdU-positive surviving cells could be detected in the pericontusional areas in both BDNF-treated rats and controls. In controls, the majority of the transplanted cells expressed GFAP or vimentin immunoreactivities, indicating differentiation to glial lineage. In contrast, the cells expressed the neuronal marker, NeuN increased significantly in the pericontusional areas in the BDNF-treated rats. In addition, the rotarod test demonstrated that attenuation of motor deficits was observed in BDNF-treated rats compared to controls. These results indicated that in situ administration of BDNF enhanced neuronal differentiation of transplanted NSCs, which may lead to the functional recovery.

P244.

BRAIN-DERIVED NEUROTROPHIC FACTOR ADMINISTERED IN GEL FOAM ENHANCES THE NEURONAL DIFFERENTIATION OF TRANSPLANTED NEURAL STEM CELLS IN RAT ABLATION MODELS.

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It has been demonstrated that the neural stem cells (NSCs), when transplanted into damaged neural tissues, the majority of the cells differentiated to into glial phenotype, but not neurons. In order to reconstruct the damaged neural circuits by NSCs transplantation effectively, promotion of neuronal differentiation is crucial. The present study was undertaken to examine whether brain-derived neurotrophic factor (BDNF)-soaked gel foam applied into ablation cavities could enhance the neuronal differentiation of transplanted NSCs. Adult male wistar rats were deeply anesthetized and the unilateral sensorimotor cortex was ablated by sucking force. Immediately after injury, BrdU-labeled rat fetus-derived NSCs (approximately 10x104 cells/ animals) were stereotactically transplanted in the area adjacent to the cavities with insertion of BDNF-soaked gel foam within the ablation cavities. Control animals were performed NSCs transplantation with saline-soaked gel foam. Two weeks after injury, survival and differentiation of transplanted cells were examined by NeuN, MAP2, GFAP, vimentin, BrdU immunohistochemistry. The BrdU-positive surviving cells could be detected in the regions in both BDNF and non-BDNF treated rats. In saline-treated rats, most of the transplanted cells expressed glial markers, little number of the cells showed neuronal marker NeuN. In contrast, in BDNF-treated rats, a large number of NeuN-positive cells existed in the area adjacent to the cavities, indicating the upregulation in neuronal differentiation of the transplanted NSCs. These findings indicates that BDNF could enhance the neuronal differentiation of NSCs, even administered exogenously.

P245.

SPATIAL AND TIME DEPENDENT DIFFERENTIATION OF NEURAL STEM CELLS TRANSPLANTED IN THE LESION INDUCED BY TRAUMATIC BRAIN INJURY.

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In contrast to in vitro studies, it has been demonstrated that transplanted neural stem cells (NSCs) showed significant glial differentiation and the neuronal differentiation was restricted, suggesting that the temporal and spatial alteration of microenvironments in vivo may strongly influence their differentiation. In the present study, we examined the temporal and spatial pattern of NSCs, transplanted into damaged brain induced by traumatic brain injury (TBI) and determine the optimal timing and sites of transplantation, which showed pronounced neuronal differentiation.

Male wistar rats were deeply anesthetized and cortical contusion was induced in the unilateral sensorimotor cortex by controlled cortical impact device. BrdU-labeled human fetus-derived NSCs (approximately 10×104 cells/ animals) were stereotactically transplanted into three individual sites (contusion core, pericontusional area, contralateral side of cerebral cortex) at 0, 3, 7, 14 days following injury. Transplanted cells survived in all animals. The marked migratory response could be observed in the animals which received NSCs transplantation at 0 and 3 days after injury, however, the majority of the cells showed glial marker, such as vimentin or GFAP. In the animals received NSCs transplantation at 7 days after injury, accumulation of the transplanted cells into pericontusional areas and a large number of cells expressed neuronal marker immunoreactivity, such as NeuN. The most prominent cell accumulation and neuronal differentiation could be observed at which the NSCs were transplanted into pericontusional areas. The present study indicates that differentiation of transplanted NSCs depends on the in vivo microenvironment and prominent neuronal differentiation achieved at subacute phase following injury.

P246.

GABA-A RECEPTOR SUBUNIT ALTERATIONS FOLLOWING TRAUMATIC BRAIN INJURY ARE NORMALIZED BY AN NMDA ANTAGONIST

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Traumatic brain injury (TBI) produces an acute phase of neuronal excitation followed by a chronic phase of depressed neuronal function. TBI-induced elevations in intracellular calcium concentrations ([Ca2+]i) may trigger mechanisms which drive changes in GABA-A receptor protein synthesis and expression. In study 1, Western blot analysis revealed no injury-induced alterations in protein expression for the GABA-A receptor $\beta 3$ subunit at 3h, 24h, or 7 days post-injury. A significant increase in a1 protein was found 24 hours following injury and persisted for at least 7 days. Since the a1 subunit is primarily located on interneurons, this may imply a dysfunctional increase in interneuronal inhibitory tone during the chronic phase of injury. In study 2, pre-injury injections of MK-801 were used to block calcium influx through the NMDA receptor. This treatment normalized $\alpha 1$ protein expression 24h following injury. NMDA-mediated calcium influx may, therefore, be responsible for triggering the cascade that results in increased GABA-A receptor a1 protein expression. These studies indicate that specific subunits of the GABA-A receptor are altered by TBI, these alterations are likely driven by excessive [Ca2+]i, and these changes may ultimately contribute to receptor dysfunction during the chronic phase of injury

P247.

CHANGES OF BENZODIAZEPINE RECEPTORS IN PATIENTS WITH NEUROPSYCHOLOGICAL DEFICITS IN THE CHRONIC STATE AFTER TRAUMATIC DIFFUSE BRAIN INJURY / PET STUDY

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<Background and Purpose> Traumatic diffuse brain injury, including diffuse axonal injury, can cause neuropsychological deficits such as problems with attention, memory, and information processing. Cortical localization of these higher brain functions has been still controversial. Conventional neuroimaging techniques such as MRI (magnetic resonance imaging) and CT (computed tomography) can not show lesions causing these deficits. We have already reported that decrease of cerebral metabolic rate of oxygen (CMRO2) was seen in frontal lobe of these patients. The aim of this study is to clarify the relationship between distribution of benzodiazepine receptors of brain (BZR) and neuropsychological impairment in the chronic state of traumatic brain injury.

<Materials and Methods> Six right-handed patients (18–63; mean 32.5 y.o.) with neuropsychological impairments and without aphasia, agnosia or motor weakness of extremities in the chronic stage (27.6 months) after traumatic brain injury, who had no abnormal lesion on MRI, were included in this study. PET scans were obtained using 11C-flumazenil to evaluate distribution of BZR of brain. The distribution pattern was compared to healthy volunteers (n = 2, 27.5 y.o.). Neuropsychological tests and 15O-gas PET study were also evaluated.

<Results> Two patients with mild dysfunction of memory and attention had no abnormal changes of BZR distribution compared to normal control. On the contrary, BZR distribution in the frontal and parietal lobe relatively decreased in patient with moderate disturbance of memory and attention (n = 2). The other two patients had severe neuropsychological deficits and also had diffuse decrease of BZR in brain. There was a tendency that areas with decreased BZR activity were corresponded to areas with decrease of CMRO2.

<Conclusion> Cerebral BZR distribution in patients with moderate or severe disturbance of neuropsychological function decreased relatively in the chronic stage. This could be a good index of higher brain dysfunction as same as decrease of CMRO2.

P248.

REGIONAL PHYSIOLOGICAL ALTERATIONS IN INHIBITORY SYNAPTIC TRANSMISSION IN FLUID-PERCUSSED MOUSE HIPPOCAMPUS

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Traumatic brain injury (TBI) patients suffer cognitive deficits including impaired learning and memory. We have used the fluid percussion injury (FPI) model of TBI to study the putative mechanism(s) underlying these impairments in mice. Previous studies have revealed regional alterations in hippocampal excitability, i.e., dentate gyrus (DG) hyperexcitability and CA1 hypoexcitability. The present study was undertaken to examine whether changes in tonic inhibitory tone may precipitate alterations in the delicate balance between exctitatory and inhibitory neurotransmission that is crucial for normal hippocampal function. Using visualized slice patch clamp techniques to test the above hypothesis, we recorded miniature inhibitory postsynaptic currents (mIPSCs) in the DG and area CA1 regions of the mouse hippocampus one week post-FPI and compared this activity to that recorded in sham and naive animals. The median mIPSC amplitude was significantly smaller in DG neurons from FPI mice than those recorded in sham and naive animals (-26.81 ± 2.7 pA; -39.04 ± 7.1 pA, FPI and controls, respectively, $p < 0.05$, unpaired t-test). The 50% decay time (T50) of mIPSCs in DG neurons from FPI animals was not significantly different from those from control animals (7.4 ± 1.5; 9.90 ± 2.0 ms, for FPI and control DG neurons, respectively). Conversely, the median mIPSC amplitude in CA1 neurons from FPI mice was significantly larger than that from sham and naive mice (-35.57 ± 0.9 pA; -28.86 ± 2.6 pA, for FPI and controls, respectively, $p < 0.05$). In similar fashion to the DG, mIPSC T50s were not significantly different in CA1 neurons from FPI animals compared to controls (7.75 ± 2.0 and 6.83 ± 1.3 ms for FPI and control CA1 neurons, respectively). These data support our hypothesis that changes in tonic inhibition in FPI mouse hippocampus may account for altered regional excitability.

P249.

PERIPHERAL NERVE TRANSPLANTATION IN THE ADULT CENTRAL NERVOUS SYSTEM: RECONSTITUTED NERVES, ALLOGRAFTS AND THE EFFECTS OF IMMUNOSUPPRESSION

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Peripheral nerve (PN) graft studies in the CNS of injured adult animals usually involve the use of autologous tissue. Clinically however, such an approach may not be optimal due to additional functional deficits that result from harvesting host PN material. We have tested new approaches to CNS repair using PN bridges, including the use of (i) freeze-thawed PN reconstituted with cultured Schwann cells (SCs), (ii) PN allografts and (iii) immunosuppression.

PN was grafted onto the cut left optic nerve (ON) of anaesthetized (halothane) young adult rats. In the reconstituted nerve study, PN was rendered acellular by freeze-thawing and was repopulated ex vivo with neonatal SCs, adult SCs, or adult olfactory ensheathing glia (OEG). These studies were performed in Fischer rats. Regeneration of axons from injured retinal ganglion cells (RGCs) was assessed with retrograde tracing methods three weeks after transplantation. No regrowth was seen in control cell-free nerves or PN reconstituted with neonatal SCs or adult OEGs. In contrast, PN seeded with cultured adult SCs supported RGC axon regrowth. These adult SCs could be either donor- or host-derived.

In allograft PN studies, nerve was taken from Dark-Agouti (RT1a) rats and grafted onto transected ON of Lewis (RT1l) rats. Without immunosuppression there was no RGC axon regeneration into allografts. However in the presence of cyclosporin-A or FK506 (given daily) axon regrowth was seen.

We were surprised to find, in control autograft studies in Lewis rats, that immunosuppression decreased the amount of regeneration into autologous PN grafts. This was a strain-specific effect; daily intraperitoneal injections of cyclosporin-A or FK506 in Fischer rats with PN-ON autografts resulted in significantly increased regrowth. These data show that there are potential alternatives to using autologous PN tissue and highlight the importance of knowing the immune status of an animal (or patient) when attempting to repair CNS injuries.

P250.

IMPROVED NEURAL REMODELING AND ENHANCED NEURAL STEM CELL PERSISTENCE AMIDST CASPASE-3 INDEPENDENT APOPTOSIS IN ADULT BAX DEFICIENT MICE FOLLOWING CONTROLLED CORTICAL INJURY

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Numerous studies have implicated apoptosis as one mechanism for cell death seen following traumatic brain injury (TBI). More recently, it has been shown that neural stem cells are involved in cortical and hippocampal remodeling induced by TBI. To examine the role of apoptosis in this process we have analyzed TBI in mice lacking the proapoptotic gene, Bax. We find that adult Bax knockout mice have similar numbers of apoptotic cells within the hippocampus immediately following controlled cortical injury when compared to wildtype littermates. However, whereas apoptosis in wildtype animals occurs via a caspase-3 mediated process, in Bax knockout mice caspase-3 activation is totally absent. We also observe significant tissue preservation in Bax deficient animals three weeks following injury. Our data demonstrate that the apparent improvement in neural remodeling seen by attenuating Bax signaling is likely due to the consequence of increased numbers of neural stem cells in the adult and not due to inhibition of apoptosis. We further provide support for the emerging notion that apoptosis following injury can occur via parallel pathways that are caspase independent.

P251.

RESPONSE OF THE SUBVENTRICULAR ZONE TO TRAUMATIC BRAIN INJURY

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The brain's ability to produce neurons throughout the lifetime of an individual (neurogenesis) is an important phenomenon to pursue in the treatment of brain injury. Previous studies in rat demonstrated that the number of cells within the subventricular zone (SVZ) of the adult brain increased as a result of degeneration in the brain. This study aims to examine this phenomenon following a controlled cortical impact (CCI) in adult mice. Adult male mice were subjected to a CCI over the forelimb region of the sensorimotor cortex. Mice were perfused 3 days post-injury, and coronal sections (30 μm) of the brain were taken and stained using cresyl violet. Digital pictures of the SVZ were obtained at 40X from sections containing a cortical injury or from corresponding sections in control mice. Using NIH Image the thickness of the SVZ was measured at different levels in both hemispheres. Results demonstrate a significant difference in the overall thickness of the SVZ in injured mice, when compared to non-injured mice (CCI = 22 $\pm$ 1mm; control = 15 $\pm$ 1 μm; $p < 0.05$). This increase was significant in the dorsal SVZ, but not in the ventral-striatal SVZ, and was limited to the SVZ ipsilateral to the injury. There was no significant increase in the thickness of either the dorsal or the ventral SVZ on the side contralateral to the injury in injured mice. These results indicate that the SVZ responds to a CCI and that this response is limited to the dorsal SVZ ipsilateral to the injury. Future studies will examine the nature of this increase by examining cell number and type as well as the migratory ability and functional roles of these cells.

P252.

DECOMPRESSIVE SURGERY FOR SEVERE TRAUMATIC BRAIN INJURY, EXPERIENCE IN HAMAD MEDICAL CORPORATION, DOHA- QATAR

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Objective: This study examines the role of non-traditional decompressive surgical procedures in treating severe Traumatic Brain Injury (TBI) with associated life-threatening intracranial hypertension. In addressing the control of the latter, the procedures, comprise temporary removal of the craniotomy bone flap with or without frontal or temporal lobectomy. Existing acute epidural, subdural or intracerebral haematomas would be removed at the same time.

Methods & Results: We retrospectively analysed the data of 529 cases of severe TBI treated in Hamad Medical Corporation in Doha-Qatar, during the period between January 1997–December 2001. The AANS Management Guidelines were followed in treating the patients who were all evacuated promptly to the hospital where the Neurosurgical team was involved in their management from the time of arrival to the Accident & Emergency Department. Out of the 82 patients who had surgical treatment (15.5% of the total), 48 underwent a decompressive surgical procedure; 27 cases had removal of the craniotomy bone flap, 10 cases had frontal or temporal lobectomy and 11 cases had both procedures. They were all males with an age range of 5 years—59 years (average; 27.1). The majority (30/48 = 62.5%) scored below 9 on Glasgow Coma Scale with 42 cases (87.5%) falling within Newcastle Outcome Prediction Groups 5 and 6 (poor groups). On follow up assessment using the Glasgow Outcome Scale, 64% of the patients were found to have had a favourable outcome (Good Recovery or Mild Disability) at 3 months to one year post injury with a 29% Mortality. This compared favourably to the predicted outcome.

Conclusion: Decompressive surgery, in the way performed in our unit, was found useful in treating life-threatening post traumatic Intracranial Hypertension as it significantly improved outcome in those selected patients whose conditions were found to be refractory to standard therapeutic modalities.

P253.

GENDER IN RELATION TO OUTCOME OF MILD-TO-MODERATE TRAUMATIC BRAIN INJURY

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Males sustain traumatic brain injury (TBI) nearly twice as frequently as women, although women are reportedly at greater risk for developing psychiatric disturbances after TBI, which may complicate recovery and outcome. Gender differences for psychiatric disorders and outcome after mild-to-moderate TBI (MTBI) were assessed 6-months postinjury, using the Structured Clinical Interview for DSM-IV, Glasgow Outcome Scale-Extended (GOS-E), Short Form-36 (SF-36), and Community Integration Questionnaire (CIQ) in 55 female and 111 male patients. The groups were comparable for Glasgow Coma Scale score, age, education, and injury severity. Rates of posttraumatic stress disorder (PTSD), depression, and postconcussion disorder were at 3%, 8%, and 9% in males and 18%, 20%, and 16% in females, respectively. Females met DSM-IV-criteria for PTSD ($p = 0.0001$) nearly six times as often as males, and DSM-IV-criteria for depression ($p = 0.040$) twice as often. Within GOS-E outcome categories, a greater proportion of females (13/19, 68%) than males (11/31, 35%) functioned at the lower level in the moderate disability ($p = 0.04$) category; no differences were noted within the good recovery category. Assessment of resumption of previous role activities (CIQ) showed that females functioned at lower levels than males for Productivity ($p = 0.013$) and Social functioning ($p = 0.037$), but had better resumption for Home functioning ($p < 0.0001$). On the SF-36, females reported their mental health functioning as worse ($p = 0.035$) than males. In summary, neuronal damage associated with MTBI may compromise the patient = s capacity for effective stress management, which may be manifested more in females and contribute to their development of psychiatric disorders and worse functional outcome.

P254.

IN VIVO AND IN VITRO EVIDENCE OF CYTOSKELETAL AND SYNAPTIC PROTEIN ALTERATIONS FOLLOWING REPEATED MILD TBI

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Neuronal cytoskeletal alterations in the cortex and brain stem following single mild TBI (sMTBI) and repeated mild TBI (rMTBI) have been well-documented. In contrast to sMTBI, rMTBI-related cognitive deficits have been shown to be more closely associated with altered hippocampal function. Changes in cytoskeletal structure are often associated with synaptic alterations (e.g., learning). However, alterations in synaptic proteins have not been investigated following rMTBI. We used in vivo and in vitro models of rMTBI to characterize cytoskeletal and synaptic protein alterations in the hippocampus following rMTBI.

Immunohistochemistry (IHC) was used to assess protein distribution, while immunoblot (IB) was used to assess protein levels in both models. IHC results showed decreased distribution of MAP2, particularly within the CA2 region. In contrast, IB expression of denatured MAP2 and NF200 showed increased levels of these proteins. IHC distribution of two synaptic proteins, synaptophysin and synaptogryn, showed overall decreased staining, but increased staining in neurons with decreased somatic distribution of MAP2. These results suggest a compensatory response of cytoskeletal and post-synaptic proteins to stabilize somato-dendritic architecture that may contribute to rMTBI-related learning and memory deficits through decreased plasticity. Supported by: BSCIRTF 2001, NWO-MW, NWO-PIONIER, NIH R01 NS39091, R01 NS40182, US Army DAMD17-99-1-9565.

P255.

CEREBRAL BLOOD FLOW AND BRAIN NITRIC OXIDE LEVELS AFTER TRAUMATIC BRAIN INJURY, HEMORRHAGE AND HYPERTONIC ARGININE RESUSCITATION

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Traumatic brain injury (TBI) reduces cerebral blood flow (CBF)(1,2) and hypotension after TBI increases mortality (3). The nitric oxide (NO) synthase substrate L-arginine or hypertonic resuscitation improves CBF after TBI and hypotension (2,4) but the effects of resuscitation with hypertonic L-arginine have not been studied.

Rats were anesthetized, intubated and ventilated with isoflurane in O2:air. Rats were prepared for TBI (2), laser Doppler flowmetry (2) and measurement of brain tissue NO levels using an ISO-NO electrode system (5). Rats ($n = 6$ per group) were randomly assigned to receive sham, moderate (2.0 atm) or severe (3.0 atm) TBI and hemorrhage to mean arterial blood pressures of 60 mmHg for 45 minutes and then resuscitation with 0.9% NaCl or hypertonic L-arginine (100 or 300 mg/kg L-arginine in 1800 mEq hypertonic saline). CBF and brain tissue NO levels were measured for 4 hrs after resuscitation.

CBF remained constant after sham-injury but decreased and remained below baseline after saline treatment. In the hypertonic arginine-treated rats after either moderate or severe TBI, CBF returned nearly to baseline during hypertonic arginine infusion and remained higher than CBF in the saline treated rats. Brain NO levels remained constant in the sham-injured rats but decreased after TBI and hemorrhagic hypotension. Hypertonic arginine increased NO levels in the severe TBI and hemorrhage group. Neither saline nor hypertonic arginine improved NO levels after moderate TBI and hemorrhage.

These results suggest that hypertonic arginine resuscitation improves CBF through mechanisms that may be unrelated to brain tissue NO levels, especially after moderate brain trauma.

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P256.

CYTOSKELETAL PROTEIN DEGRADATION AND NEURODEGENERATION EVOLVES DIFFERENTLY IN MALES AND FEMALES FOLLOWING EXPERIMENTAL HEAD INJURY

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The resulting neuropathological degeneration that occurs following a traumatic brain injury (TBI) is a consequence of both immediate and secondary neurochemical sequela. Proteolysis of cytoskeletal proteins, triggered by calcium-mediated events, is believed to be a particularly significant contributor to TBI-induced neuronal death. The objectives of this study were to 1) quantitatively describe, over a post-traumatic time course, the relationship and mechanisms of cytoskeletal degradation (Western blot) and neurodegeneration (silver staining) in male and female mice following a moderately severe weight-drop head injury; 2) to evaluate gender differences in the response to TBI and; 3) to examine the potential therapeutic window for future pharmacological treatment strategies. In male and female mice, we report a close correlation in the time courses of neurofilament M (NFM) protein degradation and α-spectrin breakdown products (SBDP 150 and 145) with the peak magnitude of neurodegeneration, as quantified by silver staining. Evidence from the increased patterns of SBDPs suggests that both calpain and caspase-3 are involved. In general, males incurred peak protein degradation and neurodegeneration within 3 days after injury, while in females, this did not occur until as late as 14 days. The neuroprotective effects of estrogen are believed to be key factors in the superior outcome of female vs. male mice following TBI. In mice, the therapeutic window of opportunity for pharmacological intervention aimed at limiting cytoskeletal degradation might be as much as 24 h following injury. Evidence of a protracted time course of cytoskeletal degradation, especially in females, suggests a potential for an extended treatment-duration following TBI.

P257.

TIME COURSE OF BRAIN TISSUE HYPOXIA IN THE PENUMBRA REGION AFTER TRAUMA.

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Brain tissue oxygen tension (PbtO2) probes measure oxygen in only a small volume of tissue. Debate continues about whether such monitors should be inserted after brain injury in "normal" regions or in tissue that is "at risk", e.g., adjacent to traumatic lesions. This study investigated PbtO2 measurements in the penumbrae of traumatic intracranial lesions (TICLs).

Data from 156 patients were analyzed for transient decreases in PbtO2 below 10 mm Hg without an identifiable cause, i.e., CPP < 60 mm Hg, SaO2 < 95%, or end-tidal CO2 < 25 mm Hg.

In 120 patients (77%), PbtO2 probes (Licox, Integra NeuroSciences) were inserted in the penumbrae of lesions: near contusions in 58, in brain underlying evacuated subdural hematomas (SDHs) in 47, or in brain underlying evacuated epidural hematomas (EDHs) in 15. In the remaining 36 patients, probes were inserted in grossly normal tissue. Twenty-five patients exhibited transient ischemic episodes beginning 25 ± 15 hours after injury (range 5–61 hours) and lasting 19 ± 19 hours (range 4–88 hours). During these episodes, PbtO2 decreased from 27 ± 8 mm Hg to 6 ± 3 mm Hg and subsequently returned to baseline. Ischemic episodes occurred only in penumbrae (12 near contusions and 13 under evacuated SDHs). MAP, ICP, EtCO2, SpO2 and SjvO2 did not change during the episodes. In all 25 patients, CT scans revealed hypodensities around the PbtO2 probes despite initially normal densities. Six months postinjury, 73% of patients with transient PbtO2 decreases had poor outcomes on a dichotomized Glasgow Outcome Scale. The mortality rate was 36%.

The occurrence of regional ischemia in the penumbra of TICL later after injury is a relatively common phenomenon and may contribute to poor outcome. Placing PbtO2 probes near contusions or in brain underlying evacuated SDHs may improve detection of transient regional ischemia.

P258.

THE INFLUENCE OF SOCIAL AND CULTURAL FACTORS ON THE INCIDENCE AND SEVERITY OF TRAUMATIC BRAIN INJURY- A COMPARATIVE STUDY OF TRAUMATIC BRAIN INJURY IN DOHA, QATAR AND NEWCASTLE UPON TYNE, UK

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Objectives: This study evaluates the geo-social factors that contribute to the incidence and outcome of the severely head injured patient by the first direct comparison of head injury outcome between a western centre and that of a developing country.

Method: A retrospective analysis of head injuries admitted to two centres (Doha and Newcastle) over a five year period (January 1997–December 2001) was carried out comparing demographic details, aetiology, severity of injury and management outcome. The mode of participation of victims was identified and the geo-social factors that contributed to the aetiology and severity of head injury were evaluated in detail. Data analysis was with Spss version 11.

Results: There was a significant male preponderance in Doha (12m:1f) compared with Newcastle (2.7m:1f). The peak age group in Newcastle for all head injuries was 0–10yrs, whereas in Doha the peak age was 20–30yrs. Road traffic accident accounted for nearly 70% of all head injuries in Doha whereas falls accounted for nearly 50% of all head injuries in Newcastle. Overall the good outcome was 74% in Doha and 81% in Newcastle (NS). The proportion of patients presenting in coma and mortality (26%) in Doha was more than twice that in Newcastle. A recent change in the law in Qatar allowing female drivers has not significantly increased the incidence of head injury.

Conclusion: The combination of powerful cars and good roads with a young population has resulted in an excessive mortality in Doha compared with Newcastle. Recent changes in the law in Qatar have not significantly altered the incidence of head injury related to road traffic accidents. The high male preponderance in Qatar reflects the relatively protected role women enjoy in this society.

P259.

HEALTH-RELATED QUALITY OF LIFE AND POSTCONCUSSIONAL DISORDER SIX MONTHS FOLLOWING MILD TO MODERATE TRAUMATIC BRAIN INJURY

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Few studies have been conducted investigating Postconcussional Disorder (PCD) using the criteria set forth in the Diagnostic and Statistical Manual, 4th Edition (DSM-IV). This study used the DSM-IV criteria for PCD to examine patients' perceptions of health-related quality of life (HRQOL) in a sample of patients with mild (N = 127) to moderate (N = 39) traumatic brain injury (TBI) six months post-injury. HRQOL was measured using the SF36 composite scores and subscales. Out of 166 patients, 19 met DSM-IV criteria for PCD. The PCD and no-PCD groups did not differ on any demographic variable. Those in the PCD group were more often involved in motor vehicle accidents (MVA) than those in the no-PCD group ($p < 0.04$). The Injury Severity Score (ISS) was significantly lower ($p < 0.03$) in the PCD group vs. the no-PCD group. The patients with PCD reported significantly poorer HRQOL on the Mental and Physical composite scales ($p < 0.001$) of the SF36. PCD patients also reported significantly poorer HRQOL on all physical and mental subscale scores ($p < 0.001$). Patients with PCD had poorer global outcome as measured by the Extended Glasgow Outcome Scale (GOS-E; $p < 0.0001$). These results suggest that PCD symptoms are reported at 6 months post-injury and represent a source of significant impairment both from the patient's perception of their HRQOL and a more objective measure of global outcome. Early interventions to reduce the severity of PCD symptoms may improve both HRQOL and global outcome following mild to moderate TBI.

P260.

SCULL BASE MISSILE INJURIES

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Missiles used in war conflicts cause extensive destructions of the scull and brain due to their higher kinetic energy. Not even directly but kinetic energy transfer trough the scull can cause "discontinuous" fracture at the distance from entry wound and not in continuity with the fracture of the vault. These wounds that traverse paranasal sinuses and destroy skull base are likely to be contaminated.The rates of the CNS infection and CSF fistulas would be expected to be higher than in other penetrating craniocerebral missile injuries. During tretament of such wounds we stress necessity of early diagnosis of CSF fistulas, and early operation with watertight dural closure.

The record of 312 casulties with missile injuries of the brain have been analyzed in the period of six years, with attention to the skull base fractures and the comlications as CSF fistulas and infections. 45 of them developed CSF fistula, 15 (33%) on the wound side, 23 (51%) presented as rhinoliqurrhoea and 7(15%) as otoliquorrhoea. 6 patients (13%)developed infectious complications. 15 developed facio-orbito-cranial injuries with scull base fractures. 6 of them (40%) died, 3 (20%)developed CSF fistula and 2 (13,3%) meningitis.

The rate of infection did not exceed the rate associated with other craniocerebral missile injuries, but the rate of CSF fistulas was twice as high.Scull base missile injuries are specific neurosurgical entity because of the high rate of CSF fistulas. They require emergency operation attempting the dura reconstruction of the scull base. With presented strategy and operative approach, the incidence of the infectious complications in scull base missile injuries remains low.

P261.

HYPERBARIC OXYGEN THERAPY IN HEAD INJURY: AN ANIMAL MODEL OF BRAIN CONTUSION

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Background and Purpose: Cerebral contusions(CC) represent therefore one of the most frequent traumatic lesions and the most common indication for secondary surgical decompression. Perilesional ischemia has been commonly proposed as one of the responsible mechanisms of deterioration. The purpose of this study was to design a reproducible and reliable model of cerebral contusion in rats, investigate the physiology of perilesional secondary brain damage and evaluate the value of hyperbaric oxygen therapy (HBO) in the treatment of these lesions.

Methods: Four groups of 5 Sprague-Dawley rats each were included in this study. All animals were prepared and operated upon under general anesthesia induced by barbiturates. A 2 mm burr hole was drilled in the parietal region and a hollow screw connected to a vacuum apparatus was attached in. A negative pressure of 0.47 ATA was applied to the unexposed cortex for 10 seconds. Animals were sacrificed after 4 days. Histological sections showed localized gross tissue loss in the cortex at the injury site, along with hemorrhages. In all cases, the severity of secondary brain damage was assessed in successive perilesional layers by numbering cells stained by Tunel and Caspase 3 preparations. The study protocol: group 1—control, group 2—HBO initiated 2 hours after injury and thereafter twice a day for 4 days (2.8 ATA for 90 min); group 3—perioperative hypoxia (POH); group 4—POH and HBO.

Results: Vacuum injury produced hemorrhagic lesions very close to traumatic CC in clinical situation. The size and morphology of the lesion at the vacuum site proved to be reproducible. At group 1, the perilesional region was characterized by a large number of apoptotic cells enhanced by Tunel and Caspase (12.24% of the cells in a distance of 0.5 mm from the necrotic area). In group 2, there were less apoptotic cells in the perilesional area (4.7%, $p < 0.0001$). Hypoxia showed to worsen the outcome (31.75%, $p < 0.001$). As in group 2, HBO therapy decreased the extent and severity of secondary brain damage (9%, $p < 0.003$).

Conclusions: Our study shows that the vacuum model of brain injury is a reproducible model of cerebral contusion and further helps understanding mechanism of secondary extension that may account for clinical deterioration in some patients. Our results suggest the HBO may limit the extent of secondary brain damage in CC and suggest further experimental studies.

P262.

PREDICTORS AND INCIDENCE OF POSTTRAUMATIC SEIZURES IN CHILDREN AFTER BRAIN INJURY

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In this study we evaluate the incidence of early and late seizures after head injury in children under 18 years admitted to our hospital during 1995 and 2001 (6 years). The purpose was to find out factors correlating with a high risk of developing posttraumatic seizures. In our study 10.9% of the children developed seizures whereas 42.3% had early (during the first week) and 57,6% late (after the first week) seizures. Factors that showed a significant higher incidence for the development of seizures and should alert the physician, were the severity of the head trauma and a GCS of 3–8. These factors correlates with those of other studies. In contrast to many studies we found out that the incidence of posttraumatic seizures was significant higher in patients older than 12 years (12–16 and 12–18). Most of the late seizures were nonconvulsive diagnosed on a snapshot-EEG during the follow-up examination of the patients. We suppose that the EEG-examination in head injured children is important to find out this patients with epileptic potentials without clinical symptoms like convulsions because the epileptic changes of the EEG could worsen the diagnosis and clinical outcome of the children in accordance to studies performed in the adult population. We do not recommend the administration of antiepileptic drugs after the occurrence of a first early or late convulsion because no one of our patients had a second one. The prophylactic use of anticonvulsants in children who show risk factors of developing seizures is recommended in other studies and was not subject of our study.

P263.

TRAUMATIC BRAIN INJURY AND HEMORRHAGIC HYPOTENSION INCREASE POST-SYNAPTIC ZINC ACCUMULATION IN RATS

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Accumulation of ionic zinc (Zn2+), which contributes to neuronal injury after experimental cerebral ischemia (1,2) and weight-drop TBI,(3) can be demonstrated by staining with N-[6-methoxy-8-quinolyl]-para-toluenesulfonamide (TSQ), a fluorescent dye with a high specificity for Zn2+.(1) We hypothesized that fluid percussion TBI would increase Zn2+ accumulation and that subsequent hemorrhagic hypotension would cause further accumulation.

Rats were anesthetized (1.5% isoflurane), prepared for fluid percussion TBI,(4) and randomly assigned to 1 of 4 groups (n = 6/group): sham TBI; hemorrhage to a mean arterial pressure of 60 mm Hg for 45 min followed by reinfusion of shed blood; moderate TBI (1.8 atm) or TBI plus hemorrhage and reinfusion. Six hours after TBI, rats were reanesthetized and decapitated. The brains were frozen and 20 um sections were stained with TSQ. TSQ-positive neurons were counted by a blinded observer.

Few TSQ-positive neurons were observed in sham-injured rats and rats subjected only to hemorrhage. TBI alone significantly increased TSQ-positive neurons in the cerebral cortex, CA3, hilus, and dentate gyrus. The combination of TBI plus hemorrhagic hypotension significantly increased TSQ-positive neurons in comparison to TBI alone in the cerebral cortex, CA3, hilus, and dentate gyrus.

These results suggest that neural injury after TBI is mediated in part by Zn2+ accumulation that is exacerbated by post-TBI hemorrhagic hypotension. Reducing Zn2+ accumulation may reduce neuronal injury after TBI.

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P264.

ANALYSIS OF GENE EXPRESSION FOLLOWING ACUTE SPINAL CORD INJURY

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Gene expression patterns offer a definable criterion on which to base the functional relevance of specific proteins to disease pathology. Our overall hypothesis is that genes whose expression is significantly/reproducibly altered following spinal cord injury (SCI) represent important candidates for intervention strategies as well as in the elucidation of mechanisms critical to cell injury/death pathways associated with SCI and other CNS disease states. We have employed the fluorescence-based quantitative method for the real time detection of PCR amplification (real time PCR) to identify gene expression patterns affected by SCI. We have selected gene families that may have a relevant impact on SCI for analysis and have evaluated the expression of over 90 known genes at acute time points post SCI in an established rat spinal cord contusion model. These families/groups include the Ephrin family of tyrosine kinase receptors and ligands; insulin-like growth factors, receptors and binding proteins; oncogenes and tumor suppressors; cell cycle regulators; interleukins and interferons; DNA binding proteins, transcription factors and regulators and extracellular cell signaling and communication proteins (i.e. growth factors, cytokines, chemokines). The gene expression patterns were evaluated at 12 and 24 h post injury compared to sham controls. 49 genes had a greater than 2 cycle number difference between at least one injury time point and control. 12 genes exhibiting the largest change with a 5 or greater cycle number difference compared to controls were also analyzed at 2 and 6 h post injury. Of the families and groups presented in this study, the most dramatic gene expression changes are observed 12 h post injury from chemokines such as the macrophage inflammatory proteins 1a and 1b. These gene expression data are evaluated within cellular pathways and gene families to determine whether gene expression patterns collectively are relevant to SCI and other disease states.

P265.

EXPRESSION OF SEMAPHORIN3A IN THE RAT SPINAL CORD TRANSECTION MODELS

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Numerous studies have provided evidences that axonal growth and guidance is regulated by attractive and repulsive molecules present in the extracellular microenvironment of the growth cone. Semaphorin3A (Sema3A) has been described to function as a chemorepulsive molecule in directing growing axons to their target. However, its role in the spinal cord injury has not been well characterized. We demonstrate here that Sema3A is acutely up-regulated after transection of the rat spinal cord.

Material and Methods: 8weeks Wistar rats were subjected to spinal cord transection and they were sacrificed 6h, 12h, 24h, 3d, 1w, 2w, and 1m after injury. Digoxigenin labeled in situ hybridization (ISH) for Sema3A and immunohistochemistry for Neu-N were simultaneously done in each cryosection. We also performed double immunofluorescence study for Sema3A and Neu-N.

Results: In ISH, positive signal for Sema3A was detected in the gray matter near the transected site 6h after injury and sustained in the same level 24h after injury. 3 days after injury, signal intensity gradually decreased and only a slight signal was detected 1m after injury. Sema3A mRNA and protein expressing cells coincided with neurons which were immunoreactive for Neu-N antibody.

Discussion: In the present study, Sema3A was acutely up-regulated in the neurons near the transected site. The time course and localization of Sema3A expression in spinal cord transection model is similar with that in middle cerebral artery occlusion model as previously reported.

P266.

"DECOY" INTERVENTION IN NF-KAPPA B ACTIVATION AFTER SPINAL CORD CONTUSION INJURY

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Spinal cord injury triggers an inflammatory response that may be responsible for the observed pathophysiology. An early lesion event is transient and robust increases in IL-1 beta levels, which contribute significantly to augmentation of cyclooxygenase 2 (COX-2) and the inducible form of nitric oxide synthase (iNOS). Both COX-2 and iNOS stimulate production of reactive oxygen species (O2., OH., and NO.). IL-1 beta stimulates NF-kappa B activation, the transcription factor known to up regulate COX-2 and iNOS translation. Using injections of "decoy" oligonucleotides at the site of contusion containing the consensus DNA sequence found in the COX-2 promoter region, we found prompt, dynamic, and transient uptake of labeled "decoys" into both the cytoplasm and nuclei of resident cells. Further, we showed a selective modulation of NF-kappa B protein binding, as seen by electrophoretic mobility shift assay, as well as selective effects on iNOS and COX-2 expression at the site of injury. These data are consistent with the hypothesis that NF-kappa B transcriptional regulation of these proteins represents elements in the pathophysiology and recovery of mammalian spinal cord after contusion injury. Supported by NINDS Grant NS-39161 and Shriners Hospital Grant 8710.

P267.

EVALUATION OF A FORCEPS COMPRESSION MODEL OF SCI IN RATS.

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A reliable, high throughput animal model of spinal cord injury (SCI) would greatly facilitate the assessment of the numerous compounds with potential for reducing secondary damage or enhancing recovery mechanisms. We have evaluated a forceps compression model of SCI in rats (based on Gruner et al., 1996) that produces a graded injury as assessed by functional and anatomical outcome measures. Following laminectomy of T9/T10 vertebrae, the spinal cord of adult rats, anesthetized with isofluorane, was injured by a brief compression to a space of 0.9, 1.3 or 1.7 mm using modified coverslip forceps. The whole operation required approximately 15 minutes from incision to closing. Control animals received a sham injury. Compression distance correlated with open field locomotor behavior, at 12 weeks post-SCI: the 0.9 mm compression produced a moderate, yet more severe injury than the 1.3 mm compression. The 1.7 mm compression resulted in a mild injury that recovered to approximately the behavioral score of sham injured animals, by 12 weeks post-injury. Histological analysis reveals that the compression injury produces tissue cavitation and degeneration characteristic of spinal cord contusion injury. In separate experiments, lesion volumes were determined at 24 hours post-SCI from spinal cord tissue sodium and potassium ion measurements using atomic absorption spectrophotometry (Young, 1992). Lesion volumes were found to correlate to injury severity in a graded manner. The forceps compression model appears to be a rapid and reliable method to induce graded spinal cord injuries in vivo that will enhance the capacity and ability to test, assess and identify agents with therapeutic benefits in SCI.

P268.

EARLY ANTI-INLFLAMMATORY TREATMENT ATTENUATES NEUROFILAMENT DEGRADATION AFTER SPINAL CORD INJURY.

D.R. Marsh, E.F. Hamilton, G.A. Dekaban and L.C. Weaver. (Robarts Research Institute, London, Ontario CA).*

The pathogenesis of spinal cord injury (SCI) involves a series of responses resulting in further destruction of nervous tissue after the primary injury. For example, lipid peroxidation and calpain proteolysis, are exacerbated by the immune response and cytokine-stimulated macrophages. Previously, we correlated improved autonomic and locomotor function with white matter sparing after an early anti-inflammatory treatment with an antibody to the aD subunit of b2 integrin. This attenuates leukocyte infiltration into the injured cord by blocking the interaction between vascular adhesion molecules and cell surface b2 integrins. We determined the effect of the aD-antibody treatment alone, or in combination with methylprednisolone (MP), on neurofilament degradation, gliosis and production of transforming growth factor (TGF)-b1. After SCI, TGF-b1 initially has pro-inflammatory actions, but by 14 days post-SCI its role becomes more neuroprotective, inhibiting proteases and cytokine/cytotoxin secretion from macrophages. Rats underwent severe clip-compression SCI at the 4th thoracic segment followed by saline, anti-aD, or anti-aD/MP treatments at 2, 24, and 48 hours. Western blot analysis of neurofilament (NF200) demonstrated a major loss of protein in the lesion at 7 and 14 days post-SCI compared to the same segment in un-injured cord. Anti-inflammatory treatment markedly reduced this loss, indicating decreased calpain-mediated degradation of NF200. Northern blot analysis of total RNA extracted from uninjured cord and lesion 7 days post-SCI revealed a significant increase in TGF-b1 mRNA levels after SCI. TGF-b1 protein, quantified by ELISA, increased from 7.4 ± 3.4 pg/mg total protein in uninjured spinal cord to 42.8 ± 11 and 28.5 ± 6.0 in the lesion at 7 and 14 days post-SCI, respectively. TGF-b1 protein levels were not altered by the anti-inflammatory treatment. In summary, anti-inflammatory treatment suppresses degradation of neurofilament protein, indicating marked neuroprotection. This effect was not mediated by modulating the changes in intraspinal TGF-b1 protein levels after SCI. Support: ICOS Corporation and Ontario Neurotrauma Foundation.

P269.

EARLY SELECTIVE ANTI-INFLAMMATORY TREATMENT BLOCKS THE DEVELOPMENT OF CHRONIC PAIN AFTER SPINAL CORD INJURY.

LC Weaver, YH Chen, JC Bruce, MA Oatway and GA Dekaban. (Spinal Cord Injury Laboratory. BioTherapeutics Research Group, Robarts Research Institute, London, Ontario CA).*

Chronic tactile allodynia is a frequent painful complication of spinal cord injury (SCI) with poorly understood mechanisms, likely involving plastic changes within the injured cord and loss of descending inhibitory pathways. Secondary damage after SCI can impact on these changes by causing death of axons and second order neurons. We proposed that a selective anti-inflammatory treatment, delivered during the first 48 hr after SCI, would minimize the development of this chronic pain. A monoclonal antibody (mAb) to the aD subunit of the B2 integrin of leukocytes was used to block early macrophage/neutrophil infiltration into the injured cord. A clinically-relevant moderate clip-compression SCI in rats was used to generate an incomplete thoracic (T12) lesion. In allodynia testing sessions, the dorsal trunk or plantar hind paws were probed ten times using 15mN Semmes Weinstein hairs. Rats were acclimated to a testing chamber and tested for allodynia prior to SCI; no behavioural responses suggesting pain were elicited. Rats were tested again at two, three and four weeks after SCI. At these three periods, stimulation of the trunk appeared noxious in untreated rats (n = 7) in response to 3 ± 1, 5 ± 1 and 6 ± 1 of 10 stimuli, shown by flinching, escaping and/or vocalizing. The mAb-treated rats (n = 8) responded only to 0.7 ± 0.5, 3 ± 1 and 4 ± 1 of 10 stimuli. Painful responses to paw stimulation appeared as abrupt withdrawal, licking or shaking of the paw and vocalization. At the three testing periods, the untreated rats responded as if 1 ± 0.4, 3 ± 1 and 5 ± 1 of 10 stimuli were noxious. The mAb-treated rats responded only to 1 ± 0.3, 1 ± 0.4 and 2 ± 0.3 of 10 stimuli. The mAb treatment also improved locomotor BBB scores from 6 ± 0.1 to 10 ± 0.3 at four weeks after SCI. In conclusion, selective inhibition of the early inflammatory response can reduce disabling chronic pain after SCI. Support: Ontario Neurotrauma Foundation and ICOS Corporation.

P270.

EVALUATION OF CONDITIONS FOR CALPAIN INHIBITON IN THE RAT SPINAL CORD: EFFECTIVE POSTINJURY INHIBITION REQUIRES INTRASPINAL MICROINJECTION

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Calpains (calcium-activated cysteine proteases) are strongly implicated in the secondary damage that follows contusion injury to the spinal cord. Calpains are activated within a few minutes following injury and their elevated activity persists for 24h, thereby providing a reasonable window of opportunity for postinjury inhibition. Previous studies demonstrated decreased axonal damage and neurofilament proteolysis with postinjury intravenous administration of relatively low concentrations of the calpain inhibitors leupeptin, E-64-D, and calpeptin. We sought to determine if conditions under which calpain inhibitors were administered in previous studies resulted in effective calpain inhibition, and to identify conditions that result in significant calpain inhibition following spinal cord injury. Contusive spinal cord injury was produced in female Long-Evans rats using the NYU impactor at the 12.5–25 mm height setting. The results demonstrate that intravenous administration of 1mg/kg E-64-D or 250 μg/kg calpeptin does not inhibit total calpain activity in the rat spinal cord, measured using a BODIY-FL labeled casein assay. Intravenous 20 mg/kg MDL28170 resulted in mild but significant calpain inhibition and a modest decrease in the proteolysis of calpain substrates a-spectrin and MAP2. Intraspinal microinjection of 50 nmoles MDL28170, either 30 min prior to or 20 min following contusion injury, resulted in a more robust inhibition of total calpain activity and significant attenuation of a-spectrin breakdown and MAP2 proteolysis. The calpain inhibition was within 2h after drug administration, but was still evident at 48h. Together, the results demonstrate that, using currently available calpain inhibitors such as MDL28170, direct microinjection is necessary to achieve the drug concentration required for effective calpain inhibition and to decrease the injury-induced proteolysis of calpain substrates.

P271.

TRANSPLANTATION OF NEUROTROPHIN-EXPRESSING FIBROBLASTS INTO CHRONIC CONTUSION CAVITIES

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Chronic contusion injuries are characterized by a cystic cavity surrounded by a spared rim of white matter. Damaged axons with retraction bulbs persist at the cavity edge and some regenerative growth occurs even 3–6 months after SCI (Hill et. al., 2001). Although most treatments for SCI focus on the acute pathology, it is possible that fibers persisting chronically at the lesion edge could be a therapeutic target. Few studies have intervened chronically, but there is evidence that application of neurotrophins to chronic injuries promotes survival and regeneration of supraspinal neurons (Grill et. al., 1997; Ye & Houle 1997; Houle and Ye, 1999). In addition, transplanted genetically-modified fibroblasts have been shown to improve behavior and enhance fiber outgrowth when applied to acute lesions (Liu et. al. 1999). Here we report the results of 2 studies in which fibroblast were transplanted into long-term stable lesions (8–9 weeks post SCI) and behavioral recovery and axonal growth were examined for 10–14 weeks following transplantation. In an initial pilot study animals received transplants of control fibroblasts (n = 5) or fibroblasts that expressed BDNF and NT-3 (n = 6). Animals in the pilot study improved in open field locomotion (mean BBB score 11.2 pre-transplantation, 13.7 post transplantation) following transplantation of fibroblasts (with or without neurotrophin expression); this result was not replicated in a subsequent comprehensive double-blind study (n = 50). These studies show successful transplantation of cells into chronic contusion sites in the rat with long-term cell survival and fiber growth into the transplants, suggesting that transplantation may be a realistic option for the treatment of chronic spinal cord injuries. (Support: ISRT, NS 38079)

P272.

DELAYED GRAFTING OF FETAL SPINAL CORD TISSUE ENHANCES EARLY GRAFT SURVIVAL AND DEVELOPMENT IN THE INJURED ADULT RAT SPINAL CORD.

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When placed into acute lesions. intraspinal grafts of fetal spinal cord (FSC) tissue exhibit a dramatic attrition of donor cells by 4d post- transplantation (PT). Surviving cells (i.e.. presumptive stem cells and lineage-restricted neuronal and glial precursors) then rebound to give rise to lesion-filling tissue masses. This loss of tissue may limit the extent of early host-graft interactions and affect the development of various neuronal phenotypes. Since it is known from other work that by introducing an interval between injury and grafting. more robust transplants can be obtained. the goal here was to determine whether a post-lesion delay can significantly reduce the degree of initial donor tissue loss seen with intraspinal FSC grafts. Adult S-D rats received E14 FSC grafts into hemilesion cavities made at spinal C4. The animals were then distributed between three transplant groups: acute. 10d delay. and 30d delay graft recipients. At 4d PT. tissue specimens were prepared for light microscopy and morphometric analysis. In acute graft hosts. the transplants consisted of small islands of cells apposed to either neighboring gray matter. vascular profiles. or the pial surface. With a 10d delay. however. the grafts were significantly larger than controls. 30d delay grafts were significantly larger than both controls and 10d grafts. It thus seems that the delayed grafting paradigm enhances the initial survival or rebound rate of these grafts. particularly with a delay of 30d. This result may be a reflection of increasing vascularity of the lesion site over time. By virtue of the stem/precursor cell nature of these fetal grafts. the present observations may prove relevant to other transplantation paradigms using other donor cell types. Studies are in progress to determine whether reduced donor tissue loss alters the developmental dynamics of these fetal transplants. (Supported by the Mark F. Overstreet Chair for Spinal Cord Regeneration Research)

P273.

NMDA RECEPTOR ACTIVATION AS A BASIS FOR INCREASED VULNERABILITY: OLIGODENDROCYTES IN CONTUSED SPINAL CORD

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Excitatory amino acid receptors play an important role in normal neurotransmission as well as in pathological changes and neural cell death that occur after spinal cord injury (SCI). Evidence also shows that spinal glia are involved in creation and maintenance of pathological pain. The purpose of this study was to investigate activation of NMDA receptors using phospho-NMDAR1 glutamate subunit expression after long-term SCI recovery(one month). A contusive injury was produced at T8 using the NYU impactor (10 gm, 12.5 mm drop). Antibodies to phospho-NMDAR1 glutamate receptor subunit (Upstate Biotechnology) were used to detect activated NMDA receptors in dual staining experiments with markers for neurons, oligodendrocytes or microglia identified cell types expressing NMDA receptors. Four weeks after SCI, more immunoreactivity for phospho-NR1 was observed in gray and white matter than in sham control animals. An increased number of oligodendrocytes was seen in both gray and white matter of the injured cord, while the number of neurons and microglia was decreased after injury. A large proportion of oligodendrocytes in the white matter were found expressing increased levels of activated NMDA receptors after injury. This observation suggests that upregulation of glutamate NMDA receptors in oligodendrocytes may play a role important in chronic pathological processes, including increased pain transmission, neural reorganization and plasticity following long-term spinal cord injury. These studies suggest a new target for pharmacological treatment of SCI and control of pain. (TIRR-Mission Connect, Spinal Cord Research Foundation, NS11255 and NS 39161)

P274.

INHERENT LOCOMOTOR DIFFERENCES IN MOUSE STRAINS IMPACT RECOVERY AFTER SPINAL CORD INJURY

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Recently spinal cord injury (SCI) research has expanded to mouse models due to the availability of transgenic and knock-out animals. Between-strain comparisons of mice found significant differences in activity level, neuroanatomy, and physiological function (Wahlsten, 2001), suggesting that differences in motor function of strains may exist. Coordinated locomotion may be an important parameter to investigate in mice because it is a sensitive indicator of injury severity. The purpose of this experiment was to determine if mouse strains use different locomotor patterns normally and which strains may be more vulnerable to SCI-induced locomotor deficits.

We examined 4 mouse strains and a hybrid cross commonly used in generating transgenic animals: Balb/c (n = 14), C57/BL6 (n = 18), C57/BL10 (n = 6), B10.PL (n = 6) and an F1 (C57/BL6 female X 129S6/SvEv male cross, n = 14). Mice received a 0.5mm contusion at T8 with the Electromagnetic Spinal Cord Injury Device. Locomotion was measured pre/post with the BBB and a subset of mice (n = 3–6/gp) was tested on a walkway apparatus that automatically calculates gait parameters. Pre-operatively, greater external rotation was noted in some strains during gross locomotion. Quantitative measures showed significant differences ($p < .02$) in diagonal forelimb-hindlimb (FL-HL) coordination between C57/BL6, Balb/c and F1s even though they walked at the same velocity, (C57/BL6: HL precedes FL by 6.34 ± 2.15%; Balb/c: HL trails FL by 4.70 ± 0.38%; F1: HL and FL move almost simultaneously 1.67 ± 0.61%). Balb/c and C57/BL6 also tended to have longer HL swing times than other strains ($p = .057$ and .069).

After SCI, C57/BL10 and B10.PL showed a greater rate and extent of recovery on the BBB than other strains. Quantitatively, C57/BL10 had greater residual deficits in FL-HL coordination than B10.PL ($p < .003$) despite similar lesion severities.

Strain differences in locomotion exist in normal and SCI mice. Attribution of behavioral differences to treatment or genetic manipulations in mice must be done with caution.

NS37846 CRPS DD1-0200-2.

P275.

FORMATION OF COLLAGENOUS SCAR IS A COMMON FEATURE FOLLOWING TRAUMATIC AND ISCHEMIC INJURIES TO THE CENTRAL NERVOUS SYSTEM

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Formation of a collagenous wound healing scar resp. basement membrane (BM) has been shown to impede axonal regeneration after mechanical transection of brain and spinal cord fiber tracts in rat. Here we show by use of specific tissue processing protocols that such a collagenous BM forms not just after transection but also following contusion and compression injuries to the brain and spinal cord as well as after ischemic insults such as focal or global stroke. These data suggest that inhibition of axonal outgrowth may underlie the same principles after penetrating injuries as well as following blunt or ischemic lesions. Therefore, we consider the collagenous wound healing scar as an obstacle for compensatory functional plasticity in traumatic and ischemic brain lesions. Suppression of collagenous scarring may thus provide a strategy to promote axonal regeneration and/or plasticity in traumatic and ischemic CNS injuries.

P276.

EFFECTS OF SEROTONERGIC DEPLETION IN BULBOSPINAL FIBERS ON LOCOMOTOR AND PUDENDAL REFLEXES IN INTACT AND CHRONIC SPINALLY CONTUSED RATS.

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In spinal cord injured (SCI) animals, distension of the external anal sphincter (EAS) produces a profound increase in muscle EMG responses. Following contusion injury (25mm; NYU device), consistent and parallel recovery of locomotor and pudendal (erectile and EAS) reflex function occurs in animals over 6 weeks post-SCI. We have reported that the reduction, and subsequent return, of fibers labeled for serotonin (5-HT) immunofluorescence mirrors the recovery of function in these animals. This study sought to determine if the observed sprouting of 5-HT fibers mediates the recovery of pudendal reflexes after contusion.

To test the efficacy of eliminating descending serotonergic inputs, intact males were administered intracisternal (IC) injections of the neurotoxin 5,7 DHT. At three days post-IC injection, the latency to the first penile erection was significantly reduced in animals with a confirmed reduction in spinal immunofluorescence. However, the magnitude and duration of EAS responses to distension were not affected. Locomotor function, as measured by BBB and computerized walkway were likewise unaffected.

Preliminary data on female rats with SCI (25 mm T10 injuries, tested at post-operative days 2, 7 and 21 for BBB locomotor function and EAS reflexes) revealed that SCI yielded a predicted disruption of locomotor function and hyperreflexia of the EAS after distension followed by recovery over time. Surprisingly, the 5-HT synthesis inhibitor p-CPA (200mg/kg IP) did not re-introduce EAS hyperreflexia but instead markedly diminished EAS responses. (Support: NIH, NS-31193).

P277.

BILATERAL HYPEREXCITABILITY OF LUMBAR DORSAL HORN NEURONS FOLLOWING UNILATERAL THORACIC HEMISECTION-BASIS FOR "PHANTOM" NEUROPATHIC PAIN

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Spinal cord injury (SCI) results in chronic central neuropathic pain (CNP) that persists in the majority of patients. One possible mechanism for the sustained hyperexcitability of dorsal horn neurons, termed central sensitization, is the maintenance of changes in excitatory amino acid and peptide elements. Another mechanism, denervation supersensitivity, produced by the interruption of tonic descending inhibition, may confer membrane voltage changes that increase the activation state of other ionic receptors. To test this, using adult male Sprague-Dawley rats (n = 8), we obtained extracellular single-unit recordings of multireceptive (MR) dorsal horn neurons from L3-L5 immediately before, and 45 minutes after (n = 10 neurons per side of the cord) a unilateral T13 hemisection. Background activity and evoked responses to innocuous and noxious cutaneous stimuli (brush, press, pinch, 3.84, 9.96, 204 mN von Frey filaments, 47°C) were recorded, and peripheral receptive fields were mapped. We report a statistically significant increase in all measured parameters on both ipsilateral and contralateral sides of the cord, caudal to the level of lesion. Ipsilaterally, the responsiveness of MR neurons was significantly increased compared to the contralateral side. We propose that one mechanism of below-level pain syndromes described by patients with CNP after SCI is release from tonic inhibition and subsequent development of hyperexcitability, as a result of receptor plasticity. (Supported by: Mission Connect of TIRR, NIH grants NS 11255 and NS 39161.)

P278.

TRANSIENT SUPPRESSION OF FIBROUS SCAR AFTER ACUTE SPINAL CORD INJURY IN RAT LEADS TO MASSIVE AXONAL REGENERATION

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Acute traumatic injury of the CNS results in formation of a basement membrane (BM) in the lesion core and a glial scar characterized by reactive astrocytes in the penumbra of the lesion. BM can be visualized by immunhistochemical staining with anti-collagen IV and anti-laminin antibodies. Due to its characteristic meshwork structure it is named fibrous scar or cicatrix and can be clearly distinguished from the glial scar with respect to spatial distribution and molecular and cellular composition. There is much evidence that fibrous scar BM serves as a scaffold to bind putative growth inhibitory and repellent molecules besides acting as a putative mechanical barrier for regrowing axons in the CNS.

We developed a strategy to suppress BM biosynthesis at the injury site in rat spinal cord. After either scouten wire knife lesions at Th8 or dorsal hemisections with microscissors, the "anti-scarring treatment" (AST) leads to a significant suppression of collagen IV deposition compared to untreated animals within the first 12 days after lesion. AST consists of multiple injections of an iron-chelator (decarboxylic bipyridine derivative BPY-DCA) into the lesion site, together with application of solid 8-Br-cAMP and longer lasting slow release of BPY-DCA through a synthetic copolymer at the top of the lesion. The 12 days time window with fibrous scar reduction proved to be sufficient to allow cortico-spinal axons to grow through the lesion site extending into the caudal spine through white and gray matter areas.

Our results show that the formation of the basement membrane in the fibrous scar after acute spinal cord injury is a major impediment for CST fiber regeneration. Supported by DFG.

P279.

VENTRAL FUNICULUS LESIONS OF THE SPINAL CORD PRODUCE LASTING SENSORY BUT NOT MOTOR DEFICITS

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Optimization of locomotion after spinal cord injury (SCI), depends on understanding the function of spared motor and sensory systems. Traditionally, descending systems in the ventral funiculus (VF) were thought to control skilled, overground locomotion but recent studies cast doubt on this role (Loy '02, Brustein '93). Making precise, permanent lesions of axons in the VF has been problematic and limits our understanding of these systems. A novel approach to VF lesions may be a zymosan-induced inflammatory response which creates discrete lesions in the CNS. The purpose of this study is to determine the cellular response, lesion development and behavioral consequences of VF zymosan injections.

Sprague-Dawley rats (n = 10) received zymosan injections (.2–1.12ul) in the VF and survived 1, 3 or 6 wks. Behavioral testing for locomotion, reflexes, and sensation was conducted preoperatively and weekly postoperatively (po). Lesioned tissue sections were stained for neurofilament, myelin, T lymphocytes and macrophages. Plastic sections (1um) were stained with toludine blue.

Macrophage and T-lymphocyte responses peaked at 3 weeks and remained above baseline levels at 6 weeks postop. Axonal loss occurred by 1 wk with lesion size peaking at 3 wks. A thin astroglial scar evident at 3 wks on plastic sections became thicker over time and contained a few remyelinating axons. Zymosan produced significant but transient declines in BBB scores 3-5 dpo ($p < .05$). Pronounced and lasting hypersensitivity developed after VF lesions using monofilaments ($p < .001$). During proprioceptive placing, knee flexion angular excursion was greater at 1 wk po ($p = .003$), indicative of hyperreflexia.

Zymosan injections into the VF produced permanent, precise lesions without damage to other areas by initiating a localized inflammatory response. Given that few sensory axons were damaged by VF lesions, the pronounced and lasting sensory changes suggest that firing thresholds of lumbar interneurons which integrate sensory and motor input are reset to lower levels. NS37846, NNS43798.

P280.

CELL PROLIFERATION AND SURVIVAL CHRONICALLY AFTER SPINAL CORD INJURY

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Following spinal cord injury (SCI), about half of the oligodendrocytes and astrocytes in the residual white matter at the injury site are lost by 24 hours. However, chronically after injury, the density of these cells in this region is equal to controls. This suggests that glial cells in the spinal cord are replaced after SCI. We have previously demonstrated that by 3 days, cell proliferation is significantly increased in the grey and white matter of the injured spinal cord as compared to laminectomy controls. To study the fate of cells dividing in response to injury, we performed SCI on adult female rats at the T8 level using a standardized contusion model. Animals received two BrdU injections (8.7 mg/kg) each day, on days 2, 3, and 4, with 2 hours in between injections. At 6 weeks after injury, spinal cords were fixed by perfusion and the tissue was analyzed using immunocytochemical detection of BrdU. We found that by 6 weeks, some cells that had been labeled 2–4 days after SCI were still present in the residual white matter, as well as in the central lesioned area. Double immunocytochemistry showed that a number of the BrdU positive cells also expressed nestin, PDGFRa, or NG2, markers characterizing glial precursor cells. These results suggests that a number of cells that are stimulated to divide in the first week after SCI do not undergo many additional cell divisions, but survive and remain in an immature state for many weeks after injury. (Supported by NIH F31 NS43019-01 and RO1 NS35647).

P281.

HYDROGEN PEROXIDE ELEVATED BY SPINAL CORD INJURY INDUCES AND A METALLOPORPHYRIN ATTENUATES OXIDATIVE DAMAGE

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We demonstrated previously that hydrogen peroxide (H2O2) concentrations increase significantly after spinal cord injury (SCI). In this study, H2O2 was administered into the rat spinal cord through a microcannula at the concentration and duration produced by SCI for 10 h. H2O2-induced oxidation of proteins and DNA was characterized by immunohistochemical staining with anti-2,4-dinitrophenyl (DNP) and anti-8-hydroxy-2-deoxyguanosine (8-OHdG) antibodies. H2O2-induced peroxidation of membrane phospholipids was determined by measuring malondialdehyde by microdialysis sampling and HPLC analysis. The numbers of DNP- or 8-OHdG-positive cells counted along the cannula track were both significantly higher in the H2O2-exposed group than in ACSF controls ($p < 0.001$ and $=0.001$, respectively). H2O2 significantly increased malondialdehyde production ($p = 0.03$). Mn (III) tetrakis (benzoic acid) porphyrin (MnTBAP)—a cell-permeable superoxide dismutase mimetic and a broad spectrum reactive species scavenger—administered through a second cannula (2.5 mM in ACSF) significantly reduced H2O2-induced oxidation of protein, DNA and membrane lipids ($p = 0.02$, 0.03, and 0.02 respectively). This is direct in vivo evidence that SCI-produced levels of H2O2 cause oxidative damage to major cellular components and MnTBAP effectively reduces H2O2-induced oxidative damage. (Supported by NIH grants NS 34048 and NS 35119).

P282.

ASSESSMENT OF POSSIBLE STRAIN—DEPENDENT DIFFERENCES IN MICE FOLLOWING SPINAL CORD INJURY

Isabella Fugaccia, Lisa M Benjamin, Stephen W Scheff. (Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY USA).

Experimental spinal cord injury (SCI) results in a rapid and significant pathophysiology throughout a large rostral-caudal extent of the spinal cord. In order to probe the molecular mechanisms of SCI, research interests have begun to employ genetically engineered mouse lines. These transgenic mice utilize a number of different background strains. The present study was undertaken to assess possible strain-related differences in response to SCI. Four different inbred strains (C57Bl/6, C57Bl/10, BALB/c, FVB/N) and one outbred strain (ICR) of mice. All animals were 7–8 wks of age when subjected to a moderate SCI (50 kdynes) at T10-11 utilizing the Infinite Horizon Impactor®. Seven days following injury, spinal cords were assessed for changes in morphology. In every injured animal there was an obvious bilateral bruising of the spinal cord at the time of injury. Initial analyses demonstrated strain-dependent differences in the injury length and volumes of spared gray and white matter. Although the age of the mice was equivalent, there were significant strain-dependent differences in the animal size and weight that might account for the observed differences. Accordingly, we assessed possible strain-related differences in the naive spinal cords and subsequently reassessed injury-induced changes compared to each strain's naïve spinal cord values. This new morphologic analysis failed to reveal any strain-related differences following SCI. These results support the idea that the basic morphologic changes observed following SCI are common to the most widely used mouse strains and underscores the need to utilize appropriate controls when assessing the results. Supported by KCHIRT #9-20 and SCRF 2085-02.

P283.

IS THERE AN ACQUIRED CHANNELOPATHY CONTRIBUTING TO AXONAL CONDUCTION DEFICITS FOLLOWING SPINAL CORD INJURY?

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Axonal ion channel dysfunction (channelopathy), as a result of immunological factors, or alterations in the expression of genes encoding ion channel properties, has emerged as an important element of the pathophysiology of conduction deficits in a variety of neurological disorders. We hypothesize that an acquired (traumatic) channelopathy, distinct from axonopathy or myelinopathy, contributes to central and peripheral conduction deficits following spinal cord injury (SCI). Evidence is drawn from immunologic studies of chronic SCI patients revealing elevated serum and/or cerebrospinal fluid titers of proinflammatory cytokines and/or autoantibodies known to alter potassium (K+), or sodium (Na++) channel conductances, as well as studies of trauma-induced modifications in the expression of genes encoding K+ and Na++ ion channel properties in animal models of SCI. The presence of axonal ion channel dysfunction would compound conduction deficits due to co-existent myelinopathy or axonopathy. If proven, the acquired channelopathy concept may help explain various clinical phenomena such as functional deficits that exceed those expected from the degree of frank neurological injury, bladder dysfunction or sensory parethesias. Acquired channelopathies may be remediable by targeted immunomodulatory therapy, gene therapies that target ion channel proteins, or agents that modify ion channel conductance, eg. 4-aminopyridine.

P284.

WHIPLASH INJURY OF THE NECK: CLINICAL SYNDROME OR MALINGERISM?

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Background: Minor spine injuries, vastly predominated by whiplash injuries of the neck, tend in later few years to occupy each year more time in the ambulatory practice of our neurosurgeons. Increasing number of these types of injury and more complicated diagnostic and therapeutic protocols represent a growing financial burden especially to the health organisations and the insurance companies protest an epidemic of financial compensation claims. The physician is so often forced into the disagreeable position to arbiter between the compensation-seeking malingerers and patients. A correct decision is almost unreachable goal in a population of these patients with predominantly subjective leading symptoms and lack of clearly distinctive criteria so esteemed by surgeons.

Aim: We could not even dare trying presenting all the aspects of the problem, but compared some data dealing with the epidemiology, diagnostic and therapeutic procedures used in clinical practice with the problem of chronic residual symptoms in medico-legal practice.

Results: The data compared referred to years 1998. and 2000. : we noticed the marked increase in number of the injured seeking the medical help in urgency, as well as in ambulatory controls (~50%). The same percentage of the litigation is registered (<20%). The data showing leading acute symptoms (pain, radicular impairment), diagnostic tools, therapy (immobilisation, non-steroid analgesics, physical treatment), work absence (~10 weeks), and residual complaints (discomfort, minor impairments of physical activities) remained similar.

Conclusion: Although aware of the both sides of the problem, we kept the "physicians attitude" to the injured: belief that most of the patients do suffer the symptoms (although somewhat aggravating) and need diagnostic and therapeutic medical approach. but we urge for clear-cut criteria both in urgency (QTF protocol, cervical spine X-rays) and in follow-up (symptom-quantification - neck muscles and mobility, radicular, cervico-cephalic, and other symptoms, and correct evaluation of instrumental findings).

P285.

HETEROGENEITY OF REGIONAL CEREBRAL BLOOD FLOW FOLLOWING SEVERE HEAD INJURY

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Local probes, such as tissue pO2 and microdialysis, are being widely used to monitor patients with traumatic brain injury (TBI). Often the probe is placed in a relatively uninjured area of the brain, assuming that this will provide a measure of global CBF. The purpose of this study was to analyze the distribution of cerebral blood flow (CBF) after TBI to provide an understanding of what information could be expected from a local monitor.

CBF was measured using xenon-enhanced computed tomography (CT) in 76 patients within 12 hours after severe TBI. For each patient, rCBF was calculated in 80 standard cortical regions of interest (ROIs). The distribution of CBF in these ROIs was analyzed.

The average CBF for all ROIs studied was 35 ml/100g/min, but the values ranged widely from 1 to 151 ml/100g/min. The type of injury was the most important factor in determining the distribution of CBF. With Diffuse injury 1, CBF was normally distributed, and was between 30–60 ml/100g in 72% of ROIs. CBF was below 20 ml/100g/min in only 8% of ROIs. With Diffuse injury 2 and 3 and with mass lesions, the median CBF was lower and the range of CBF in the ROIs was wider. With Diffuse injury 4, CBF was uniformly decreased, with 90% of all ROIs having a CBF between 10 and 30 ml/100g/min. Other factors that determined the distribution of CBF were age and the level of ICP at the time of the CBF measurement.

CBF within the brain varies widely in most patients after TBI. Depending on the placement of a local probe within the brain, the values obtained may or may not reflect global CBF values.

P286.

COMPARISON OF SINGLE VOXEL AND MULTIVOXEL MR SPECTROSCOPY IN PREDICTING 3 AND 6 MONTH NEUROLOGIC OUTCOME AFTER TRAUMATIC BRAIN INJURY

Barbara A. Holshouser, Karen A. Tong, Lori Shutter. (Loma Linda University Medical Center, Loma Linda, CA US).

Methods: We reviewed 27 patients, aged 15 to 79 (mean 33 years), at an average of 7.2 days after sustaining severe TBI (initial Glasgow Coma Score £ 8). Seven control subjects, aged 17 to 49 (mean 27 years) were also evaluated. With a 1.5T MR scanner, two SVS (8 cc) were acquired in normal appearing brain regions: mid-occipital gray matter (GM) and parieto-occipital white matter (WM), using a short echo time (TE = 20msec) stimulated echo acquisition mode (STEAM) sequence. SVS spectra were processed using LCModel software to obtain peak areas for N-acetylaspartate (NAA), creatine (Cre), choline (Cho) and metabolite ratios.

MRSI was acquired using a long echo time (TE = 144 msec) point resolved spectroscopy sequence (PRESS) in a 10 mm thick slice at the level of the corpus callosum. Spectra for each voxel were processed to obtain mean metabolite ratios, then averaged to obtain a pooled mean metabolite ratio (Total) for each patient and control. Three clinical outcome groups (good recovery, moderate disability and severe disability/vegetative state (VS)) based on the Glasgow Outcome Score (GOS) were assigned at 3 and 6 months following injury.

Results: For 3 and 6 month outcomes: SVS metabolite ratios (GM and WM NAA/Cho and Cho/Cre) showed significant differences between controls and the severe/VS group only. (ANOVA; $p = .01–.05$); MRSI "Total" metabolite ratios (NAA/Cre, NAA/Cho, Cho/Cre) showed significant differences between controls and all three outcome groups ($p = .001–.05$). Additionally at 6 months, MRSI could distinguish between disability groups.

Conclusion: The MRSI pooled metabolite ratios were better able to distinguish outcome groups than SVS ratios, particularly at 6 month outcomes.

P287.

SIGNIFICANCE OF A REDUCED CEREBRAL BLOOD FLOW WITHIN 12 HOURS AFTER SEVERE HEAD INJURY.

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Posttraumatic hypoperfusion is a well-known feature of traumatic brain injury pathophysiology. "Ischemic" levels (<18 ml/100g/min) of cerebral blood flow (CBF) occur in [1/3] of patients within 6–12 hours after injury. However, the underlying cause of this hypoperfusion is not so clear. The purpose of this study was to examine the factors associated with an early reduction in global CBF.

77 patients with severe head injury who underwent measurement of CBF using xenon enhanced computed tomography (XeCT) within 12 hours after injury were included in this study. Global CBF, physiological parameters at the time of CBF measurement, and outcome measures were analyzed.

Global CBF averaged 35.8 ± 16.4 ml/100g/min. Nine patients had an average global CBF < 18 ml/100g/min (11.8 ± 5 ml/100g/min); the remaining 67 patients had a global CBF of 39 ± 15 ml/100g/min. Initial ICP was greater than 20 mmHg in 90% of patients, and greater than 30 mmHg in 80% of patients in group with CBF < 18 ml/100g/min, compared to 33% and 16%, respectively, in the nonischemic patients. Mortality was 90% at the time of ICU discharge, and at 6 months post-injury in patients with CBF < 18. Mortality in the patients without global ischemia was 17.9% at discharge and 19.4% at 6 months after injury. In contrast, the major factor associated with a reduction in global CBF in the range of 18–40 ml/100gm/min was the volume of tissue with rCBF < 18 ml/100g/min (i.e. the volume of regional ischemia).

In patients with CBF < 18 ml/100g/min, intracranial hypertension plays a major causative role in global ischemia. Treatment would most likely be directed at controlling ICP, but the early, severe intracranial hypertension probably indicates a very severe brain injury. For levels of CBF between 18 and 40 ml/100g/min, the presence of regional ischemia was a more important factor in reducing global CBF.

P288.

SERIAL QUANTITATIVE PROTON SPECTROSCOPIC FINDINGS IN SEVERELY BRAIN INJURED PATIENTS CORRELATE WITH OUTCOMES

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Intro: Proton magnetic resonance spectroscopy (MRS) is a sensitive non-invasive technique used to measure changes in brain metabolites. The purpose of this study is to quantitate serial metabolite changes following severe traumatic brain injury (TBI) to determine if MRS is useful to predict long-term outcome.

Methods: We prospectively studied 42 patients, aged 14 to 79 (mean 34 years), who sustained severe TBI (initial Glasgow Coma Score £ 8). Using a 1.5T scanner, MRS was obtained within 16 days after injury and repeated at 6 months (n = 32). Two single voxel spectra were acquired in normal appearing brain: one in occipital gray matter (GM) and a second in parieto-occipital white matter (WM). All spectra were processed using the LCModel technique to quantitate peak areas for N-acetyl-aspartate (NAA), creatine (Cre), choline (Cho), glutamate/glutamine (Glx) and myo-inositol (Ino). Peak area metabolite ratios were also calculated. MRS data were correlated with the Glasgow Outcomes Score (GOS) at 6 months following injury. Patient outcomes were divided into two groups: favorable outcome (good recovery and moderate disability) and poor outcome (severe disability, vegetative state and death).

Results: Results of logistic regression analysis on initial MRS data showed that elevated GM Cho ($p < 0.05$), WM Cho ($p < 0.01$) and GM Glx ($p < 0.01$) quantitative levels are significantly correlated with poor outcomes with odds ratios of 5.4, 2.2 and 5.8 respectively. Elevated GM and WM Cho/Cre, and decreased WM NAA/Cho also showed significant correlation with poor outcomes ($p < 0.01$). Follow-up MRS showed continued NAA decline and persistent Cho and Ino elevation in the poor outcome group. Linear discriminant analysis found that GM and WM NAA, Cre and Cho correctly predicted 86% of outcomes. Combining MRS data with clinical information raised the predictive ability to 93%.

Conclusion: MRS correlates with long-term outcome, and provides additional information for patient management.

P289.

ASSESSMENT OF FLOW VOLUME IN THE INTERNAL CAROTID ARTERY. CORRELATION WITH 133XENON CEREBRAL BLOOD FLOW

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Objective: Monitoring of cerebral blood flow in acutely brain injured patients is an essential component of critical care monitoring. The purpose of this study was to evaluate a new device coupling angle independent dual beam flow and digital Doppler technology (FlowGuard) for the assessment of the blood flow volume (BFV) in the carotid artery (ICA) in normal volunteers and acutely brain injured patients.

Methods: ICA-BFV (ml/min) and diameter (mm) in 30 healthy volunteers were measured by means of FlowGuard and duplex ultrasound and compared. Then BFVs in the ICA assessed by FlowGuard were compared with measurements of the cerebral blood flow (CBF, ml/100g/min) obtained using the clearance of Xe133 and AVDO2 measurements. Sixteen CBF studies performed in eight acutely brain injured patients were compared to BFV rates in the ICA.

Results: BFV was satisfactorily recorded in 28 normal subjects (6.6% failure). ICA mean BFV was 277 ± 25 ml/min (range: 239–338) with a mean diameter of 5 ± 0.5 mm (range: 4.1–6.1). ICA-BFV proved to be significantly higher (N = 17, 284 ± 21 ml/min) in subjects younger than 35 years than in subjects older than 35 years (N = 11, 267 ± 28 ml/min, $p = 0.041$). ICA diameter measured by the FlowGuard correlated with the results of the Duplex ultrasound ($r = 0.94$, $p = 0.0001$). In head injured patients, BFV showed a strong correlation with global CBF measurements ($r = 0.91$, $p = 0.0001$) as well as hemispheric CBF, right $r = 0.89$, $p = 0.0001$, left $r = 0.82$, $p = 0.0001$. The mean error in CBF estimation by means of BFV was 13.6 ± 8.5% (range 3–31%). The BFV also correlated with the AVDO2, $r = -0.51$, $p = 0.08$ while CBF $r = -0.39$, $p = 0.22$.

Conclusions: This study showed that BFV measurements using the FlowGuard can be easily performed and implemented in the critical care environment. This preliminary study suggests that CBF can be estimated at patient's bedside with a reasonable accuracy.

P290.

IMPROVED MRI DETECTION OF HEMORRHAGIC SHEARING INJURIES IN ADULTS USING SUSCEPTIBILITY WEIGHTED IMAGING (SWI): CORRELATION WITH SEVERITY AND OUTCOME.

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Purpose: To compare a new high resolution susceptibility weighted imaging (SWI) technique to conventional gradient recalled echo (GRE) imaging in the ability to detect hemorrhage suggestive of diffuse axonal injury (DAI), to determine severity of injury and to predict outcome after traumatic brain injury (TBI).

Material & Methods: Eleven patients were imaged early after trauma. A standard MR protocol was performed, including conventional gradient echo (GRE) imaging. A high resolution SWI technique was also performed using a 3D GRE sequence that incorporates post-processing to enhance signal loss from hemorrhage. Number and volume of hemorrhagic lesions demonstrated by both methods were compared. Extent of hemorrhage was also compared to initial Glasgow Coma Score (GCS) as well as Glasgow Outcome Score (GOS) at 1, 3 and 6 months after trauma.

Results: Hemorrhagic lesions on SWI were more visible than on conventional GRE. SWI detected approximately 3.8 times more lesions and 2.5 times more lesion volume than GRE. Number of SWI lesions had an inverse relationship with initial GCS. In addition, number of SWI lesions were related to severity of clinical outcome at 1 month: patients in a vegetative state (n = 3), with severe disability (n = 5), and with moderate disability (n = 3) had a mean number of 157, 77, and 51 lesions respectively. When dichotomized into two outcome groups, the mean number of lesions on the initial MRI study were consistently higher in the poor outcome group compared to the good outcome group, when assessed clinically at 1, 3, and 6 months after injury.

Conclusion: The SWI technique significantly improves visibility of hemorrhagic injuries. Extent of hemorrhagic lesions were related to initial GCS as well as severity of subsequent outcome, suggesting that SWI can improve diagnosis of hemorrhagic brain injuries and potentially predict outcome after TBI.

P291.

CEREBRAL HEMODYNAMICS AFTER CORTICAL IMPACT INJURY IN THE eNOS KNOCKOUT MOUSE

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Nitric oxide (NO) generated by endothelial nitric oxide synthase (eNOS) plays an important role in regulating basal cerebral blood flow (CBF). The purpose of this study was to investigate the role of eNOS in maintaining CBF after controlled cortical impact injury (CCI).

Three groups of animals were studied: wild-type mice (C57BL6) (WT; n = 12), eNOS-deficient mice (eNOS-/-; n = 12), and eNOS-deficient mice treated with L-arginine (eNOS-/-Arg; n = 12). The mice were anesthetized with isoflurane, intubated, and mechanically ventilated prior to CCI (3 m/sec, 1.5 mm deformation). Five minutes after injury, saline was administered to the WT and eNOS-/- groups; L-arginine (300 mg/kg) was administered to the eNOS-/-Arg group. Arterial blood pressure (ABP), intracranial pressure (ICP), and laser Doppler-CBF (LD-CBF) at the impact site were monitored for two hours after injury.

Baseline ABP was significantly higher in eNOS-/- animals than in WT animals (89 ± 10 mm Hg vs 69 ± 6 mm Hg, $p < 0.001$) and remained higher even after injury (P of group effect < 0.001, time effect < 0.001, group × time effect < 0.001; two-way repeated measures ANOVA), with no difference between eNOS-/- and eNOS-/-Arg groups. Although baseline ICP was the same in all groups, ICP after injury was significantly higher in the eNOS-/- animals (group < 0.001, time < 0.001, group × time < 0.001). Immediately after CCI, LD-CBF decreased to 34 ± 13% of baseline in WT animals and to 19 ± 11% of baseline in eNOS-/- animals. LD-CBF was consistently lower in eNOS-/- animals compared to WT animals (group $<$ 0.001, time < 0.001, group × time < 0.001), with no effect of L-arginine administration.

NO generated by eNOS plays an important role in the maintenance of CBF following CCI. Absence of eNOS prevents L-arginine from effecting an improvement in CBF after CCI.

P292.

BEHAVIORAL DEFICITS FOLLOWING LATERAL FLUID PERCUSSION INJURY IN THE RAT PUP DUE TO CELLULAR DYSFUNCTION AND NOT CELL DEATH.

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Traumatic brain injury (TBI) is the number one cause of pediatric death and disability in the United States. Surprisingly, research into the mechanism of developmental post-TBI pathophysiology is lacking. Evidence in several adult models of TBI suggests that hippocampal cell death and behavioral dysfunction are correlated. Following a mild percussion injury (FPI), however, dysfunction can be detected without obvious neuronal death. Elevated glutamate is well characterized during the acute phase following FPI in developing rats. The CA3 region of the hippocampus, having the highest glutamate receptor density and an elevated resting membrane potential, is particularly susceptible to glutamate excitation and excitotoxic neuronal death. To test the hypothesis that deficits following FPI in post-natal day 19 pups are not related to neuronal loss, stereological cell counts were performed in the CA3 region. Estimation of the total number of cells in CA3 of injured (n = 8) and control (n = 3) pups two weeks following injury revealed no difference between groups (260285 ± 62982 and 294529 ± 60684 respectively). Similarly, within individual animals there was no difference in neuronal number between the ipsilateral and contralateral CA3 (260285 ± 62982 and 245233 ± 48695 respectively). This data supports the hypothesis that FPI in the developing rat leads to cellular dysfunction and indicates that at least some of the deficits reported following TBI are not related to cell death.

P293.

CELLULAR LOCALIZATION AND ALTERATIONS OF INHIBITORS OF APOPTOSIS AFTER TRAUMATIC BRAIN INJURY.

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Regulation of cell type expression of inhibitors of apoptosis (IAP) was examined in the normal rat brain and in brains subjected to moderate traumatic brain injury (TBI) using parasagittal fluid-percussion brain injury model (1.7–2.2 atm). Immunohistochemistry combined with confocal microscopy were used to determine distribution and cell type expression of XIAP and cIAP-1. Quantification of XIAP-positive cells in the hippocampus of normal and traumatized rats was performed by stereological techniques. XIAP was present exclusively in neurons and localized to the perinuclear region and cell soma. cIAP-1 was expressed in cell processes and in the cell soma of large neurons in cortical layer IV, whereas hippocampal and thalamic neurons demonstrated differential cellular expression. Some oligodendrocytes in the corpus callosum expressed cIAP-1, but this IAP was undetectable in other glial cells. Traumatized brains showed dramatic redistribution and alterations in cellular and regional expression of XIAP and cIAP-1. XIAP immunoreactivity decreased significantly ($P < 0.001$ vs sham) in both hemispheres early after injury. By 24 hours the levels of XIAP increased significantly ($P < 0.001$ vs 1hr and 6 hr groups), but did not reach those of sham controls. In contrast, cIAP-1 expression increased immediately after injury in neurons located primarily around the lesion epicenter. Astrocytes in the cortex and hippocampus showed robust cIAP-1 immunostaining. Expression arrays (GEArray, Superarray, Bethesda, MD) of apoptotic genes demonstrated increased mRNA expression of cIAP-1, cIAP-2, tumor necrosis factor-receptors-1 and -2, casper (FLICE-like inhibitory protein) at 24 hours after TBI. Our data demonstrate that in the normal adult rat brain XIAP and cIAP-1 are expressed in a lineage-specific manner and in different brain regions. TBI induces redistribution and alterations in levels of mRNA and proteins in the apoptotic and anti-apoptotic pathways that may contribute to the pathophysiology after injury. Supported by AHA Grant 0215133B to G.L., and by NS 30291-10 to W.D.D.

P294.

THE EXPERIMENTAL OBSERVATION ON DELAY NEURONAL DEATH AFTER ACUTE BRAIN TRAUMA

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OBJECTIVE: In order to observe the phenomenon of delayed neuronal death. METHOD: These experiments were mainly based on rat diffuse brain injury model. Cultured newborn rat hippocampal neurons were tested for delayed neuronal death in vitro. The conditions of tissue and cell injury were observed under the microscopy and electromicroscopy. The neuronal DNA injury in cortex and hippocampal was observed by DNA-Ladder and TUNEL stain. RESULT: Histological examination showed that the neurons presented degenerative change; electromicroscopy examination showed that the neuronal apoptosis and necrosis would be seen in the cortex and hippocampal, most serious on 24 hours. DNA Ladder would be seen and most distinct on 24 hours after severe injury. Mostly neurons with positive TUNEL stain represent neuronal apoptotic change. In vitro, 24 hours after neuron thrust injury, the neuron died by necrosis; some neurons died by apoptosis when the normal neuron cultured by the culture liquid from the injury neuron. CONCLUSION: Delayed brain injury happened after acute brain injury due to lot of second injury factors, and mostly exhibited by necrosis and apoptosis, given priority to apoptosis.

P295.

AGE-DEPENDENT RESPONSE TO SCALED CORTICAL IMPACT IN THE PIGLET

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Introduction: To investigate whether maturational stage influences the brain's response to mechanical trauma, a scaled cortical contusion model was developed which delivers a rapid volume of indentation proportional to changes in brain mass and dimensions with growth. Piglets of three different ages at injury were studied using serial magnetic resonance imaging (MRI) scans, histology, and immunohistochemistry. Methods: Anesthetized piglets at 5 days (infant). one month ("toddler"), and four months of age (adolescent) underwent craniectomy, dural opening, and direct frontoparietal cortical impact with the scaled indentation device. Injury magnitude was chosen to cause a clinically asymptomatic but visible lesion. Serial MRI studies at 24 hours, 7 days, and one month post-injury were obtained. Histology and immunohistochemistry at 6 hours, 7 days, and one month post-injury were performed to investigate processes relevant to cell death, repair, and regeneration. Lesions were compared among ages by expressing size as a ratio between the lesion and the contralateral uninjured hemisphere. Results: Lesions involving the cortex, white matter, and periventricular region were seen on histology and MRI. Despite comparable injury inputs, at seven days post-injury, histologic lesions were smallest in the youngest subjects, intermediate in the "toddler" age group, and largest in the adolescents. Differences in the time course and magnitude of swelling were seen on MRI, with the youngest (infant) subjects having the earliest peak, the "toddler" group having the most marked swelling, and the adolescent age group having the latest peak. Age-dependent differences in repair and regeneration processes were also seen. including generation of new cells in the subventricular zone. Conclusion: The response of the gyrencephalic brain to focal mechanical trauma differs with age at injury. These differences have implications both to clinical care and to strategies in influencing cell death pathways and in promoting repair and regeneration after traumatic brain injury.

P296.

DIFFERENCES IN ICP-AND CARDIOVASCULAR RESPONSE IN DEVELOPING VERSUS ADULT RATS FOLOWING DIFFUSE TRAUMATIC BRAIN INJURY

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Objective: Diffuse brain swelling and malignant brain edema following traumatic brain injury is a unique feature in the pediatric age group. In the present study the influence of diffuse traumatic brain injury on intracranial pressure (ICP) changes and cardiovascular response is investigated in developing rats and compared to findings in adult rats. Methods: Diffuse brain injury was produced in intubated and ventilated 19–23 days old Sprague-Dawley rats ($N = 8$) using a modification of the Marmarou-model (1.5m/100g). Mean arterial blood pressure recordings and intracranial pressure recordings were performed continuously. The results were compared to readings in adult animals ($N = 10$) subjected to a 1.5 m/500 g injury. Results: In the developing rat MABP decreased from 77.1 ± 16.8 mm Hg to 50.9 ± 28.5 mm Hg immediately following injury. Within 3 min it started to recover without reaching base-line levels within one hour (56.3 ± 17 mm Hg). No significant ICP-increase was determined and mortality rate was 50% within one hour following injury. In the adult rat only a minor decrease of MABP was determined 3 min following injury (from 139.5 ± 9.3 mm Hg to 119.5 ± 11.5 mm Hg) reaching base line levels within 15 min. All adult animals recovered following trauma with no relevant ICP-increase within one hour post-trauma. Conclusions: The results of the present study indicate a pronounced affection of the brainstem in developing versus adult rats resulting in a higher mortality. These findings may contribute to the different response of the young brain to traumatic brain injury.

P297.

MICROARRAY ANALYSIS OF CELL TRAFFICKING GENES AFTER TRAUMATIC BRAIN INJURY: THE EFFECTS OF HYPOTHERMIA AND HYPERTHERMIA

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Several lines of evidence suggest a pathogenic role of inflammation in brain trauma. Recent data have reported the importance of temperature on the early accumulation of polymorphonuclear leukocytes (PMNL) after traumatic brain injury (TBI). Mechanisms underlying these temperature effects remain to be clarified. The purpose of this study was to utilize expression arrays (GEArray, Superarray, Bethesda, MD) of cell trafficking genes to screen for changes in expression of these genes after TBI. In addition, the effects of post-traumatic hypothermia (33 C) and hyperthermia (39.9 C) were investigated. Male Sprague-Dawley rats underwent moderate fluid percussion brain injury (1.8 to 2.2 atmospheres). Four hours after injury, the injured cortex was removed and total RNA extracted, 32P labeled cDNA probes were generated by reverse transcriptase and hybridized to the arrays. Following exposure, signals were analyzed by Phosphoimager. Expression of the genes on the arrays was then compared between the various experimental groups. At 3 hrs after TBI, a number of genes were shown to decrease compared to sham operated controls. For some genes, post-traumatic hypothermia appeared to alleviate the injury-induced decrease in gene expression. These include Ncam2, NCAM, Lamb1, integrin βx, cathepsin B, collagen β2, caveolin, catenin β, and basigin. In contrast, post-traumatic hyperthermia appeared to increase gene expression of integrin βx, cathepsin B and collagen β2 compared to normothermic levels. Finally, expression of some genes was increased by both hypothermia and hyperthermia compared to normothermic TBI including tenascin C, Timp1 and CD44. Taken together these results suggest that microarray analysis of gene expression may be useful in elucidating the molecular mediators of inflammation after TBI and clarify specific genes that are sensitive to post-traumatic temperature manipulations. NS42133 and NS30291.

P298.

EXPRESSION OF P2 PURINERGIC RECEPTORS IN RAT CORTEX AFTER MODERATE TRAUMATIC BRAIN INJURY.

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Extracellular levels of ATP are increased after brain trauma. This increase in ATP release is thought to be involved in the initiation of reactive gliosis via stimulation of ATP/P2 purinergic receptors. However, little is known about the expression of these receptors after traumatic brain injury (TBI). In the present study, we have investigated the expression of metabotropic P2Y and ionotropic P2X receptor subtypes in rat cortical tissue 1 (n = 9). 3 (n = 8). and 7 (n = 9) days after TBI or sham (n = 8) procedures. Fluid percussion brain injury (1.8–2.1 atm) was produced in anesthetized Sprague Dawley rats. At various periods after TBI rats were killed and cortical samples for ipsilateral and contralateral hemisphere dissected and frozen for analysis. Messenger RNA (mRNA) levels of P2Y1, P2Y2, P2Y4, P2X1, P2X3 and P2X7 were measured either with ribonuclease protection assay (RPA) or relative quantitative reverse transcription-polymerase chain reaction (RT-PCR). P2 receptor expression in cortical tissue from the injured side was compared with the uninjured side. After moderate TBI, there were no statistically significant changes in the mRNA levels of the P2Y receptor subtypes studied (P2Y1, P2Y2, P2Y4). although an increase in P2Y1 and P2Y2 was observed at day 3. For the P2X subtypes studied, a significant increase in expression of P2X1 and P2X7, but not P2X3, receptors was observed at day 1. These results indicate that the expression of distinct P2X receptor subtypes is increased by moderate TBI and suggest that this response may be involved in reactive gliosis in vivo.

P299.

THE INFLAMMATORY RESPONSE AFTER TRAUMATIC BRAIN INJURY IN MALE AND FEMALE RATS AS ASSESSED BY cDNA ARRAYS

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Pro-inflammatory cytokines such as TNF-a, and IL1-β have been shown to increase rapidly after traumatic brain injury (TBI) and to be involved in mediating the inflammatory response. However, it is unknown whether there are gender differences in the inflammatory response after TBI. In this study, inflammatory response cytokine expression arrays (GEArray, Superarray, Bethesda, Md.) were used to screen for changes in expression of 23 known genes in the inflammation pathway. The use of a gene array specifically targeting components of the inflammatory cascade provides a useful tool for determining future targets of this cascade for therapeutic intervention.

Male and female Sprague-Dawley rats underwent moderate fluid percussion brain injury (1.8–2.2 atm). Three hours later, the injured cortices were removed and total RNA extracted. 32P labeled cDNA probes were generated by reverse transcriptase and hybridized to the arrays. Following exposure, signals were analyzed by Phosphoimager. Expression of the genes on the array was compared to a sham operated male.

Members of the interleukin superfamily (including IL-1a, IL-1β, IL-2, IL-6, IL-10), transforming growth factor family members (TGF-a, TGF-β1, TGF-β2, TGF-β3), chemokine GRO-1, transcription factor MCP-1, and lymphotoxin B (LT-b) were upregulated in the TBI animals as compared to sham controls at 3 hours after TBI. In reference to potential gender differences, TGF-b1 and MIF were higher and IL-2 and LT-β lower in traumatized females versus males. Traumatic brain injury initiates a complex inflammatory cascade that involves the increased expression of many different genes. Whether these responses act beneficially, detrimentally, or in combination remains to be elucidated. The time course of this response as well as the influence of gender on the expression of these and other genes is being studied. Supported by NIH grants NS42133, NS30291, and Eli Lilly & Co.

P300.

INTERLEUKIN-16 RELEASE FROM CD8-POSITIVE T LYMPHOCYTES FOLLOWING TRAUMATIC BRAIN INJURY.

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Interleukin-16 (IL-16) is expressed in a number of pathological conditions, including autoimmune disease and infection with human immunodeficiency virus. IL-16 induces the chemotaxis and regulates the activation of CD4 positive cells. Using flow cytometry to detect intracellular cytokine and enzyme-linked immunosorbent assay to quantitate cytokine in patient plasma, we report that IL-16 is released from pre-formed intracellular depots by peripheral blood CD8-positive T cells immediately after severe traumatic injury. Both the extent of T cell release of IL-16 and peripheral blood IL-16 levels were found to be greater in more severely-injured patients, particularly those suffering a traumatic brain injury. In addition, the kinetics of IL-16 release was found to coincide with a transient decrease in peripheral blood CD4/CD8 T cell ratio. Release of IL-16 from CD8-positive T lymphocytes in vitro could be demonstrated by culture of normal donor peripheral blood leukocytes with epinephrine. T lymphocyte function is known to be compromised after severe traumatic injury, presumably to limit the adaptive immune response to self tissues. Release of IL-16 by peripheral blood CD8-positive T cells may be a mechanism to regulate CD4 T lymphocyte functional activity in the post-traumatic peripheral circulation.

P301.

S100 BETA PROTEIN RESPONSE IN ASTROCYTES AFTER HUMAN BRAIN CONTUSION

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S100 beta protein, a calcium-binding protein, present mainly in astrocytes, that exerts paracrine trophic efects of several neuronal populations. After human brain injury serum concentration of S100 beta increases. However, there is not direct information about the S100 beta protein response in the brain after Central Nervous System injury. Contused brain tissue were obtained from 18 consecutive patients undergoing surgery for trauma contusion 6 hours to 6 days after trauma. S100 beta protein were analysed by immunohistochemistry. Twelve hours after trauma S100 beta protein was first detected in astrocytes and then increased progressively until 6 days, both in temporal and frontal contusions. Therefore, astrocytes can increase S100 beta protein expression after human brain contusion according to the serum concentration increase. The knowledgement of trophic response after brain trauma can be helpfull in development of new therapeutic approaches that can modulate glial and neuronal responses. Acknowledgements: Laboratory of Neuroregeneration. Department of Anatomy. Institute of Biomedical Sciences. University of São Paulo. São Paulo. Brazil.

P302.

HYPOTHERMIA REDUCES THE ACTIVITY OF NF-kB AFTER PARASAGITTAL FLUID-PERCUSSION BRAIN INJURY.

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Post-traumatic hypothermia has previously been reported to attenuate the early inflammatory response to brain trauma. We investigated whether hypothermia exerts its effect by inhibiting the activation of the nuclear factor kappa B (NF-kB), a transcriptional activator that is essential to the expression of genes that are involved in the development of inflammation in the brain. Moderate traumatic brain injury (TBI) (1.8-2.2 atm) was induced in rats by a fluid percussion (F-P) device. In the first phase: intracerebral expression of NF-kB was studied using the electrophoretic mobility assay at 3- and 7-days after injury. In the second phase: rats underwent moderate F-P brain injury followed immediately by 4 hr of post-traumatic normothermia (37°C) or hypothermia (33°C)and were then killed. Ipsilateral and contralateral cerebral cortical regions were then assayed for NF-kB activation. Results indicate that on post-trauma days 3 and 7, the activity of NF-kB was increased in the ipsilateral cortex at 3-days after TBI and at both ipsilateral and contralateral cortices at 7-days after TBI. Post-traumatic hypothermia reduced NF-kB activity at both time points in ipsilateral and contralateral sites tested ($p < 0.05$). Post-traumatic temperature is a critical factor for determining NF-kB binding activity after brain trauma. Impairment of stimulus-induced transcription factor activity may contribute to the reduced inflammatory response and the neuroprotective effects of early post-traumatic hypothermia. (Supported by NINDS # 1P50NS30291).

P303.

TRAUMATIC FRONTAL LOBE INJURY IN RATS CHRONICALLY AFFECTS T-MAZE ALTERNATION: EFFECTS OF CYCLOSPORIN A.

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Experimental injury of frontal lobes using a controlled cortical impact (CCI) model results in necrotic cavitation in frontal and medial frontal cortex, sparing hippocampus. In an effort to establish a cognitive assay sensitive to cortical damage in the absence of hippocampal damage, we tested Long Evans hooded rats in a working memory paradigm following frontal cortex damage. A T-maze alternation task was selected for its sensitivity to medial frontal cortex injury. Rats were pre-trained to run the alleys, and beginning a week after bilateral frontal lobe injury they were trained to alternate alley choice for a food reward (10 trials daily for a maximum of 24 days). Rats were required to meet or surpass a criterion of 90% alternation on 2 consecutive training days. Total trials-to-criterion and total errors-to-criterion were measured. Performances of CCI rats were significantly impaired when compared to sham controls; committing more errors and requiring more trials to learn the task. Our laboratory has reported significant neuroprotection with post-injury administration of Cyclosporin A (CsA). To determine if CsA treatment may affect recovery on this cortically dependent cognitive task, CCI rats were separated into 2 groups: CCI alone, and CCI + CsA (at a dose regimen previously shown to be neuroprotective in the CCI model). CCI + CsA treated rats required no fewer trials and made no fewer errors to criterion than CCI alone rats. We conclude that this task is sensitive to enduring cortical damage in the absence of hippocampal damage and is therefore a good candidate for inclusion in a battery of tests of cortically dependent cognition in the rat. Supported by NIH NS39828 & KSCHIRT #9-20.

P304.

LONGITUDINAL ANALYSIS OF THE DICHOTOMIZED GLASGOW OUTCOME SCALE SCORE

Charles F. Contant, Delvida Long, Steve Pluth, H. Julia Hannay. (Baylor College of Medicine, Houston, TX US).*

The Glasgow Outcome Scale score (GOS) is well known. It is often used as the outcome measure for clinical studies, including clinical trials. The purpose of these analyses was to examine factors related to the GOS as measured at three time periods, instead of at a single time point. We have used a method that is well known in the statistical literature, but not often applied in neurotrauma.

The GOS was assessed at one, three and six months after injury. The following admission data were evaluated: age, pupillary reactivity, gender, presence of a gunshot wound, Marshall classification of the ER CT scan, and the ER motor component of the GCS. The GOS was dichotomized into two groups: Poor (Dead, PVS and SD) and Good (MD and GR).

A multi-level model was fit to the data. The logit of Poor outcome used as the dependent variable, where Poor outcome was assumed to follow a binomial distribution, resulting in a logistic regression like model. The predictors were all included as "fixed" effects, but a random baseline probability of being in the Poor outcome group was given to each patient. Data from 144 patients treated at Ben Taub General Hospital were used. In the initial analysis, age, gender and gunshot wound were removed from the model. The remaining variables were then evaluated using a Markov Chain Monte Carlo (MCMC) method. This is a simulation method for evaluating complex models of longitudinally measured dichotomous variables. Following 300,000 simulations, stability in the estimated coefficients was obtained, and these were evaluated. Pupillary reactivity, ERCT and ER GCS were all found to be significantly associated with Poor outcome. A significant time effect was found. The random baseline probability was also significant. While computationally intensive, the MCMC can provide valid estimates for a dichotomy evaluated over time.

P305.

ASSESSMENT OF TRAUMATIC AXONAL INJURY IN THE CORPUS CALLOSUM: A COMPARISON OF THE CORONAL AND SAGITTAL PLANES.

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Objectives: In conventional coronal sections of corpus callosum the automated assessment of axonal bulb size is complicated by the presence of filled axons. We have investigated axonal pathology in the sagittal plane to determine if this reduces the confounding factors for image analysis.

Materials and methods: Paraffin sections were cut in the coronal and sagittal planes from the corpus callosum of 7 cases of closed head injury (survival times from 12 hrs to 5 mths). Sections were immunostained using an antibody raised against amyloid precursor protein (APP). Digital images were captured and the size of axonal swellings determined using a previously developed algorithm.

Results: Assessment of the immunostaining in the sagittal plane revealed that the majority of profiles were round or oval in shape. with few axonal "tails". However, a new confounding factor was the heterogeneity of the staining intensity within individual axons, which posed a different set of problems for image analysis. Furthermore, quantitative analysis revealed no change in axonal diameter with increasing survival time in this small sample.

Conclusions: The use of sagittal sections of the corpus callosum does not confer any advantage over coronal sections in the measurement of axonal swelling size.

P306.

LONGITUDINAL ANALYSIS OF THE DISABILITY RATING SCORE FOLLOWING TRAUMATIC BRAIN INJURY

H. Julia Hannay, Steve Pluth, Delvida Long, Charles F. Contant. (Baylor College of Medicine, Houston, TX US).*

The Disability Rating Scale (DRS) described by Rappaport has been suggested as a possible outcome measure for clinical studies, including clinical trials. The DRS is a thirty-point scale with death at one end (30) and no disability at the other (0). The purpose of these analyses is to evaluate the relationship of the DRS to the generally used predictors of outcome that are derived from studies using the Glasgow Outcome Scale (GOS) as the outcome.

The DRS was measured at one, three and six months following injury in 144 patients from Ben Taub General Hospital. Extensive quality assurance of the DRS and GOS was performed. The DRS scores were used as the dependent variable in a multilevel longitudinal linear model. The independent variables were age, pupillary reactivity in the Emergency Center (EC), gender, presence of a gunshot wound (GSW), the Marshall classification of the EC CT scan and the motor component of the GCS measures in the EC (ECGCSM). Random effects of time were included in the model so that each patient had their own "slope" over time.

The model containing all the independent variables was fit, and those variable which were not significant at the $p < 0.10$ were removed and the model refit. Gender ($p = 0.59$), GSW ($p = 0.44$) and age ($p = 0.37$) were removed. The final model was highly significant. The effects of pupillary reactivity ($p < 0.001$), EC CT ($p < 0.01$) and ECGCSM ($p < 0.08$) were retained in the model. These relationships are consistent with those found in multivariable logistic regression models using the GOS, and indicate the DRS may have utility as an outcome measure. The use of the longitudinal modeling with random effects provides increased statistical power and further insight into the changes over time.

P307.

GENDER SPECIFIC ACTIVITY AND FOOT-FAULT PERFORMANCE ON THE GRID TASK AFTER CASTRATIONS AND OVARECTOMIES

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During locomotion on the grid-footfault task, we have female rats to be significantly more active than there male counterparts. This increase in activity is correlated with an increased number of foot-faults during the trial. Previous literature suggests that the difference in activity may be hormonally mediated. In order to test this hypothesis we took male and female Long-Evans rats, approximately 100 days of age and performed castration procedures on male rats and ovarectomies on female rats. Additional subjects were used as normal male and female controls. After a 30-day recovery period the subjects were tested on the grid-footfault task along a regular two-month testing schedule. During the sixty-second videotaped trial the total activity, as measured by line crossings, and number of footfaults per limb were quantified. Results of line-crossing activity showed female rats, either normal (Mean of summed activity 46.8) or operated (Mean sum 46.8), to be more active than normal male (Mean sum 33.5) or operated male (Mean sum 42.6) counterparts. For left forelimb fault performance, normal females showed mean sum of 15.0, and normal males 8.5. Operated females presented a mean sum of 18.8 and operated males 18.0. Right forelimb fault performance was similar, with normal females presenting a mean sum of 14.4 and normal males 9.5. Operated females presented a mean sum of 18.8 and operated males 13.6. The data show a trend for operated males to show a slight increase in activity compared to normal males, and operated subjects, both male and female, to trend towards greater forelimb fault performance on the grid task. Supported by a research grant from the New York College of Osteopathic Medicine.

P308.

CEREBRAL AMYLOID ANGIOPATHY AND TRAUMATIC BRAIN INJURY: THE EFFECT OF APOLIPOPROTEIN E GENOTYPE

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Objectives: Possession of APOE e4 is associated with poor outcome after traumatic brain injury (TBI). Cerebral amyloid angiopathy (CAA) is characterised by the accumulation of A_ peptide in the walls of cerebral blood vessels. The major clinical manifestation of CAA is spontaneous intracerebral haemorrhage for which e4 is also a risk factor. We hypothesised that e4 carriers have worse outcome after TBI in part due to CAA related susceptibility to haemorrhage.

Materials and methods: We have determined the frequency of CAA and the extent of the haemorrhagic pathology in relation to APOE genotype in 88 autopsy cases of TBI.l Results: CAA was present in 7 of 40 e4 carriers compared with 1 of 48 non-e4 carriers ($p = 0.021$) with 6 (4 with CAA) of the 40 e4 carriers being homozygotes. There was also a tendency for patients with CAA to have more severe contusion injury (median contusion index 19 versus 14.5. $p = 0.23$).

Conclusions: Among head-injured patients (i) CAA occurs predominantly in APOE e4 carriers (ii) patients with CAA tend to have more severe contusions and (iii) this association may explain in part why e4 is associated with poor outcome after head injury.

P309.

TETRAHYDROBIOPTERIN AND L-ARGININE AFTER CORTICAL IMPACT INJURY IN RATS

L. Cherian, R. Hlatky & C.S. Robertson. (Baylor College of Medicine, Houston, TX US).*

This study compared the effects of tetrahydrobiopterin (BH4), an essential co-factor for nitric oxide synthase (NOS), and L-arginine on cerebral blood flow after severe controlled cortical impact injury (CCII) in rats.

Fasted Long Evans rats were anesthetized with isoflurane and subjected to severe (5 m/sec, 3 mm deformation) CCII. Rats received L-arginine (300mg/kg), BH4 10mg/kg, or saline 5 min after injury. Laser Doppler flow (LDF) and nitric oxide (NO) were measured at the impact site for 2hr after injury.

In saline-treated rats, LDF decreased to 28% of preinjury values and NO decreased by 20+ 3.8nM. L-arginine increased LDF to 65% of preinjury levels. BH4 augmented LDF to 75% of preinjury values. Both L-arginine and BH4 normalized tissue NO concentrations.

Since both L-arginine and BH4 restore tissue NO levels after CCII, this study suggests that uncoupling of NOS contributes to the low tissue levels of NO that occur in the contused tissue. An increase in CBF accompanies the restoration of normal NO levels suggesting that the low NO levels have a role in the secondary ischemia that occurs in contused tissue.

P310.

A META-ANALYSIS TO DETERMINE THE SIGNIFICANCE OF SKULL FRACTURE AS A RISK FACTOR FOR INTRACRANIAL PATHOLOGY IN THE PAEDIATRIC POPULATION

Joel Desmond, John Batchelor. (Manchester Royal Infirmary, Manchester, Manchester UK).*

Objectives: Triage of minor head injuries in children in the UK relies heavily on the skull radiograph. This has been largely replaced by Computed Tomography in the USA. We sought to perform a meta-analysis of the paediatric literature to assess the significance of skull fracture and intracranial Pathology (ICP).

Methods: The literature was searched using Medline, Embase and the Cochrane Database. Reference lists were crosschecked. Once all papers had been searched a common odds ratio was determined.

Results: 12 papers (see poster for references) were identified as satisfying criteria for inclusion in the meta-analysis. Data was extracted from these papers relating to either positive CT scan or positive ICP and the presence of a skull fracture. An Odds Ratio was calculated for each paper and a common odds ratio was calculated using the Mantel-Haenszel test with a pooled estimate.

The Pooled results of the papers gave a total sample size of 12,684. The common odds ratio was 2.151 (1.85 to 2.48) therefore suggesting a positive correlation between skull fracture and intracranial pathology.

The pre-test probability of intracranial pathology was 7.2%. After a SXR the post-test probability of intracranial pathology if skull fracture was not found was 4% and if a skull fracture was found the post-test probability was 16%.

Conclusions: There is a significant correlation between skull fracture and intracranial pathology shown in the literature but due to the low incidence of intracranial pathology the finding of a fracture only increases the probability of ICP from 7.2% to 16%, which in our opinion is of little use in the triage of children with minor head injury.

We are currently completing a prospective study of 22,000 children with head injury in the UK to provide an alternative decision rule to replace the SXR based protocol.

P311.

EFFECTS OF DIETARY CREATINE ON NEUROCHEMICAL MAKERS OF SECONDARY INJURY FOLLOWING TRAUMATIC BRAIN INJURY

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Biochemical alterations following traumatic brain injury (TBI) include lactate acidosis and phospholipid degeneration, leading to the generation of free fatty acids (FFA) and lactic acid, markers of cellular injury. We have previously shown that animals fed a creatine (Cr) enriched diet demonstrate enhanced neuroprotection following TBI. To further characterize the neuroprotective qualities of dietary creatine we studied neurochemical changes in cortex and hippocampus following a moderate injury. Adult rats were fed either a control or Cr-supplemented diet (0.5%, 1%) for two weeks prior to TBI. At 30 min or 6h after injury, animals were subject to in situ brain freezing and tissue processed for levels of lactic acid and FFA. At 30 min post TBI, lactate was significantly increased in all tissues ipsilateral to the injury. Animals fed Cr-diet had significantly lower levels although elevated compared to sham controls. Accumulation of FFA was also significantly diminished at 30 min post injury in Cr-diet animals and in many cases not significantly different from sham controls. At 6h post-injury, levels of lactic acid were significantly elevated following TBI. Creatine-fed animals showed less lactate than animals fed the control diet. Levels of FFA were significantly higher at 6h in the control diet animals with Cr-diet animals less than control diet but above sham levels. In most regions, animals fed a 1% Cr-diet demonstrated lower levels for both lactate and FFA than animals fed a 0.5% Cr-diet. These results support the idea that a Cr-enriched diet can provide substantial neuroprotection in part by suppressing the accumulation of lactic acid and FFA. The fact that a 1% Cr-diet was more protective and a 0.5% diet suggest a possible dose response intervention. Supported by NIH NS39828 and KSCHIRT #9-20.

P312.

THE PITFALL OF BRAIN HYPOTHERMIA MANAGEMENT IN NEUROTRAUMA PATIENTS

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Purposes: The effectiveness of hypothermia to the brain injury has been demonstrated in many experimental animal models. However, clinical trials of hypothermia for severe brain injury are still controversial. Why these different results occur? Without understanding of these mechanisms, the clinical hypothermia treatment will be fail. We have studied about pitfall on the brain hypothermia treatment in experience of 11 years, retrospectively.

Clinical studies: One hundred fifty cases of GCS < 6 neurotrauma patients were treated by brain hypothermia. All of these patients were cared with monitoring of brain tissue temperature, internal jugular venous temperature, core temperature, SjO2, cardiac output, oxygen delivery and extraction ratio, ICP, BBB dysfunction (CSF / serum albumin ratio < 0.01), and hemoglobin function. The brain hypothermia, in 30 cases, managed with monitoring of brain tissue glutamate, lactate, glucose, and glycerol by micro-dialysis technique and also hypothalamus-pituitary adrenal (HPA)-axis neurohormones changes in CSF and blood were studied. The pitfall of brain hypothermia was focused all of brain hypothermia management.

Results: The brain injury mechanism is not similar with human to experimental animal models in severe brain injury. The 20–100 times severe catecholamine surge was recorded that was not observed anesthetized experimental animal models. This excess stress to HPA axis produced more than 230mg insulin resisted hyperglycemia, and no effect of oxygen inhalation by difficult release of oxygen from binding hemoglobin with reduced hemoglobin DPG. Therefore, normal control of ICP, CPP, CBF and PaO2 is not enough management. The precise control of serum glucose between at 120–140mg/dl prevents these pitfalls. Especially, metabolic shift from glucose to lipid at lower than 34Åé of brain temperature, produces more easily increasing of serum glucose increasing and brain tissue lactate. Uncontrolled serum glucose make much worse at 32-33Åé than 34Åé of brain temperature. The complication of pneumonia under presence of severe BBB dysfunction (CSF / serum albumin ratio < 0.01) fails the hypothermia treatment. Proinflammatory cytokines easy go into the injured brain tissue and produces uncontrollable increasing of neuron toxic glutamate. The prevent of immune dysfunction by intermittent control of brain temperature between at 32-34Åé, replacement of pituitary hormones, and control of serum albumin >3.5g/dl are important. The 2 days short duration of hypothermia make stop of progression of brain damage than restoration. The re-progression of injury mechanism at rearming make much worse by over lapping of rewatming stress in severe brain injury. Without evidence of recovery of brain damage, rewarming is not indicated.

Conclusion: The management of brain hypothermia with control of above pitfalls produces excellent clinical results.

P313.

TREATMENT OF COLD INJURY-INDUCED BRAIN EDEMA WITH A NONSPECIFIC MATRIX METALLOPROTEINASE INHIBITOR MMI270 IN RATS

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Blood-brain barrier (BBB) disruption is a critical event leading to vasogenic brain edema after cold injury-induced brain trauma. Matrix metalloproteinases (MMPs), proteolytic enzymes which degrade the extracellular matrix, are implicated in BBB disruption in this model. This study was conducted to examine the effects of MMI270, a synthetic nonspecific MMP inhibitor, on cold injury-induced brain edema in rats. Cold injury was induced by applying a metal probe cooled with liquid nitrogen on the skull for 30 seconds. Treatment of MMI270, a bolus injection at a dose of 30 mg/kg, was started immediately after the induction of cold injury and was continued for 24 hours (40 mg/kg/day) using an intraperitoneal osmotic minipump (n = 7). In the untreated control group (n = 7), rats were administrated a vehicle and implanted with a vehicle-containing osmotic pump. Two percent Evans Blue (EB) in saline (1 ml/kg) was administrated intravenously immediately after the cold injury in another group of rats, 6 of which were untreated and 6 of which were treated with MMI270 at the above dose. Compared with the untreated control group, treatment with MMI270 significantly reduced the brain water content in the ipsilateral core area (83.9 ± 0.5 vs. 83.0 ± 0.7%; $p < 0.05$) and protect the BBB integrity to EB in the ipsilateral core area (65.3 ± 18.1 vs. 33.5 ± 20.0 ng/g wet tissue; $p < 0.05$) at 24 hours after the cold injury. These results indicate that treatment with MMI270 in rats exhibits protection in acute brain edema formation by attenuating the BBB permeability after cold injury.

P314.

BONE MARROW STROMAL CELL TRANSPLANTED TO TRAUMATICALLY INJURED RODENT BRAINS MAY AID IN THEIR RECOVERY THROUGH PRODUCTION OF NERVE GROWTH FACTOR

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Recent reports demonstrated that bone marrow transplantation to brain parenchyma improved neurological outcomes in a rodent model of traumatic brain injury (TBI) (NeuroReport 12:559–63, 2001). We found that intraventricular injection of bone marrow stromal cells (BMSCs) in TBI mice reduced the brain lesion. However, the underlying mechanisms for the beneficial effects of BMSC transplantation are not clear. In this study, 4 groups of 5 ICR mice were used: 1) TBI with BMSC injection; 2) TBI with PBS injection; 3) sham-injury with BMSC transplantation; 4) sham-injury with PBS injection. BMSCs were harvested and cultured from the green fluorescence protein (GFP) transgenic mice and were transplanted (2 × 105/10 ul PBS/mouse) into the ipsilateral ventricle at 5h post cortical impact injured mouse. At 13 and 45 days post-transplantation, mice were euthanized, CSF was collected and frozen brain sections were processed for data analyses. By 45d, the transplanted BMSCs migrated to the boundary of the injured area. Histological analyses showed that tissue lesion volume was significantly ($P < 0.05$) reduced in group1 compared with group2. NGF ELISA demonstrated significantly ($P < 0.01$) higher NGF levels in CSF samples from group 1 and 3 at either time point compared with group 2 and 4, respectively. We conclude that NGF production may contribute to the protection effects of BMSC transplantation in TBI mouse brains. Characterization of NGF producing BMSC is currently in progress. (Supported by NIH grant RO1-NS35502-05 and ATP grant 004949-0074).

P315.

THE SPECIAL CONSIDERATION OF MANAGEMENT OF VEGETATION IN BRAIN HYPOTHERMIA TREATMENT

Nariyuki Hayashi. (Nihon University School of Medicine, Itabashi-ku, Tokyo JP).

Purposes: We have experienced brain hypothermia treatment 400 cases in severe trauma stroke and cardiac arrest. Neuronal recovery without memory and emotional disturbances is important. We have hypotheses that selective radical damage of dopamine A10 nervous system produces mind and memory disturbances. previously. In this paper. mechanism of vegetation. diagnostic method of reversibility of vegetation. and special consideration of brain hypothermia management for protection and restoration of vegetation are studied.

Clinical studies: One hundred cases of GCS less than 6 were treated brain hypothermia treatment. The brain tissue temperature controlled at 32-33Åé for 7–10 days. The management is focused on control of ICP. brain edema. CBF and also management of oxygen carrier hemoglobin function. maintaining of lipid metabolism with 120–140mg/dl of serum glucose. and hazard of hypothalamus-pituitary-adrenal (HPA) excess hormones. The prevent of excess release of hypothalamus dopamine in acute stage. and maintaining of dopamine A10 nervous function by in chronic stage were treated for management of vegetate state. The diagnosis method of reversibility of vegetate state was studied by responsiveness of neurotransmitter using micro-dialysis and biochemical CSF analysis.

Results:: In cute stage. early induction of brain hypothermia into the 32–33Åé brain hypothermia combined with administration of Metoclopramide are very effective for prevent of NO radical production. The 34Åé of brain hypothermia could be prevent only 50% of NO2 production. The responsiveness of no need outer stimulation consciousness such as volition. emotion. thinking. understanding. and love was diagnosed by increasing of CSF Dopamine / Ploractin ratio. The reversibility of vegetate state was evaluated with simultaneous responsiveness of CSF norepinephrine. dopamine. serotonin and Dopamine / Ploractin ratio. The combination therapy of pharmacological treatment of Leodopal and Amantadin. Estrogen Patch. Median nerve stimulation with 10–20mA/20sec.on/30sec.off. 30pulse/sec.. duration time; 300mm/sec.. and music therapy were effective for restoration of vegetate state. Good recovery was recorded in GCS; 3. 4. 5 and 6 was 8%. 29%. 26% and 34% respectively. The vegetation was very few in GCS; 3.4.5 and 6 was 0%. 5%. 7% and 5%. respectively. The mortality was in GCS; 3. 4. 5. and 6 was 84%. 52%. 26% and 42%. respectively.

Conclusion: The early induction brain hypothermia lower than 34Åé and combined management of dopamine A10 are very useful for prevent of vegetation. The special management technique for damage of dopamine A10nervous system is developed.

P316.

EFFECTS OF HYPOTHERMIA AND ALKALIZING AGENTS ON BRAIN INJURIES IN RATS WITH ACUTE SUBDURAL HEMATOMAS

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Brain ischemia is the leading pathophysiological mechanism in the development of secondary brain damage after acute subdural hematoma (SDH). Hypothermia has been employed as an effective cerebroprotective treatment on brain injuries, but the control of the general condition is very difficult under hypothermia, and various severe complications have been reported. Cerebral acidosis in the ischemic area is one of the important factors augmenting the brain edema formation. Tris-(hydroxymetyl)-aminomethane (THAM) has been used as an alkalizing agent for acidosis on brain injury and is reported to be effective. In the present study, we used a rat acute SDH model to assess the effect of mild (35 centigrade) hypothermia and THAM combined treatment on brain water content, brain ischemia, and blood-brain barrier (BBB) permeability at 4 hours after hematoma induction. Mild hypothermia did not significantly reduce the brain water content beneath the hematoma (79.5%) compared with normothermia (80.2%), but mild hypothermia combined with THAM presented a significant reduction (78.7%: $p < 0.01$). Combined with mild hypothermia and THAM treatments significantly reduced the Evan's blue extravasation (35ng/g wet tissue: $p < 0.05$) compared with normothermia (63ng/g wet tissue). Furthermore, volume of infarction at 24 hours after the hematoma induction (54mm3: $p < 0.01$) was significantly smaller by the combined treatment compared with normothermia (70 mm3). The present findings indicate that mild hypothermia of 35 centigrade combined with THAM presents a potent cerebroprotective strategy. The protection of the BBB is one of the possible cerebroprotective mechanisms in this rat acute SDH model.

P317.

DIETARY CREATINE SUPPLEMENTATION ENHANCES COGNITIVE RECOVERY FOLLOWING EXPERIMENTAL RAT BRAIN INJURY

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The food supplement creatine is neuroprotective against several different brain insults including, ischemia, oxidative stress and traumatic brain injury. In rats, chronic administration of creatine significantly reduces the extent of brain damage caused by a cortical contusion injury (CCI), possibly through stabilization of mitochondrial integrity. This study tested whether the tissue sparing effect of creatine is associated with enhanced functional recovery following brain injury. Adult male rats received dietary supplementation with 1% creatine for 2 weeks prior to a 1.5mm CCI injury, 2 weeks following CCI or in both the pre-and post-surgical intervals. Starting on the 15th day following CCI, the animals were tested in the Morris Water maze (MWM) for 4 trials per day, on 5 consecutive days. Following the last trial on the fifth day, the platform was removed and animals were retested. CCI caused significant impairment in MWM performance, which was attenuated by creatine when administered in the combined pre-and post-surgical intervals. Creatine enhanced performance in both the acquisition and probe test phases of testing. Creatine supplementation did not enhance cognitive performance in sham-operated animals. Following behavioral testing, brains were prepared for quantitative analysis of alpha 7 nicotinic receptor expression. 1.5mm CCI caused a significant reduction in the density of hippocampal and cortical alpha 7 nicotinic receptor binding, which was reversed by chronic creatine administration. These results indicate that the neuroprotective effects of creatine are associated with enhanced cognitive recovery following CCI. Attenuation of CCI-induced neurochemical changes may contribute to creatine-induced functional recovery. Supported by the Kentucky Spinal Cord and Head Injury Research Trust and NIH (NS39828 to SWS and NS42196 to JRP).

P318.

AGE RELATED EFFECTS OF ACUTE NMDA BLOCKADE ON FUNCTIONAL OUTCOME AFTER CONTROLLED CORTICAL IMPACT IN IMMATURE RATS

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Rationale: Anti-excitotoxic strategies have been shown to paradoxically exacerbate neuronal death after traumatic injury in the developing brain. We sought to investigate the age-related effects of N- methyl, D- aspartate (NMDA) blockade on functional outcome after experimental traumatic brain injury (TBI) in the developing rat.

Methods: Using our contemporary models of controlled cortical impact (CCI), postnatal day (PND) 7 and 17 Sprague Dawley rats were injured (left, frontoparietal, 4 m/sec, 1.75 or 2.0 mm deflection, and 3 or 6 mm tip respectively) and treated acutely with a single dose of MK-801, 30 min pre-CCI, i.p. (0.25, 0.5, or 1.0 mg/kg) vs vehicle. Morris water maze (MWM) performance was then evaluated on post-injury days (PID) 11- 17.

Results: In PND 7 rats, while a single, pre-injury dose of 0.25 mg/kg neither worsened nor improved MWM performance after CCI, higher doses of 0.5 and 1.0 mg/kg significantly worsened MWM performance. In contrast, in PND 17 rats, dose escalation from 0.25 to 0.5 mg/kg significantly improved MWM function as compared to vehicle; though this beneficial effect was lost with further dose escalation to 1.0 mg/kg.

Conclusion: Acute treatment of PND 7 rats following CCI with an NMDA antagonist adversely impacted functional outcome while NMDA blockade with MK-801 in PND 17 rats improved MWM function. These findings further support our hypothesis that there are critical age-related injury responses to therapies. Potential therapeutic modalities for pediatric TBI must be evaluated experimentally across a broad range of developmental ages prior to clinical trials.

(Supported by NIH [NS01809/ NS40049] -PDA)

P319.

ENDOTHELIN-1 CONTRIBUTES TO AGE DEPENDENT G PROTEIN IMPAIRMENT AFTER BRAIN INJURY

William M. Armstead (University of Pennsylvania, Philadelphia, PA US).

Previous studies have observed that endothelin-1 (ET-1) concentration is elevated in CSF and contributes to impaired cerebral hemodynamics following fluid percussion brain injury (FPI) in an age dependent manner. This study was designed to characterize the effects of FPI on the vascular activity of two activators of a pertussin toxin sensitive G protein, mastoparan and mastoparan-7, as a function of age and the role of ET-1 in such effects in newborn (1–5 days old) and juvenile (3–4 weeks old) pigs equipped with a closed cranial window. Mastoparan (10-8, 10-6M) elicited pial artery dilation that was blunted more by FPI in newborn vs juvenile pigs (9 ± 1 and 16 ± 1 vs 3 ± 1 and 5 ± 1%, newborn; 9 ± 1 and 15 ± 1 vs 6 ± 1 and 9 ± 1%, juvenile). Similar results were observed for mastoparan-7 but the inactive analogue mastoparan-17 had no effect on pial diameter. BQ123 (10-6M), an ET-1 antagonist, partially restored impaired mastoparan dilation after FPI in the newborn but not in the juvenile (3 ± 1 and 5 ± 1 vs 7 ± 1 and 11 ± 1%, newborn; 6 ± 1 and 9 ± 1 vs 6 ± 1 and 10 ± 1%, juvenile). These data show that G protein activation elicits cerebrovasodilation that is blunted following FPI in an age dependent manner. These data suggest that ET-1 contributes to G protein activation induced dilator impairment post insult in an age dependent manner.

P320.

INCIDENCE AND PROGRESSION OF INTERCELLULAR CA2+ WAVES IN ASTROCYTES SURROUNDING AREAS OF MECHANICAL INJURY

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One possible avenue of communication between astrocytes during mechanical injury is through calcium waves, which are propagating increases in cytosolic calcium concentrations in cells. In this study, we examine the calcium waves that occur in astrocytes populations adjacent to regions of astrocytes subject to mechanical stretch. Astrocytes were cultured on flexible membranes and loaded with the calcium fluorescent dye fura-2. Cells were stretched at one of three magnitudes-low (1–2%), moderate (3–6%), and high (12–17%). Cells stretched at the low magnitudes experienced no significant increase in cytosolic calcium levels ($p > .144$) but were able to induce calcium waves in cells adjacent to the injury area, leading to a 15.9 ± 1.2 fold increase in fluorescent ratio in the unstretched cells ($p < .001$). Cells stretched at higher levels undergo an ATP-independent calcium rise and also initiate calcium waves in the adjacent region. Apyrase, which hydrolyzes ATP, attenuated the calcium wave in the unstretched region but had no effect on calcium changes in the stretched cells. The dramatic increase in astrocytic [Ca2+] at very low levels suggests that astrocytes can coordinate a response even under the mildest forms of injury, and pose and additional factor that can affect neuronal signaling following mechanical injury. Funds were provided by NIH NS 35712 and HD 41699.

P321.

THE EFECTS OF VITAMIN B3 (NICOTINAMIDE) ON BEHAVIORAL OUTCOME FOLLOWING BILATERAL FRONTAL CORTEX CONTUSION INJURY IN THE RAT.

M.R. Hoane and S.L. Akstulewicz. (Brain Injury Laboratory, Department of Psychology and Program in Neuroscience, East Carolina University, Greenville, NC USA).*

Previous studies have shown that administration of vitamin B3 (B3) in stroke models significantly reduced the size of infarction and improved functional recovery. The present study evaluated the effect of administration of B3 on recovery of function following traumatic brain injury (TBI), incorporating the bilateral medial frontal cortex contusion injury model. Groups of rats were assigned to B3 (500 mg/kg) or saline (1.0 ml/kg) treatment conditions and received contusion injuries or sham procedures. Drug treatment was administered 15 min and 24 hr following injury. Rats were examined on a variety of tests to measure sensorimotor performance (bilateral tactile removal), skilled forelimb use (staircase test), and cognitive ability (reference and working memory) in the Morris Water Maze. Preliminary results indicated that administration of B3 following injury significantly reduced the behavioral impairments observed on the bilateral tactile removal test, but not on skilled forelimb use. The acquisition of reference and working memory tests were also significantly improved compared to saline-treated rats. Examination of the brains revealed that administration of B3 significantly reduced the size of the lesion compared to treatment with saline. In addition, examination of glial fibrillary acidic protein (GFAP) expression around the lesion revealed that B3 significantly reduced the number of GFAP+ astrocytes. Our results indicate that B3 administration significantly improved behavioral outcome following injury, reduced the size of the lesion, and reduced the expression of GFAP. These findings suggest that B3 may have therapeutic potential for the treatment of TBI. This research was supported by an ECU Faculty Senate Research/Creative Activity Grant.

P322.

THE EFFECT OF AGE ON SENSORIMOTOR AND COGNITIVE RECOVERY FOLLOWING BILATERAL FRONTAL CORTEX CONTUSION INJURY IN THE RAT

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The elderly are one of the most at risk populations for sustaining traumatic brain injuries (TBI). However, the effect of age is rarely studied in animal models of TBI. The present study evaluated the effect of age on recovery of function following bilateral medial frontal cortex injury. Groups of young (2.5 months old) and old (~14 months old) rats received either bilateral frontal cortex contusions or sham procedures. The rats were tested on a variety of tests to measure sensorimotor performance (bilateral adhesive tactile removal test), skilled forelimb use (staircase test), and the acquisition of a reference and working memory task in the Morris Water Maze (MWM). Preliminary results indicated that bilateral frontal cortex injury produced significant impairments on the bilateral adhesive tactile removal test, staircase test, and on the acquisition of a reference and working memory task compared to sham controls. Aged rats that received cortical contusions were significantly impaired on the bilateral adhesive tactile removal test, staircase test, and on the acquisition of a reference memory task compared to young rats. There was no effect of age on the acquisition of the working memory task; however, the aged rats had received extensive pre-operative spatial memory training 4–5 months prior to injury. This pre-operative training may have prevented the acquisition impairment in working memory in the MWM. Our results indicate that aged rats respond to brain injury differently than young rats. An ECU Faculty Senate Research/Creative Activity Grant supported this research.

P323.

SECONDARY CEREBRAL ISCHEMIA-INDUCED CA1 HIPPOCAMPAL CELL DEATH: LATERAL VS CENTRAL FLUID PERCUSSION INJURY IN RAT.

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Secondary insults after fluid percussion injury are known to exacerbate cell damage particularly within the hippocampus. Secondary cerebral ischemia has been shown to enhance cell death following central fluid percussion injury (CFPI). However, markedly different results have been reported following lateral fluid percussion injury (LFPI) (Otori, J. Neurotrauma 18:1131, 2001). To compare these models, male Sprague-Dawley rats were subjected to moderate CFPI or LFPI and 8 min forebrain ischemia was carried out at 1h following injury. The animals were divided into the following 4 groups (1) LFPI+ischemia, (2) CFPI+ischemia, (3) LFPI alone and (4) CFPI alone. Mean arterial blood pressure and blood gases were monitored. To assess the neuronal cell damage in the CA1 region, animals were sacrificed at 7 days following injury and the surviving cells were counted stereologically in cresyl-violet-stained sections. In all injured animals, secondary ischemia resulted in additional cell loss within the CA1 region of the hippocampus only when PCO2 levels during the secondary insult were below 30 mmHg. Within these animals, CFPI+ichemia resulted in more cell death within the hippocampus compared to LFPI+ischemia (number of surviving cells; Left. 71 ± 19 vs 131 ± 48, Right. 88 ± 32 vs 279 ± 34). Not surprisingly, LFPI+ischemia resulted in more cell death within the ipsilateral hippocampus compared to the injury alone (number of surviving cells; 131 ± 48 vs 247 ± 29, $p < 0.05$). Secondary cerebral ischemia never resulted in more surviving cells compared to injury alone. Preliminary work indicates that the FPI results in low levels of ATP in the CA1 region up to 2h. Additional ATP measurement using bioluminescence will confirm if secondary ischemia creates an additional loss of energy, which may explain the injury-induces vulnerability to secondary ischemia. [Supported by NS27544, NS30308, NS37363]

P324.

CEREBRAL SPINAL FLOW (CSF) DYNAMICS IN PATIENTS WITH POSTTRAUMATIC HYDROCEPHALUS: PHASE-CONTRAST MRI DATA.

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Objective: to demonstrate the efficiency of phase-contrast MRI with cardiogating for controlling results of ventriculoperitoneal shunting (CSF dynamics evaluation) in patients with open posttraumatic hydrocephalus and in the nearest postoperative period.

Material and methods: All MR-examinations were performed using high-field MRI before and after surgery: MR study included: T1-W1, T2-WI, phase-contrast MRI in sagittal orientation and across aqueduct. The values of linear and volume velocities of CSF in 3-d ventriculostoma and cerebral aqueduct were evaluated. We examined 11 patients with posttraumatic open hydrocephalus. All examinations were performed before and immediately after shunting, and during following 2 weeks in every 3-rd day.

Discussion: Results of dynamic examination of the patient with posttraumatic hydrocephalus: mean linear velocity amplitude (LVA) was 22,1 cm/sec before and 12,3 cm/sec after shunting (5,6 ± 0,7 cm/sec in norm). The mean CSF amounts in cerebral aqueduct, moving per one cardio-cycle, – stroke volume (SV) were 560 ml before surgery, 170 ml after surgery (56 ± 25 ml in norm). Clinically positive reaction was marked. In our experience CSF pulsation considerably exceeded the normal value ($P < 0,001$), thus making it possible to prognose favourable outcomes after surgery.

Conclusion: Phase-contrast MRI at the level cerebral aqueduct showed the decreasing of CSF pulsation after shunting for open posttraumatic hydrocephalus. These alterations remained for 2 weeks after surgery in all patients.

P325.

DIFFUSE AXONAL INJURY IN INTENTIONAL INFANT INJURY SYNDROME VICTIMS IS ACCOMPANIED BY EVIDENCE OF EXTERNAL TRAUMA TO THE HEAD

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Although intentional brain injury in infants was initially postulated to be due to violent shaking of the head, the need for head impact has recently been argued. The phrase "shaken baby syndrome" has been used frequently while others have preferred "shaken impact syndrome." We prefer "intentional infant injury syndrome" (IIIS) since it does not presuppose a mechanical mechanism. Pathological findings in non-survivors of IIIS commonly include subdural (SDH) and subarachnoid hemorrhages (SAH), bilateral retinal hemorrhages (RH), cerebral edema and diffuse axonal injury (DAI) with minimal signs of external trauma. Antibodies to beta-amyloid precursor protein (b-APP) have reliably detected DAI in adults and we sought to determine whether b-APP immunostaining might detect DAI in suspected IIIS. Hospital records and police reports were used to identify abuse cases. Autopsies on 10 children documented external and internal evidence of head injury. IIIS was suspected in 9 cases. One death was due to motor vehicle crash (MVC) and one was due to sepsis. B-APP immunostaining of selected brain regions was performed without knowledge of whether injury was intentional or not. b-APP staining was not observed in the MVC victim or in one suspected IIIS victim with skull fracture. Little b-APP staining was found in the sepsis victim. Of the remaining 7 cases, b-APP staining of dystrophic axons and retraction bulbs was observed in corpus callosum and thalamus. All 7 victims presented with head or facial contusions, but without skull fractures. SDH and/or SAH and brain edema was seen in all cases. Bilateral RH was noted in 6 of 7 cases.

Conclusion: b-APP immunostaining can identify DAI in suspected cases of IIIS. While the post mortem hallmarks of IIIS were found, they were invariably accompanied by external evidence of abuse. This preliminary study suggests a positive correlation of DAI with external impact to the head.

P326.

QUANTIFICATION OF SECONDARY BRAIN DAMAGE AFTER CONTROLLED CORTICAL IMPACT IN MICE

Christian Erös, Seong Woong Kim, Klaus Zweckberger, Ricarda Zimmermann, Alexander Baethmann and Nikolaus Plesnila. (Institute for Surgical Research, Munich, DE).*

Introduction: The most important constituents of secondary brain damage from traumatic brain injury (TBI) with contusions are brain edema formation and delayed contusion expansion. In order to quantify secondary brain damage after the controlled cortical impact (CCI) model we investigated contusion volume and brain edema formation during the first 24 h after trauma.

Materials & Methods: Male C57/Bl6 mice (n = 48) were craniotomized and subjected to CCI (8 m/s. 1 mm). The craniotomy was closed thereafter. Brain water content and contusion volume were assessed 6. 12. 24. and 48h and 15 min. 6. 12. and 24h after trauma.respectively.

Results: Brain water content increased continuously from 78.1 ± 0.4 % in sham operated animals to a maximum of 81.1 ± 0.7 % ($p < 0.05$) in the ipsilateral hemisphere 24 h after CCI. No significant increase was detected on the contralateral side. The contusion was clearly demarcated already 15 min after trauma (19.4 ± 4.0 mm3). 6 and 24 h later the contusion increased to 131% (25.4 ± 3.1 mm3; $p < 0.05$ vs. 15 min) and 171% (33.2 ± 3.0 mm3; $p < 0.05$ vs. 15 min and 6h) of its initial volume 15 min after CCI (100%). respectively.

Conclusion: The size of a cortical contusion is expanding significantly after closed head CCI. This secondary contusion expansion is paralleled by brain edema formation. Our data demonstrate on a quantitative basis that parenchymal loss in the vicinity of a cortical contusion is an ongoing process and amendable to therapy due to its delayed character.

P327.

ROLE OF BRADYKININ B2 RECEPTORS FOR SECONDARY BRAIN DAMAGE AFTER TRAUMATIC BRAIN INJURY IN MICE

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Introduction: Bradykinin B2 receptors may be involved in the pathophysiology of traumatic brain injury (TBI). However. direct evidence is missing yet. In the present study we investigated contusion volume. brain edema and functional outcome of bradykinin B2 receptor knock out (B2 Ko) and wild type (WT) mice after experimental TBI.

Materials & Methods: B2 Ko and WT mice (n = 7 each) were subjected to controlled cortical impact injury (CCI. 8 m/s. 1 mm indentation). Brain water content and contusion volume were assessed after 24 h and after 7 days. respectively. Hind paw misplacements during beam walking were counted daily 4 days before and 7 days after CCI. WT mice had a contusion volume of 7 days after CCI.

Results: Deletion of the B2 gene resulted in a reduction of contusion volume by 33% as compared to WT mice (9.1 ± 1.4 mm3 vs. 13.5 ± 4.5 mm3; $p < 0.02$). In B2 Ko mice cerebral water content 24 h after trauma was reduced from 81.1 ± 0.7% in WT mice to 79.6 ± 0.4% (−51%; $p < 0.05$). Functional outcome was significantly better on day 5 post trauma in B2 Ko mice as compared to WT mice (6.7 ± 3.2 and 12.2 ± 5.8 foot misplacements. respectively ($p < 0.05$).

Conclusion: Bradykinin B2 receptors mediate brain edema formation. loss of brain parenchyma. and loss of function after TBI. Therefore the bradykinin B2 receptor might represent a good target molecule for the development of drugs against secondary brain damage after TBI in man.

P328.

INCREASED HIPPOCAMPAL CA3 VULNERABILITY TO LOW LEVEL GLUTAMATE ANALOGUE, FOLLOWING LATERAL FLUID PERCUSSION INJURY.

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It is still debated whether high extracellular levels of glutamate are a cause or a consequence of secondary neuronal damage. We used a sublethal dose of the glutamate analogue kainic acid (KA) to determine whether a secondary acute increase in neuronal activity exacerbates anatomical damage in vulnerable hippocampal regions following a mild lateral fluid percussion (LFP) injury.

KA (9mg/kg) was injected intraperitoneally in sham (n = 7) and LFP (n = 16) injured rats 1 hour following injury. An equivalent volume of saline was injected in LFP injured (n = 5) rats. Histological damage (7 days) in the dorsal hippocampus (CA3, CA4, and hilar regions) was assessed (LFP+KA: n = 8, LFP+saline: n = 5, sham+KA: n = 5, and naive: n = 3) by two dimensional cell count. Seizures were rated by Racine classification in the same subgroup. Hippocampal activation 15 minutes following KA injection was assessed by glucose metabolic rates (CMRglc; umol/100g/min) using [18F]-fluorodeoxyglucose in LFP+KA (n = 4) and sham+KA (n = 2) rats. Following FPI+KA the ipsilateral side exhibited a 62.7, 75.7 and 52.1% decrease in CA3, CA4 and hilar neurons respectively compared to naive rats. These CA3 and CA4 neuronal counts were also significantly decreased compared to LFP+saline and sham+KA groups. The median Racine score in LFP+KA and sham+KA groups was 4 and 2 respectively ($p < 0.015$). CMRglc in CA3 following LFP+KA was 121.8 ± 3.9 (mean ± SD) ipsilaterally and 71.5 ± 10.8 contralaterally ($p < 0.0012$). No changes were found in the BBB permeability measured by 14Cα-aminoisobutyric (AIB) in CA3, CA4 and hilar regions.

We conclude that the low level presence of kainic acid acutely after LFP dramatically increases the extent of hippocampal activation and induces a striking loss of ipsilateral CA3 pyramidal neurons. (NINDS 30306; NS02089).

P329.

INTRACELLULAR CALCIUM SIGNALING IS PERTURBED IN ASTROCYTES AND MICROGLIA ISOLATED FROM HYDROCEPHALIC RATS.

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Hydrocephalus is often a secondary pathology associated with traumatic brain injury (TBI). Despite the prevalence of hydrocephalus in many neurological conditions. we know very little about the biochemical alterations in brain cells that lead to the development of hydrocephalus. In these experiments. mixed organotypic cultures of brain cells were isolated from 1–2 day old rat pups from either spontaneously hydrocephalic H-Tx strain or control Sprague-Dawley rats. Organotypic cultures contained a mixture of all brain cells. including astrocytes. microglia. and neurons. Using Fura-2 microspectrophometry and high speed digital imaging. calcium-mediated signal transduction pathways were examined in astrocytes and microglia from H-Tx and control rats. Astrocytes in cultures prepared from H-Tx rats exhibited a dramatically increased intracellular free calcium ([Ca2+]i) elevation in response to glutamate. as compared control astrocytes. suggesting that glutamate-mediated [Ca2+]i signaling is enhanced in H-Tx rats. Similar results were observed in microglia from H-Tx rats. in which the [Ca2+]i elevation elicited by glutamate was also increased as compared to controls. Intracellular calcium store-mediated signaling was examined with thapsigargin. which elicits release of calcium from intracellular stores in the endoplasmic reticulum. followed by capacitative influx of extracellular calcium. Astrocytes and microglia cultured from H-Tx rats displayed an increased thapsigargin-stimulated [Ca2+]i elevation. as compared to controls. suggesting that the capacitative calcium signaling pathway is enhanced in hydrocephalus. These results provide insight into the signal transduction mechanisms activated in hydrocephalus and suggest potential targets for intervention. Supported by NS40490 and Wade Center at HRI.

P330.

A COX2 INHIBITOR ATTENUATES CASPASE-3 ACTIVATION AND COX2 EXPRESSION FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT

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Understanding the details of neuronal "death pathways" may lead to new treatment paradigms in diseases from Alzheimer's and Parkinson's to cerebral vascular accidents and traumatic brain injury (TBI). Neural cells are isolated from the periphery by the blood brain barrier, use limited substrates for energy, and have dynamic electrophysiological traits. Consequently, many genes expressed in the central nervous system function differently than in the periphery. Cyclooxygenase-2 (COX2) is one such gene that has recently become the subject of intense investigation. However, the role of COX2 in neural development and neuropathology has yet to be determined.

Increased COX2 expression has been observed with TBI, cerebral ischemia, seizures, as well as chronic neurodegenerative conditions. We have found that DFU, a COX2-specific inhibitor, improves functional recovery in a rat model of TBI. In addition, this inhibitor protects neurons from glutamate-mediated neurotoxicity in cerebellar granule cell cultures. Our molecular findings indicate that glutamate receptors mediate COX2 mRNA induction in these neurons. We hypothesize that COX2 contributes to excitotoxic cell death following brain injury. Thus, a COX2 inhibitor should reduce cell death in vivo. Using the model of lateral cortical impact TBI, we show that treatments with DFU that improve behavioral recovery are neuroprotective. Immunohistochemistry shows an attenuation of caspase-3 activation. In addition, both IHC and immunoblot results indicate an attenuation in COX2 gene expression in brain regions associated with functional deficits following traumatic brain injury. This combination of in vitro and in vivo preclinical studies suggest exciting potential for this agent in the pharmacological treatment of TBI, and support the consideration of a Phase I clinical trial.

P331.

PATHOGENESIS OF "BRAIN LOW T3 SYNDROME" IN PATIENTS WITH SEVERE BRAIN INJURIES

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OBJECTIVES: A conceptualization of the crucial role of thyroid hormones in adult brain based on the discovery of the thyronergic system and strict brain thyroid homeostasis (BTH) regulation has involved in recent years. The goal of this study was to evaluate changes in BTH (based on TSH, T4, T3, free T4, free T3, TBG) and to correlate changes with biochemical markers of tissue injury (protein S100, neuron specific enolase) and the acute inflammatory response (TNFa, IL-1b, IL-6).

METHODS: Markers of thyroid function, tissue damage and inflammation in serum and CSF were evaluated by radioimmunoassays in 128 patients with traumatic brain injury (GCS <8) and in 75 patients with aneurysmal subarachnoid hemorrhage (48% Hunt-Hess score III-V). Patients were evaluated in both the acute and chronic phases of injury.

RESULTS: A significant decrease in T3 ($p < 0.001$) was the most consistent finding across all patients especially during the periods of hematoma expansion, ischemia/hypoxia, vasospasm and brain edema, as documented by CT, MRI, TCD and oximetry (SjO2) data. There was a direct correlation between T3 level and the patients' clinical condition (GCS, speech and motor function, presence of meningeal signs ($p < 0.05$). Low T3 levels correlated with increasing plasma and CSF cytokine, S100 and NSE levels ($p < 0.001$). FT3 level normalization was strongly correlated with a favorable clinical outcome, ranged by Glasgow Outcome Scale and by Burdenko Psychiatric Assessment Scale ($p < 0.001$).

CONCLUSION: The changes of T3 levels indicate significant disturbances in BTH following severe brain injury. Good outcomes correlate with restoration of BTH as indicated by a normalization of FT3 and T3 levels. On the basis of these findings we propose the idea of 'Brain Low T3 Syndrome', which is independent from whole organism stress response. Besides our results emphasize T3 monitoring and corresponding hormonal therapy may contribute neuroprotective effect in the management of seriously brain injured patients.

P332.

CEREBRAL OXYGENATION AND RESPONSE TO HYPEROXIA IN ACUTE BRAIN DAMAGE

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Introduction: Cerebral oxygen tension (PtiO2) is measured to assess oxygen availability to the brain. PtiO2 depends on various factors, as probe positioning and arterial oxygen tension.

Aim of the study is to assess PtiO2 and PtiO2-response to hyperoxia in patients where PtiO2 probe was placed close to focally damaged tissue at the CT scan (Focal), compared with patients in whom the probe was in normally appearing tissue (Non focal).

Materials and Methods: Twenty-seven patients (14 Females, 43 + 17 years old) suffering from TBI (12) or SAH with a median motorGCS of 5 were studied. Monitoring included ICP, MAP, SjO2 for AVDO2 calculation and tissue oxygen tension catheter (Licox GMS, Germany and Neurotrend, Codman UK). PtiO2 probe position was assessed by CT scan. Sixty-three hyperoxia tests were performed by increasing inspired oxygen fraction to 100%. PtiO2 has been indexed for PaO2 (PtiO2/PaO2 index) and PtiO2 response to hyperoxia was calculated as (PtiO2 plateau—PtiO2 at baseline)/(PaO2 at plateau—PaO2 baseline)

Results: The PtiO2/PaO2 index was 0.13 +0.08 in the focal group (12 patients) compared to 0.24 +0.16 in the non focal group (15 patients; $p < 0.05$). There was a relation between the magnitude in PtiO2 response and baseline PtiO2/PaO2 values ($R2 = 23.3\%$ $P = 0.011$ Slope 0.67). PtiO2 response was 0.09 + 0.06 in 10 focal and 0.25 + 0.16 in 15 non focal patients monitored ($p = 0.005$).

Conclusions: PtiO2 was lower and PtiO2 hyperoxia response was weaker when PtiO2 was measured at the margin of focally damaged tissue compared to CT normally appearing tissue. This difference may reflect a greater amount of oedema, and therefore an increased intercapillary distance, in perifocal tissue. Although Hyperoxia tests could help in better understanding cerebral regulation, this response can be largely predicted by baseline PtiO2 values.

P333.

MILD FLUID PERCUSSION INJURY LOWERS THE THRESHOLD TO KAINIC ACID-INDUCED SEIZURES WHICH IN TURN ELICIT RECURRENT INCREASES IN GLUTAMATE AND ENERGY DEMAND IN VULNERABLE TISSUE

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It is hypothesized that early seizures may precipitate adverse events in the traumatically injured brain primarily due to an increase in energy demand. We used a low dose of kainic acid (KA) to address the role of neural activity with respect to metabolic changes following a mild lateral fluid percussion (LFP) injury. KA (9mg/kg) was injected intraperitoneally in sham (n = 5) and LFP (n = 6) injured rats 1 hour following injury. An equivalent volume of saline was injected in LFP injured (n = 6) rats. Two CMA/12 microdialysis probes were placed into the cortex and perfused with saline (2microl/min). Samples were collected at 10 minute intervals, 1h before and 4h after LFP. Electroencephalogram (EEG) was recorded simultaneously. No EEG evidence of spontaneous seizures after LFP was detected. LFP resulted in a glucose dialysate decrease ranging from −13 and −33% (duration: 4h) and in lactate dialysate increase up to 137% (duration: 30 min), indicating an increase in glycolytic metabolism. A glutamate spike up to 441% was detected immediately following injury. KA-induced ictal activity occurred in 3/5 sham+KA animals but was not associated with significant changes in neurochemistry. However, EEG seizures were detected in all LFP+KA acid animals and were associated with multiple glutamate spikes up to 154% (duration: 50 min) and further lactate increase up to 109% (duration: 70 min). We conclude that LFP injury lowers the threshold to KA-induced seizures. In this phase, the EEG-seizures induce glutamate release and an additional demand for energy and may play a detrimental role on cells already in a state of metabolic derangement. (Support: NINDS 30306; NS02089).

P334.

ROLE OF DECOMPRESSION CRANIOTOMY FOR SECONDARY BRAIN DAMAGE AFTER TRAUMATIC BRAIN INJURY IN MICE

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Introduction: Decompression craniotomy is a well known clinical treatment option for increased intracranial pressure (ICP), however, its role for the prevention of secondary brain damage, e.g. delayed contusion expansion, is not known on a quantitative basis.

Materials & Methods: Male C57/Bl6 mice (BW 25–28g; n = 36) were subjected to controlled cortical impact injury (CCI, 8 m/s, 1 mm indentation). In half of the animals (n = 18) the craniotomy was left open, in the other half it was closed. Animals (n = 6 per group) were sacrificed 15 min, 24 h and 7 days after CCI for quantification of contusion volume. A functional test battery was performed daily.

Results: 15 min after CCI contusion volumes were not different in animals with open or closed craniotomy (22.1 ± 1.4 mm3 vs. 22.1 ± 4.4 mm3, respectively). 24 h after CCI the contusion volume of the mice with intact skull increased by 37% ($p < 0.05$), while the craniotomized animals did not have larger contusions (18.3 ± 5.3 mm3; n.s.) as compared to 15 min after CCI. After 7 days the mice had large cavities at the site of CCI making direct comparisons with the findings after 15 min and 24 h difficult. Function (beam walking, nesting) was improved in craniotomized animals.

Conclusion: The volume of a cortical contusion expands during the first 24 h after CCI only in animals with closed skulls where ICP can develop. In craniotomized mice where an increase in ICP does not occur secondary contusion expansion is completely prevented. Early craniotomy may therefore be one of the most potent procedures for the prevention of secondary contusion expansion.

P335.

THE CNS MICROVASCULAR PERICYTE RESPONSE TO HYPOXIA

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In response to fluctuations in environmental oxygen the cells of the blood brain barrier (BBB) undergo a number of complex adaptive measures in order to maintain tissue homoeostasis and hemostasis. These adaptive responses are particularly important when the balance of oxygen availability and utilization is altered as a result of the pathophysiology of CNS disease. At the microvascular level oxygen responsive signaling mechanisms involving both the endothelial cell (EC) and the pericyte (PC) regulate angiogenesis, vascular permeability and metabolism. We have investigated very early responses of the CNS PC following in vitro exposure to hypoxia. Freshly isolated rat cerebral microvessels were either cultured or sub-cultured to produce primary PC and EC. Cells or microvessel fragments were exposed to low oxygen for vary periods of time using the GasPak 100 hypoxia system (Becton Dickinson and Company Sparks, MD.). Within 15 minutes of exposure to low oxygen (1%) PC synthesize and release the cyclopentenone prostaglandin PGD2. Increased PGD2 and the dehydration product D12PGJ2 were detected by HPLC and by immune techniques. PGD2 was not synthesized by EC. Using PCR technology we have discovered that PC use the hematopoietic form of PGD synthase (PGDase) rather than the lipocalin form. PC constitutively express three of the five alternate splice variants of vascular endothelial cell growth factor (VEGF) mRNA. Exposure to low oxygen did not significantly alter mRNA levels but did increase synthesis and release of VEGF protein. Addition of either D12 PGJ2 or 15-deoxy D12,14 PGJ2 to PC under normoxic conditions increased the synthesis and release of VEGF protein in a dose dependent manner. In conclusion, results suggest that PGD produced by the CNS PC is an early signaling molecule in regulation of the angiogenic response to hypoxia.

P336.

ACID-SENSING ION CHANNELS IN ACIDOSIS-INDUCED NEURONAL INJURY

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Acidosis is a common feature of ischemia and traumatic brain injury. Our previous studies have shown that activation of acid-sensing ion channels (ASICs) likely contributes to acidosis-induced neuronal injury. Here we explored the possibility that ischemic treatment may in turn modulate the activity of ASICs. Cultured mouse cortical neurons were subjected to oxygen-glucose deprivation (OGD) in anaerobic incubator and the currents through ASICs were recorded in both control and OGD-treated neurons. Following 1h OGD treatment, the amplitude of ASIC currents was markedly increased, while desensitization of the currents was significantly decreased. In addition, OGD treatment induced a leftward shift of pH dose-response curve. Similar potentiation of ASIC currents and the shift of pH dose-response curve were observed in the same neurons following simple glucose removal or addition of metabolic inhibition agents such as NaCN (0.1–3.0 mM), rotenone (10 μM) or oligomycin (2.5 μg/ml). Substituting glucose with 2-deoxyglucose, a non-hydrolysable analogue of glucose, mimics the enhancement by glucose removal. An increase in intracellular calcium is not required in the potentiation of ASIC currents as the inclusion of 10 mM BAPTA in the pipette solution did not eliminate the potentiation. The enhancement by the addition of NaCN or glucose removal was however diminished in outside-out patch configuration, indicating the involvement of second messenger in the modulation of ASICs. With functional homomeric ASICs expressed in cos-7 cells, removal of glucose or addition of NaCN only potentiated the currents mediated by homomeric ASIC1a, without affecting the currents mediated by ASIC1b, ASIC2a or ASIC3 subunits. LDH assay demonstrated that addition of NaCN (1 mM) substantially potentiated the neuronal injury induced by incubating neurons with pH6 solution. Enhancement of ASIC responses by metabolic inhibition suggests that activation of ASICs in ischemic conditions may cause more injury than in acidic condition alone.

P337.

OVEREXPRESSION OF RAT HEAT SHOCK PROTEIN 70 REDUCES NEURONAL INJURY AFTER TRANSIENT FOCAL ISCHEMIA, TRANSIENT GLOBAL ISCHEMIA, AND KAINIC ACID-INDUCED SEIZURE

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Our lab has previously demonstrated that mice overexpressing rat heat shock protein 70 (HSP Tg mice) showed less infarction than wild type controls after permanent focal ischemia. The purpose of this study was to determine whether neuronal injury is reduced in HSP70 Tg mice after transient focal and global ischemia, and kainic acid (KA)-induced seizure.

Adult male mice (28–36 g) were used for this experiment. Transient focal ischemia was produced by middle cerebral artery occlusion (MCAO) using intraluminal suture cannulation (n = 18). Infarct volume was assessed 24 hours after 30 minutes MCAO. Transient global ischemia was produced by 25 minutes bilateral common carotid occlusion (BCCAO) (n = 16). KA (30mg/kg) was administered subcutaneously and seizure activity was monitored (n = 20). . The number of eosinophilic neurons was assessed in CA1 72 hours after BCCAO and in CA3 24 hours after KA administration.

Infarct volume after transient MCAO was significantly less in HSP70 Tg mice than in Wt mice (9.1+5.7 mm3 vs. 22.4+16.8 mm3: $P < 0.05$). The number of eosinophilic neurons in CA1 after BCCAO was significantly decreased in HSP70 Tg mice than in Wt mice (949.1+1095.5 vs. 2406.9+1380.3:$P < 0.05$). The number of eosinophilic neurons in CA3 after KA injection was significantly reduced in HSP70 Tg mice compared with Wt mice (33.8+45.3 vs. 119.4+112.1: $P < 0.05$).

Results suggest that HSP70 is neuroprotective and reduces excitotoxic cell death after transient ischemia, and after KA-induced seizure. Induction of DNA laddering in HSP70 Tg mice indicated that HSP70 reduced apoptosis in these in vivo injury models.

P338.

HYPOXIA CHANGES AKT PHOSPHOLYLATION IN SUPERFUSED RESPIRING NEONATAL RAT CEREBROCORTICAL SLICES

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The serine-threonine kinase Akt (protein kinase B), which can critically alter the balance between survival and apoptosis, is activated by phosphorylation. Phospho-Akt is also involved in the regulation of glucose metabolism. Western blot quantifications of phospho-Akt, which prevents apoptosis by inactivating caspases and other targets, were evaluated after hypoxia in superfused, respiring, neonatal rat cerebrocortical slices.

Using a protocol approved by the UCSF Committee on Animal Research, 350 μm thick slices were acquired from P7 Sprague-Dawley rats and superfused with 37°C oxygenated artificial cerebrospinal fluid (ACSF). Thirty minutes of hypoxia were induced by stopping the ACSF flow. Recovery occurred during 4 h superfusion with oxygenated ACSF.

Western blot intensities of phospho-Akt were moderate before hypoxia (control), nearly undetectable at the end of hypoxia, clearly detectable after 0.5 h of recovery, and greater than control after 1.5 and 4 h of recovery. Total Akt (phosphorylated and unphosphorylated) showed no change during and after hypoxia. Parallel 31P NMR studies at 14.1 Tesla were done with respiring superfused slices to examine phosphocreatine (PCr) and ATP levels at times corresponding to Akt measurements. PCr and ATP, nearly undetectable at the end of hypoxia, recovered quickly but incompletely after hypoxia. Reductions in phospho-Akt during hypoxia were consistent with a general unavailability of high energy phosphates from PCr and ATP. Reasons for the time course of high levels of phospho-Akt in the recovery period after hypoxia require further study. The following NIH support is gratefully acknowledged: R01 GM34767 (Litt), P50 NS14543 (Chan) and R01 NS25372 (Chan).

P339.

ACCUMULATION OF CALPAIN AND CASPASE-3 CLEAVED aII-SPECTRIN BREAKDOWN PRODUCTS IN CSF AFTER MIDDLE CEREBRAL ARTERY OCCLUSION IN RATS

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Although numerous biochemical markers of brain injury are correlated with outcome, a major limitation of current biomarkers is an inability for identifying specific neuropathological cascades operative in the injured brain. Identification of biomarkers elevated in CSF in response to brain injury that offer insight into specific pathological neurochemical events will provide critical information for emergency triage and will guide administration of therapeutic compounds. Non-erythroid aII-spectrin is a cytoskeletal protein cleaved by calpain and caspase-3 proteases to signature spectrin breakdown products (SBDPs). Although calpain-specific SBDPs are detected in CSF after traumatic brain injury (TBI) (Pike et al., J. Neurochem., 2001, 78:1297–1306), CSF levels of SBDPs has never been examined after cerebral ischemia. Methods: Transient focal cerebral ischemia in rats was produced by middle cerebral artery occlusion (MCAO) for 2 h followed by reperfusion. Ipsilateral (injured) and contralateral (uninjured) cortex and CSF were collected at 24, 48, and 72 h post occlusion. Results: Following MCAO, native aII-spectrin protein was decreased in brain tissue and increased in CSF up from 24 h to 72 h after injury. Calpain- and caspase-3-specific SBDPs were increased in brain (ipsilateral side) and CSF after injury. Levels of calpain-specific SBDP were greater at each post-injury time point than the caspase-3-specific SBDP. Levels of these proteins were undetectable in CSF of uninjured control rats. Conclusion: Transient focal MCAO injury results in increased brain and CSF levels of calpain- and caspase-3-specific SBDPs. Importantly, MCAO injury resulted in greater CSF levels of caspase-3 SBDPs than was observed after TBI by our laboratory. Thus, use of protease-specific SBDPs as surrogate biomarkers may provide a powerful tool for discriminating concussive vs. ischemic injury and provide critical insight into specific patterns of protease activation after CNS injury. (Supported by DAMD17-99-1-9565, DAMD17-01-1-0765, NIH R01 NS39091, NIH R01 40182 and USAMRMC)

P340.

TISSUE-TYPE TRANSGLUTAMINASE EXPRESSION FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION

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Tissue-type transglutaminase (tTG) has been implicated in neurodegenerative diseases and in protein aggregation associated with neurodegenerative disease. In this study, we have demonstrated induction of tissue-type transglutaminase in response to ischemic injury achieved by transient occlusion of the right middle cerebral artery. The area of infarcted tissue was revealed by decrease in TTC staining. Maximum decrease in TTC staining was observed 3 days after injury with recovery of TTC staining after 3 days. This suggests that maximum infarction was 3 days after occlusion. Western blot analysis has demonstrated increased expression of TG-L (79Kda) protein with no detectable expression of tTG-S (70Kda) after ischemia. In ipsilateral cortex, peak induction was observed 5 days after injury (525% ± 10% of control), while lesser tTG-L protein induction was observed in hippocampus after five days (196% ± 8% of control). To measure the mRNA transcript levels of TG-L and TG-S in rat cortex and hippocampus after traumatic brain injury a semiquantitative PCR was used. Results show that tTG-L and tTG-S mRNA transcripts are induced after injury. In ipsilateral cortex both forms of tTG peaked on day 5 after injury with tTG-L transcript level being higher (990% ± 130%% of control) than that of tTG-S transcript (690% ± 90% of control). However, in hippocampus the peak induction of both forms of tTG was on day 1 after injury and to a lesser level than that in ipsilateral cortex, tTG-L transcript level was 200% ± 20% of control and that of tTG-S was 175% ± 11% of control. These results demonstrate that the tTG mRNA and protein levels are increased much later than the occurrence of maximum infarction. The temporal profile of tTG induction after ischemia was similar to that observed after traumatic brain injury, suggesting similar role of tTG in both conditions. (Supported by DAMD 17-99-1-9565 and NIH RO1 NS 39091)

P341.

NUCLEAR FACTOR-KAPPA B DECOY OLIGODEOXYNUCLEOTIDES CAN REDUCE THE ISCHEMIC SPINAL CORD INJURY OF RAT

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Purpose: Recently, it is reported that the cis element decoy oligodeoxynucleotides (ODNs) against nuclear factor -kappa B (NF-kB) block the activation of genes, which mediate ischemic injury. To evaluate the effect of NF-kB decoy ODNs on the spinal cord ischemia, we studied with rat spinal cord ischemia / reperfusion model.

Methods: Female Wister rats were used (B.W. = 200 to 250 g, n = 30). The animals were anesthetized with intraperitoneal injection of pentobarbital (50mg/kg). Transient spinal cord ischemia (SCI) model was produced with the method described by Kanellopoulus. A 2Fr Fogarty catheter was inserted into the descending aorta via left carotid artery then the balloon was inflated for 10 min. Hemagglutinating virus of Japan -liposome complex with fluorescein isothiocyanate-labeled NF-kB decoy ODNs was injected through the femoral artery during ischemia of spinal cord. Three hours, 3 days, 7 days after SCI, the spinal cords were removed at the lumbar enlargement level. The mRNA levels of factors related with ischemic-reperfusion injury at three hours were estimated by a real-time polymerase chain reaction method. Immunohistochemical study was performed using anti MAP2 antibody and anti ED-1 antibody to evaluate the degree of the neuronal damage and the infiltration of macrophages.

Results: The strong signals of the fluorescein were recognized in the spinal cord. The mRNA levels of tumor necrosis factor-alfa, interleukin-1 beta, intracellular adhesion molecule 1 and cyclooxygenase 2 were significantly reduced by the NF-kB decoy. The administration of NF-kB decoy reduced the number of infiltrated macrophages about 44% on 3days and the infarct area about 30% on 7days after SCI.

Conclusions: The introduction of NF-kB decoy reduced the spinal cord damage after ischemic injury. This strategy with NF-kB decoy may provide a useful tool for ischemic injury to the central nervous system.

P342.

DIFFERENTIAL EFFECTS OF HYPERBARIC OXYGENATION ON TISSUE NECROSIS AND ATP CONTENT FOLLOWING FOCAL CEREBRAL ISCHEMIA

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Objective: Failure of energy metabolism and ATP synthesis may play a pivotal role in tissue necrosis following cerebral ischemia. The aim of the present study was to determine the neuroprotective effect of normo- and hyperbaric oxygenation and its relationship to the ATP content of the tissue.

Methods: In male SD rats anesthetized with isoflurane a focal ischemia was produced by an 8 hour unilateral occlusion of the middle cerebral artery (MCAO) using an intraluminal thread. After MCAO animals were kept in normal air (control), 100% oxygen (normobaric oxygenation, NBO) or 100% oxygen including a 1 hour hyperbaric period (HBO). Brains were serially cut, developed for planimetric analysis of tissue necrosis and ATP content and subjected to volumetric analysis.

Results: Lesion volume was 303 ± 54 mm3 (mean ± SD) in controls and 295 ± 56 mm3 in NBO animals. Volume of ATP loss amounted to 72.9 ± 19.7% of necrotic tissue. In the HBO group tissue necrosis was significantly smaller than in controls (187 ± 67 mm3, $p < 0.05$) while the volume of ATP loss was markedly increased to 208.6 ± 38.4% of necrosis ($p < 0.01$ vs. NBO).

Conclusion: Normobaric oxygenation during an 8 hour period of permanent MCAO in rats does not salvage tissue from necrotic death. In contrast, an intermittent period of HBO protects tissue from early ischemic death and simultaneously increases the tissue volume displaying low levels of ATP. The pathophysiological meaning of this dissociation may reflect increased consumption resulting in neuroprotection or delay of ischemic cell death with ATP loss heralding growth of ischemic damage.

P343.

SELECTIVE HIPPOCAMPAL CA1 NEURONAL ACIDOPHILIA (RED CELL CHANGE) IN CASES OF SUDDEN DEATH FROM TRAUMA

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Neuronal red cell change, the hallmark of hypoxic-ischaemic injury, is considered to be only reliably identified in immersion formalin fixed brains after a period of 4–12 hours. The nuclear changes and cytoplasmic eosinophilia seen in red cell change may be the result of excitotoxic calcium mediated activation of endonucleases and proteases. We reviewed the neuronal appearance in the CA1 region of the hippocampus in 74 patients who had died suddenly as a result of trauma, and compared them to a group of 77 patients who had died in hospital as a result of non-traumatic medical and surgical conditions. Two independent observers examined haematoxylin and eosin (H&E) stained paraffin sections of the CA1 region in the hippocampus in each case and determined whether the neurons appeared normal, showed typical red cell change or were abnormal but without the typical features of red cell change. Neuronal red cell change was present in 58.0% of cases of sudden death due to trauma, compared to 10.7% in control cases; in contrast the CA1 neurons appeared normal in 56.3% of control cases but only 14.5% of trauma cases. These findings suggest that neuronal red cell change may develop over a very short period of time in cases of sudden traumatic death and is probably related to acute global cerebral ischaemia as the change occurred as frequently in the group who died of non-brain related injuries as in the group who died of traumatic brain injury (61% and 59% of cases respectively).

P344.

DNA MICOROARRAY ANALYSES OF GENE EXPRESSION CHANGES UNDERLYING CHRONIC CENTRAL PAIN IN SPINAL CORD INJURY

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The rodent model of chronic central pain (CCP) is assessed using somatosensory tests of paw withdrawal responses to mechanical punctate (von Frey hairs), and measurements of response threshold for mechanical stimuli. By using K-means clustering, we divided injured rats in two groups: only one showing statistically significant increases in mechanical allodynia ("pain group") 28 days after contusion spinal cord injury (SCI). Locomotor recovery, measured in open field-tests (BBB scores) for both groups of rats were indistinguishable, suggesting that the injuries to SC were equivalent for all rats. To characterize the gene expression changes underlying the development of CCP in SCI, we used Affymetrix DNA microarrays to analyze injured spinal cords (above the site of injury, T8) and thalami of rats (n = 4) in the "pain" group and compared them with expression profiles of injured spinal cords and thalami of rats in the "non-pain" group. Transcriptional changes in SC and thalami of rats with CCP included upregulation of inflammatory molecules, downregulation of molecules with "analgesic" effects (IGFII, somatostatin, opioids) and changes typical for tissues exposed to severe oxidative stress. Especially complex was the alteration in ion channels/transmitter receptors composition in spinal cords of rats showing mechanical allodynia. Novel findings were that neuropeptides involved in olfaction, hormone receptors and regulators of cell adhesion/neurite outgrowth may have a role in CCP development.

Supported by NINDS NS 39161.

P345.

DATA MINING IN SCIGENES, THE DATABASE OF SPINAL CORD INJURY-RELATED GENES

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SCIgenes is a searchable database containing information about genes whose expression is affected by spinal cord injury or nerve injury. It combines information about sequences, injury type, and protein function. Each entry includes links to sequences, fields describing the type of injury, the cell types affected, the polarity of the change in gene expression, and the temporal aspects of this response. It also includes links to functional information about each gene product. It supports data mining operations, making it possible to look for patterns of gene expression changes across all entries. For example, a search strategy for genes whose expression increases within the first two days after peripheral nerve transection returns a set of growth factors and their receptors, transcription factors, and neuronal plasticity genes. SCIgenes is updated continuously, and users are encouraged to submit their own data at http://scigenes.uky.edu. Supported by an award from the Kentucky Spinal Cord and Head Injury Research Trust.

P346.

REDUCING THE T LYMPHOCYTE RESPONSE TO SPINAL CORD INJURY DECREASES SECONDARY DEGENERATION AND FUNCTIONAL DEFICIT

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Injury to the central nervous system (CNS) is followed in all instances by secondary degeneration, which leads to progressive tissue loss and cystic cavitation. Cellular and humoral immune responses have been implicated as mediators of secondary degeneration, and the expression of specific leukocyte chemoattractants has been shown to precede immune cell influx into the injured CNS. However, regulation of the cascade of proinflammatory molecule expression and immune cell recruitment into the traumatized CNS is poorly understood. Here we show that the lymphocyte chemoattractant CXC chemokine ligand (CXCL) 10 is upregulated following dorsal hemisection and crush injury to the adult mammalian spinal cord, and that antibody neutralization of CXCL10 in injured animals dramatically reduces the CD4+ T lymphocyte invasion that normally occurs after trauma. This treatment resulted in a near elimination of secondary degenerative tissue loss and significantly reduced locomotor deficits. We conclude that CXCL10 plays a critical role in the recruitment of CD4+ T lymphocytes to sites of spinal cord injury, and that a reduction of the robust CD4+ T lymphocyte response to CNS injury significantly benefits tissue preservation and functional outcome following spinal cord injury. This project was funded by the Reeve-Irvine Research Center and the Roman Reed Foundation.

P347.

CHARACTERIZATION OF A RAT CERVICAL CONTUSION MODEL

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Numerous animal injury models currently exist that attempt to reproduce the pathophysiology of human spinal cord injury (SCI). However, these various paradigms do not represent the most prevalent type of SCI, contusive trauma to the cervical spinal cord. Whereas several cervical contusion studies have been performed, the number is a small percentage of the research conducted using SCI models. Because both (1) the different ways of injuring the spinal cord (compression, contusion, transection) induce different processes of tissue damage and (2) the architecture of the spinal cord is not uniform, there is a need to use a model that is more clinically applicable to human SCI. Therefore, in the beginning study we have characterized a rat model of contusive, cervical SCI using the Electromagnetic SCI Device (Ohio State University) to induce injury by spinal cord displacement. The moderate contusion injury was performed at cervical level C6, using the circular flap tip of the impactor (made of methylmethacrylate, 4mm diameter) to transduce a force of 3 Kdyn (as indicated by the force transducer). This results in slight dimpling of the dura dorsally and provides a consistent starting point from which displacement was measured (0.80mm displacement injury with a single, brief displacement of <20 msec). Analysis of the histopathological and behavioral consequences of SCI was performed over a 9-week period. Traumatized animals developed severe forelimb and hindlimb paralysis. Quantitative assessment of motor performance included BBB evaluation, hanging, climbing, and gripping tests for upper body strength and inverted plane, gridwalk and footprint analysis. Over the study period some degree of improvement in motor function was observed. Histological assessment demonstrated a reproducible pattern of gray and white matter necrosis in terms of lesion size and cellular composition. This model of cervical SCI should allow the testing of novel neuroprotective and reparative therapies. (Supported by NIH PO1 38665)

P348.

IMPLANTATION OF SKIN-ACTIVATED BLOOD-BORNE MONOCYTES TO SPINALLY CONTUSED RATS: RECOVERY OF MOTOR ACTIVITY AND REDUCED CYST FORMATION.

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Severe spinal cord injury leads to irreversible sensory and motor deficits due to the primary insult and to secondary degeneration that it causes. It was shown that in rats with transected spinal cords local implantation of peripheral nerve-activated blood-borne monocytes can induce functional motor recovery. In the present study we locally implanted skin-activated blood-borne monocytes to contused spinal cord of adult rats. The activated blood-borne monocytes, when compared to non-activated monocytes showed increased production of cytokines and elevated expression of surface molecules characteristics of antigen presenting cells. The implantation of skin-activated monocytes resulted in an improved recovery of motor activity and in a reduction of cyst formation when compared to spinally contused non-treated rats. The effect was noticed when the activated monocytes were injected even two weeks after the contusion. The results of this work further support the contention regarding the role of the inflammatory response, if well controlled, in recovery from CNS insult.

P349.

THE SERUM AND CEREBROSPINAL FLUID ELASTASE ACTIVITY DURING TREATMENT OF SPINAL CORD INJURY BY PERFTORAN

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The previous experimental researches show that the blood substitute with gas transporting function, known as "Perftoran"(Pf) is capable of reducing spinal cord (SC) damage and protecting medullar tissue against ischemia and secondary metabolic degeneration in acute spinal cord injury (SCI). Pf is emulsion of perftormethyl cyclohexil-pyperidin stabilized by Proxanol 268. It oxygen solubility is 6–7 vol. %. Pf accelerate the process of O2 delivery and CO2 elimination, increase cerebral blood flow. The clinical studies showed that the immediate local SC oxygenation and i/v infusion by Pf in acute SCI after decompression surgery results recuperation of the leg motor function to 12 of 20 paraplegic patients in contrast of 5 of 18 same patients without Pf treatment.

The aim of the present study was to clarify the relationship between such positive results and the level of SCI patient's serum and cerebrospinal fluid (CSF) elastase activity (EA). The EA was measured before and 3 weeks after treatment by L.Visser and E.Blout (1972) method. The SCI patients classified into 3 groups: macrodex solution subdural and i/v infusion treatment of acute SCI (1st group, 18 patients); oxygenated Pf subdural and i/v infusion treatment of acute SCI (2nd group, 20 patients); 3–4 months post-injury only i/v Pf treatment (3 group, 14 patients).The EA was detected as 150-/+10.3 nM/min/ml in normal serum and was no detected in normal CSF. The EA was detected as 315-/+56.1 in serum and 21-/+11.2 nM/min/ml in CSF after SCI. The EA was of no change significantly in mostly patients of 1st group. The serum EA was declined to 182.4-/+ 58.1nM/min/ml and to 4.3-/+1.2 in CSF in cases of the successful Pf treatment in 2nd group. The return of walking ability was observed 2–4 weeks post-surgery in these cases. The level of liquor EA was no change in unresult cases. A decrease of serum EA till 213-/+ 38.1 nM/min/ml was observed in 3 chronic conditions group without real clinical results. Conclusion: The EA may be reduced by Pf high absorb capacity. On the other side, the early tissue cord oxygenation and recuperation of SC microcirculation by Pf treatment perhaps limit antimyelin and antiendothelial damage activity of neutrophil elastase during first hours after the SCI.

P350.

DOES MILD INTRAOPERATIVE HYPOTHERMIA LEAD TO INCREASED COMPLICATIONS IN ELECTIVE SPINAL SURGERY?

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INTRODUCTION: Spinal cord surgery carries risks of neurological injury. Neuroprotection is desirable, but no strategies have achieved broad clinical acceptance. Hypothermia, resulting from general anesthesia, is a readily achievable neuroprotective strategy. Moderate hypothermia (32.5–35.5 C) carries smaller clinical risks than deep hypothermia (<32.5C). We investigated the association between incidental moderate hypothermia and complications in a review of 50 adults undergoing complex spinal procedures.

METHODS: Surgical procedures included: 41 cervical, 6 thoracic, 1 occipitocervical, 1 cervico-thoracic, and 1 thoracolumbar procedure. Systemic hypothermia followed induction of anesthesia; esophageal or bladder temperature was monitored.

We plotted time-temperature (T/T) curves to derive mean temperature, nadir temperature, time course of hypothermia, and the hypothermic integral (dose) [projected normothermic area under the curve (AUC)—actual T/T AUC]. Patients with and without complications were compared for age, blood loss, anesthetic duration, and temperature measures.

RESULTS: Complications (14) included: one accidental durotomy, one hematoma, two wound infections, one collapse of a vertebrae adjacent to a fusion, one SVT, one brief intraoperative asystole, one episode of postoperative pulmonary edema, one episode of delerium tremens, one postoperative death (DVT), two transient radiculopathies, and two cases of transient long-tract dysfunction.

Comorbidities were not significantly linked to complications. Patients with (n = 14) and without (n = 36) complications were compared. P-values were significant for anesthetic duration (0.007), blood loss (0.005), and hypothermic integral (0.004). Neither mean nor nadir temperatures were statistically associated with complications. Anesthetic duration and blood loss were linked (r = 0.62). T/T profiles showed no uniform pattern.

CONCLUSION: Anesthesia duration was linked to complications and blood loss. Regarding hypothermia, neither mean nor nadir temperatures were linked to complications, but the "dose" of hypothermia was linked. Extended exposure to moderate hypothermia (>5 hours) is associated with increased risks.

Meaningful neuroprotective use of hypothermia during spinal surgery will require more precise delimitation of the exposure.

P351.

CHRONIC CENTRAL PAIN IS ATTENUATED BY EXOGENOUS LEUKEMIA INHIBITORY FACTOR (LIF) AFTER SPINAL CORD INJURY (SCI).

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After SCI, chronic central pain (CCP) develops in a majority of patients. We wished to test if the CCP following SCI may be reduced by intraparenchymal injection of LIF, a key modulator of neuropathic pain that is thought to play an anti-inflammatory role. We used a rodent model of SCI, unilateral hemisection at T13, and tested for the development of mechanical and thermal allodynia. Male Sprague-Dawley rats (225–250 gm) were anesthetized, and at the time of spinal hemisection, 100 ng of LIF in 1.0 ml of artificial cerebral spinal fluid (ACSF, pH-7.4) was injected 1 mm rostral and 1 mm caudal to the spinal hemisection. LIF treated hemisected rats (n = 8) were compared to vehicle treated hemisected rats (n = 8) by comparing postsurgical behavior at 30 days with presurgical behavior to test if LIF treatment attenuated mechanical and thermal allodynia. Treatment with LIF produced statistically significant attenuation of both mechanical and thermal allodynia. Thus, we hypothesize that central sensitization in CCP is attenuated by LIF. Specifically, we propose that LIF treatment inhibits the invasion of neutrophils and lymphocytes both of which produce chemokines, cytokines and other factors, including NGF, known to produce sensitization.

P352.

ENHANCED REGENERATION INTO SCHWANN CELL BRIDGES IMPLANTED INTO THE COMPLETELY TRANSECTED SPINAL CORD FOLLOWING CYCLIC AMP INJECTION OR SUPERFUSION INTO THE STUMPS IS NOT ACCOMPANIED BY IMPROVED BEHAVIORAL RESTITUTION.

Pereira, F.C., Pearse, D.D., Tresco, P.A. and Bunge, M.B. (University of Miami, The Miami Project, Miami, Florida US).*

Cyclic nucleotides are able to modulate the behavior of growth cones, promote the growth of axons over certain inhibitory environments, and act as cues capable of influencing axon-turning responses in vitro. Can these important intracellular molecules then promote regeneration after spinal cord injury (SCI) in vivo? The current study asked if the administration of different doses of dibutyrl-cAMP (db-cAMP) injected or superfused rostrally and caudally to a Schwann cell (SC) bridge implanted into the completely transected spinal cord (following removal of a 4mm block of tissue) could promote axon regeneration into and across the SC bridge. These bridges were composed of 5 × 106 SCs, which were mixed with fluid matrigel, and then injected into an empty poly(acrylonitrile-vinylchloride) tube, which had been pre-implanted between the spinal cord stumps. Solidification of the matrigel then occurred at 370C.

Doses of 25 mM or 50 mM cAMP significantly increased the number of myelinated axons that were able to grow into, but not out of, the SC bridge after 10 weeks, compared to SC-only bridges. However, no differences were observed behaviorally between these animals and the controls when they were tested for open-field locomotion using the BBB score. These studies indicate that cyclic AMP can increase regeneration into a Schwann cell bridge implanted into the completely transected spinal cord. However, the elevated levels of cyclic AMP are probably too transient after a single, acute administration, due to their hydrolysis by phosphodiesterases within the spinal cord, to promote the long distance axonal growth that is required for behavioral restitution. Future studies aimed at continuous administration of cyclic AMP or the co-administration of cyclic AMP with agents capable of inhibiting its hydrolysis may enable the long distance growth of axons to their targets that is needed to improve functional restitution after SCI.

P353.

TRANSPLANT-MEDIATED REMYELINATION AND LOCOMOTOR RECOVERY OF THE MHV MODEL OF MULTIPLE SCLEROSIS

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Demyelinating diseases such as multiple sclerosis (MS) are characterized by recurrent episodes of focal demyelination and progressive neurological impairment. To address the complex and reactive CNS environment of a neurodegenerative disease state, we explored the remyelinating capability of early neural progenitor cells following transplantation into lesions in the demyelinating mouse model of MHV infection. Striatal stem cells were isolated from postnatal day 1 mice and grown on a nonadherent substrate in defined media with EGF, then differentiated into oligodendrocytes and astrocytes, but not neurons. These findings indicate that this stem cell preparation results in the restriction of cells to a glial lineage. Seven day-old undifferentiated, BrdU labeled floating neurospheres were implanted into the thoracic spinal cord of actively demyelinating MHV mice. After 21 days, alternating 1 mm blocks of the spinal cords were plastic embedded and frozen sectioned. Behavioral analysis showed locomotor recovery starting at two weeks after the transplantation. In the non-transplanted animals, numerous demyelinated axons were present amongst vacuoles, myelin debris, activated macrophages, lymphocytes and necrotic cells. In contrast, transplanted animals showed large areas of remyelinated axons. The transplanted BrdU labeled cells were present after 21 days. These studies confirm that remyelination can take place during pathogenesis and indicate that transplanted glial-committed progenitors are capable of extensive remyelination of regions of demyelination in the MHV model of multiple sclerosis. The success of remyelination following transplantation into this inflammatory environment is of central importance in approaches that aim to enhance remyelination in MS lesions. Supported by: Reeve-Irvine Research Center.

P354.

THE NEURONAL-SPECIFIC RNA-BINDING PROTEIN HUD IS UP-REGULATED AND COLOCALIZED WITH GAP-43 MRNA IN THE FACIAL NUCLEUS OF THE MOUSE DURING REGENERATION.

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The Growth-Associated Protein, GAP-43, is highly expressed during development of the nervous system and is re-expressed during regeneration of the peripheral nervous system (PNS). In vitro, the GAP-43 mRNA is highly labile and its? half-life is regulated post-transcriptionally, in part, by the neuronal-specific RNA-binding protein HuD. In vivo, however, the regulation of GAP-43 mRNA is poorly understood. To begin to analyze the molecules involved in the in vivo regulation of GAP-43 mRNA, HuD protein expression was examined following a PNS lesion in which successful regeneration occurs. C57Bl/6 mice were given a unilateral crush injury of the facial nerve and were allowed to survive for various time intervals. In situ hybridization studies revealed high levels of GAP-43 mRNA in the ipsilateral facial nucleus one week following injury. At the same time, immunohistochemistry demonstrated HuD protein to be colocalized with GAP-43 mRNA in the motor neurons of the facial nucleus ipsilateral to the injury. Neither HuD protein nor GAP-43 mRNA were detectable in the contralateral facial nucleus. These results suggest that HuD protein may play a role in the in vivo regulation of GAP-43 mRNA and may account for the prolonged expression of GAP-43 following a PNS lesion during which successful regeneration does occur. Supported by the NIH NS-41710 (KDA) and NIH NS-32280 (OS).

P355.

THE ROLE OF OSP/CLAUDIN-11 IN OLIGODENDROCYTE PROGENITOR CELL MIGRATION FOLLOWING DEMYELINATION OF THE ADULT SPINAL CORD

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Oligodendrocyte-specific protein (OSP)/claudin-11 is a major protein of CNS myelin, forming tight junctions within myelin sheaths. OSP/claudin-11 is involved in membrane interactions with the extracellular matrix and appears to modulate proliferation and migration of oligodendrocytes, a process essential for myelination and repair. Using an anti-NG2 antibody to identify oligodendrocyte progenitors (OPs), we have previously shown an acute increase in the number of NG2+ cells in normal white matter surrounding a region of antibody-induced demyelination in the adult rat spinal cord. These NG2+ cells incorporated BrdU, illustrating a local proliferation of cells. An absence of NG2+/BrdU+ cells 2 weeks after demyelination suggested these cells had migrated into the demyelinated area to become remyelinating oligodendrocytes. In the present study, 16% of the NG2+ cells surrounding a region of demyelination in the adult spinal cords of OSP/claudin-11 homozygous knockout mice were still BrdU+ 2 weeks after demyelination, suggesting restricted migration of OPs into the demyelinated area. No NG2+/BrdU+ cells were seen in normal white matter of wild type mice. In addition, the total number of BrdU+ cells in the dorsal column of knockout mice was 40% higher compared to wild type mice, supporting a role for OSP/claudin-11 in proliferation. We examined the extent of NG2 colocalization with PDGFR, another marker for OPs involved in proliferative ability. More NG2+/PDGFR+ cells were seen in normal white matter surrounding a region of demyelination in OSP/Claudin-11 knockout mice, further suggesting limited migration of OPs into the demyelinated area. These data indicate that OSP/Claudin-11 knockout mice display restricted migration of proliferating OPs into a region of demyelination, in support of a role for OSP/Claudin-11 in migration. Current investigations are underway to assess the extent of remyelination in both knockout and wild-type mice. These studies were supported by Multiple Sclerosis Society of Canada and NIH Neural Repair Training Grant.

P356.

LOCMOTOR TRAINING IN A RODENT MODEL OF INCOMPLETE SPINAL CORD INJURY

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Locomotor recovery was assessed in 14 female adult Long-Evans rats with incomplete thoracic spinal cord injury (iSCI, T10 moderate contusion) with ($n = 9$) and without ($n = 5$) quadrupedal treadmill step training (15 minutes/day, 5 days/week, starting 1 week post-injury for 12 weeks) using 2-D and 3-D kinematics of gait, and the BBB locomotor score. Mean hindlimb stance width (SW), eversion angle (EA), and stride length/velocity (SLv) were obtained from 4–5 passes of overground walking in a track pre-injury and every week post-injury if the animal could plantar step. 3-D analysis determined hip, knee, ankle, shoulder and elbow angles for all four limbs during 4–20 cycles of treadmill locomotion. The effects of training, the BBB score (<14 (11.67 ± 0.15 (sem) or $>=14$(16.69 ± 0.28)), and time post-injury were examined (3-factor, repeated measures, $p < 0.05$). For all iSCI rats the SW, EA, and SLv were significantly greater post-injury. Also, they were significantly different between the training groups or based on the BBB score. EA of the trained group for the first 4 weeks post-injury was larger than that for the last 5 weeks and at all times smaller than that for the untrained group. For BBB$>=14$, both SW and EA were significantly smaller in the trained group. 3-D analysis indicated that 13 weeks post injury the untrained group had a significantly increased ankle extension and range ($124.8 \pm 7.0\%$; $164.7 \pm 11.0\%$ with a loss of double burst pattern) and reduced knee extension, knee range and elbow range ($81.0 \pm 4.2\%$; $50.3 \pm 6.0\%$; $61.9 \pm 15.4\%$). Preliminary analyses of trained rats ($n = 3$) indicated only a reduced knee range ($49.0 \pm 3.6\%$). These quantitative kinematic indices indicate that injury alters hindlimb as well as forelimb function. They suggest that the degree of injury influences the kinematic impairment and the effects of step training on locomotor recovery. (Support: KSCHIRT-09A and HD-40335).

P357.

GENETICALLY TARGETED ASTROCYTE SCAR ABLATION RESULTS IN LIMITED, LOCAL GROWTH OF CORTICOSPINAL TRACT AXONS AFTER SPINAL CORD INJURY.

J.R. Lomonaco-Faulkner. (UCLA, Huntington Beach, CA US).

Genetically targeted astrocyte scar ablation results in limited, local growth of corticospinal tract axons after spinal cord injury. J.R. Lomonaco-Faulkner, J. Herrmann, N. B. Doan and M.V. Sofroniew. Department of Neurobiology and Brain Research Institute, UCLA, Los Angeles CA 90095-1763.

After spinal cord injury (SCI), scar tissue formed by reactive astrocytes is thought to prevent axon regeneration. We used a genetic targeting strategy to ablate reactive astrocytes after SCI. Transgenic mice that express herpes simplex virus thymidine kinase (HSV-TK) from the mouse glial fibrillary acidic protein (GFAP) promoter were given the antiviral agent gancilovir (GCV). Transgenic and non-transgenic mice received a bilateral lesion of the corticospinal tract (CST) at T9/10. Non-transgenic mice exhibited dense astrocyte scars. Transgenic mice given GCV exhibited substantial ablation of scar-forming astrocytes. Areas depleted of astrocytes exhibited a statistically significant, 5-fold increase in the density of nerve fibers detected by immunohistochemistry of neurofilament M, suggesting the sprouting and growth of local nerve fibers. CST axons were assessed using biotinylated dextran amine (BDA) injected unilaterally into the sensory motor cortex. In non-transgenic mice, many large BDA-labeled retraction bulbs were evident proximal to the glial scar and no labeled fibers were observed within or distal to the lesion. Transgenic mice given GCV had fewer retraction bulbs, and areas depleted of astrocytes exhibited many fine BDA-labeled fibers. In some cases, finely beaded and branched CST fibers grew across and beyond the lesion for a moderate distance. Supported by Christopher Reeve Paralysis Foundation, NIH grant #NS07479, and CA State Roman Reed Initiative for SCI Research.

P358.

HP184, A COMBINED SODIUM AND POTASSIUM CHANNEL BLOCKER, IMPROVES LOCOMOTOR SCORES 35 DAYS AFTER A MODERATE SPINAL CORD INJURY IN THE RAT.

Michel Rathbone, Shucui Jiang, Mohammad Khan, Yao Lu, Josef Buttigieg, David Lee, Jesse Harvey, Kristen Paulseth, Rani Bain, Adeel Safdar, Suzie Wang, Jay Saoud, Dave Rampe, Margaret Petty and Craig P. Smith (Division of Neurology and Neuroscience, McMaster University, Health Sciences Centre, Hamilton, Ontario and Aventis Pharmaceuticals, Inc., Bridgewater, NJ USA).*

There are currently no available therapies for restoring function to patients with chronic spinal cord injury (SCI), a population estimated at >250,000 in the USA. Recent literature suggests that clinically significant neurological improvements may be obtained with 4-AP (4-aminopyridine). However, the therapeutic use of 4-AP may be limited by various side effects, which include restlessness, confusion, and infrequently reported findings of generalized tonic-clonic seizure. HP184 is an analog of 4-AP which is a voltage-dependent blocker of potassium currents in PC12 cells and a use- and frequency dependent blocker of sodium channels. This combination of activities allows high levels of HP184 to be administered without danger of convulsion. Consistent with other sodium channel blockers, HP184 also has neuroprotective properties, and pre-dosing with 10mg/kg, po, attenuates the reduction in infarct volume caused by permanent middle cerebral occlusion in mice. Also, we have observed efficacy in well established SCI. In spinal cord injured rats, HP184 significantly improves open field walking in long-standing (35 day) spinal cord injury of moderate intensity (0.3, 1.0 & 3 mg/kg, po).

P359.

PRO-CYSTEINE COMPOUND (OTC) DECREASES THE NUMBER OF ACTIVATED MACROPHAGES/MICROGLIA FOLLOWING SPINAL CORD INJURY

Kamencic H, Kelly M, Damant A, Schültke E, Griebel RW, Paterson PG, Juurlink BHJ. (University of Saskatchewan, Saskatoon, SK CA).*

In this experiments we have examined the effect of L-2-oxo-thiazolidine-4-carboxylate (OTC) administration, which promotes glutathione synthesis, on inflammatory responses, especially accumulation of activated macrophages/microglia, and myeloperoxidase activity within first 24-hr following severe clip-induced spinal cord injury. The spatial localization and morphology of activated macrophages/microglia were described quantitatively at 24-hr after injury through injured segment and segments directly adjacent, rostral and caudal, by using immunohistochemistry. OTC at 12 mmoles/kg initially followed every 12 hr with 4 mmoles/kg was administered intraperitoneally.

Administration of OTC significantly reduced the number of ED1 (antibody against activated macrophages/microglia)-positive cells in the ventral white matter at the site of injury ($p < 0.03$). We found high accumulation of ED1-positive cells epidurally, intra- and perivascularly within necrotic tissue at the epicentre of the lesion. Myelin was severely vacuolized not only at the site of injury but also rostral and caudal to the damage after the first 24-hr. Longitudinal sections through central canal showed a decrease in the number of activated macrophages/microglia by ~30% in the gray matter at the site of injury following OTC treatment. Saline-treated animals have higher accumulation of ED1-positive cells in the gray matter up to 3 mm rostral and caudal to the site of injury. We also analyzed myeloperoxodase (MPO) activity, an enzyme predominantly located in neutrophils, and found that OTC administration significantly decreased ($p < 0.0001$) MPO activity in two groups, in male rats 12-hr and in female rats 24-hr following injury.

In conclusion, our data suggest that OTC administration decreases neutrophils and activation of microglia and/or extravasation of monocytes and prevents much of the secondary damage following spinal cord injury. Supported by the Christopher Reeve Paralysis Foundation and H. Kamencic holds an HSURC Saskatchewan Post-Doctoral Fellowship.

P360.

THE ADMINISTRATION OF VARIOUS DOSES OF L-2 OXOTHIAZOLIDE CARBONATE TO PROMOTE RECOVERY FROM NEUROTRAUMA

Kelly MEB, Griebel RW, Kamencic H, Schültke E, Paterson P, and Juurlink BHJ. (University of Saskatchewan, Saskatoon, Saskatchewan CA).*

Background: Decreasing oxidative stress by maintaining tissue glutathione following spinal cord injury improves functional outcome in a rat model. We are analyzing the dose response to various levels of L-2 oxothiazolidine-4-carboxylate (OTC) after rat spinal cord injury.

Methods: An extradural aneurysm clip with a calibrated force of 50 grams was applied to the rat spinal cord at the T6 level. Intraperitoneal administration of OTC was performed. The functional recovery of the rats was assessed for six weeks after receiving either 1) saline, 2) 1, 2 and 4 mmol/kg OTC 30 minutes after injury and then every 12 hr for 5 days, 3) 10 mmol/kg OTC for one dose 30 minutes after injury and 10 mmol OTC/kg 30 minutes after injury followed by 1 mmol/kg bolus at 12 hours. Functional recovery was assessed using standard techniques BBB behavioural scoring.

Results: A statistically significant improvement in functional recovery (36 % of animals walked) was seen in the animals receiving 1 mmol/kg OTC and 4 mmol/kg OTC for five days. Only 25% of animals receiving the 30 minute bolus of 10 mmol/kg OTC and 10 mmol/kg OTC with a 12 hour 1 mmol/kg bolus recovered ^{3} 10 on the BBB score (walking ability). Not one of the saline vehicle-treated animals ever achieved a BBB score greater than 9.

Conclusions: Spinal cord injury results in a significant increase in oxidative stress at and distant to the site of injury. The administration of OTC in various doses significantly improves functional recovery in a rat model over saline controls. Research supported by the Christopher Reeve Paralysis Foundation.

P361.

CEREBRAL METABOLIC AND BLOOD FLOW DIFFERENCES BETWEEN TRAUMATIC HEAD INJURED PATIENTS: INFLUENCE OF COCAINE

JL Benae, TC Glenn, P Vespa, F Song, DF Kelly, DA Hovda, NA Martin. (UCLA Neurosurgery, Los Angeles, CA US).*

Purpose: Cocaine use can be high in patients with traumatic brain injury (TBI). Recent studies with dogs have shown that cerebral blood flow (CBF) responsiveness to carbon dioxide may be affected by cocaine. The purpose of this study was to determine if CBF and metabolism differences exist between patients who did or did not test positive for cocaine on admission to the hospital following severe TBI.

Methods: We studied prospectively, 23 age-matched, consented head injured patients (median admission GCS = 7, M:F 15:8, mean age 27.8 ± 2.9, mean days studied 4 ± 3). Seven patients tested positive for cocaine upon admission. Arterial and jugular venous samples were collected during daily 133Xenon CBF (ml/100g/min) studies and were analyzed for oxygen and carbon dioxide and cerebral metabolic rates calculated. Additionally, microdialysis catheters were placed in 5 of the cocaine positive patients and 9 of the cocaine negative patients.

Results: Following injury, CBF was higher in cocaine positive patients mean CBF 41.4 versus 37.9, $p = 0.22$. Additionally, CBF in the gray matter (GIS) was significantly greater in the cocaine positive patients 66.6 versus 53.6, $p = 0.03$. Arterial CO2 levels correlated more strongly with CBF but not GIS in cocaine positive patients: CBF $r = 0.65$, $p = 0.0001$ versus $r = 0.45$, $p = 0.0001$, GIS $r = 0.48$, $p = 0.003$ versus $r = 0.52$, $p = 0.0001$. CMRO2 was significantly less in cocaine positive versus cocaine negative patients, 1.4 versus 1.2 ml/100g/min, $p = 0.04$, respectively. Microdialysis showed that cocaine positive patients had significantly lower levels of glucose (mmol), 0.61 versus 1.05, $p = 0.009$, lactate (mmol) 0.32 versus 0.63, $p = 0.003$, and pyruvate (umol) 21.9 versus 37.3, $p = 0.008$.

Summary: This study shows that TBI patients who tested positive for cocaine exhibit unique cerebral blood flow and metabolism characteristics compared to age and injury severity-matched cocaine negative patients. Thus drug use may affect hemodynamic and metabolic responses to TBI and potentially responses to therapy.

P362.

ACUTE METABOLIC DEVIATION FROM NORMAL PREDICTS LONG TERM OUTCOME AFTER TBI

W. John Boscardin, Ph.D., Daniel F. Kelly, M.D., Thomas C. Glenn, Ph.D., David L. McArthur, Ph.D., Paul Vespa, M.D., David A. Hovda, Ph.D., Neil A. Martin, M.D. (UCLA, Los Angeles, CA US).

Object: Metabolic dysfunction after TBI is a complex phenomenon; available data are characterized by uneven numbers of studies at varying collection times. The purpose of this study was to develop a novel statistical methodology to quantify metabolic abnormality after moderate or severe TBI and relate these measurements to 6 month Glasgow Outcome Scale.

Methods: Serial assessments of cerebral metabolic rates for glucose (AVDglu and CMRglu), oxygen (AVDO2 and CMRO2) and lactate (AVDlac and CMRlac) were performed using a modified Kety-Schmidt method, with bedside 133Xenon CBF. Forty-two patients, (mean age 37 ± 17 years, median GCS 6, 71% male), were studied from post-injury days 0 to 5. Indices of metabolic deviation from normal were derived using a demographically similar sample of 28 healthy volunteers (mean age 34 ± 8 years, 70% male). For each metabolic study in the trauma cohort database, a multivariate Mahalanobis distance from the normal data was computed. This metric, takes into account correlation of measurements in the normal metabolic state, and thus quantification of abnormality examines both the magnitude of deviations of individual components from normal and the extent of pairwise "uncoupling".

Results: A three component abnormality measure using CBF, AVDO2, and AVDlac was strongly associated with 6 month GOS ($p < 0.001$) and with survival ($p < 0.002$). The patients with GOS of 1 (death) had the highest degree of abnormality and those with GOS of 5 (good recovery) had the lowest. These associations remained strong after controlling for known prognostic factors such as GCS, pupillary status, CT score, CPP and ICP ($p < 0.01$ for both).

Conclusions: During the first 5 days after moderate or severe TBI, the combined degree of abnormality in CBF, AVDO2, and AVDlac is strongly associated with poor longterm outcome. The deviation from normal methodology identifies multivariate measures of metabolic dysfunction that are predictive of outcome.

P363.

EXTRACELLULAR CALCIUM FLUCTUATIONS AFFECT VASCULAR TONE IN ISOLATED RAT MIDDLE CEREBRAL ARTERIES

TC Glenn, YC Lee, JS Hwang, DA Hovda, NA Martin, SM Lee. (UCLA Neurosurgery, Los Angeles, CA US).*

Rationale: Following traumatic brain injury extracellular levels of calcium (Ca+2) have been shown to fluctuate in both humans and rats. The purpose of this study was to determine if minor fluctuations of extracellular calcium affect vascular tone in isolated rat middle cerebral arteries (MCA).

Methods: MCA were isolated from male Sprague-Dawley rats (250–300g) and mounted in an isolated vessel chamber (Living Systems, Burlington, VT) which was perfused with phosphate buffered saline, temperature 37°C, pH 7.39, aerated with 20% oxygen, 5% carbon dioxide, and balance nitrogen. Vessel diameter was measured by a video dimension analyzer and data stored in a data acquisition program. Extracellular Ca+2 concentrations were modified from normal concentrations of 1.6 mM, to a minimum of 0.6 mM and a maximum of 3.1 mM. Pressure within the vessel was maintained at 60 mm Hg. Results: Altering extracellular Ca+2 caused the MCA to dilate at low concentrations and constrict at higher concentrations. At 0.6 mM calcium the arteries dilated to 123 ± 8% of the 1.6mM concentration, $n = 7$, $p < 0.05$, ANOVA. Extracellular Ca+2 at 3.1 mM caused the MCA diameter to constrict to 78 ± 5% of the baseline diameter, $n = 7$, $p < 0.05$ ANOVA. Both vasodilatory and vasoconstrictive effects of Ca+2 were inhibited by the L-type calcium channel antagonist verapamil at 10-6 and 10-5 M, but not at 10-7 M. However, the N-type calcium channel antagonist omega-conotoxin did not affect the Ca+2 mediated vascular responses.

Summary: Minor alterations in extracellular calcium affect vascular tone in an isolated MCA preparation. Following traumatic brain injury, extracellular calcium concentration may change due to excitatory amino acid activation, adenosine fluxes, depolarization, and other pathophysiological events. Thus, the hemodynamic status of the cerebral vasculature may be modulated by and fluctuate along with brain extracellular calcium concentration.

P364.

CYCLOSPORIN A DOES NOT AMELIORATE THE ANAEROBIC GLYCOLYSIS RESPONSE TO VIBRISSA MOTOR CORTEX STIMULATION FOLLOWING TRAUMATIC BRAIN INJURY

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We have demonstrated that the traumatically injured brain can respond metabolically to vibrissa motor cortex stimulation. This study examines the neurochemical response to stimulation following lateral fluid percussion injury (LFPI). Adult male Sprague-Dawley rats were studied at 1d ($n = 7$) and 7d ($n = 8$) following LFPI or sham injury ($n = 5$) conducted under isoflurane anesthesia. A microdialysis probe was placed 2 mm from the stimulating electrode in vibrissa motor cortex ipsilateral to LFPI. Stimulation (100–200 mA. 0.3 Hz. 40–60 mV) elicited a vibrissa response and a corresponding increase in both extracellular glucose (10%) and lactate (23%) concentrations in the sham-injured group. In both LFPI groups, stimulation elicited significantly greater increases in extracellular lactate (55–63%) and a significant decrease in extracellular glucose (2–17%). These results suggest that the injured brain relies on anaerobic glycolysis to fulfill the increased energy demands of stimulation. In an attempt to improve oxidative metabolism, cyclosporin A (CsA: 20 mg/kg. i.p.) was administered at 15 minutes and 1d following LFPI. CsA in these stimulated animals did not improve the anaerobic glycolysis response. In contrast. FK506 (1 mg/kg. i.p., 15 min and 1d) reduced both metabolic and neurochemical responses to stimulation. In determining the consequence of this secondary energy demand. we noted that CsA increased the number of Fluoro-Jade positive neurons in a defined area of posterior, medial cortex seen with stimulation following injury whereas FK506 had no such effect. This CsA-enhanced Fluoro-Jade response was reduced with co-administration of PBN (100 mg/kg. i.p., 15 min). suggesting a role of oxygen free radicals. These results suggest that CsA does not ameliorate the anaerobic glycolysis response to stimulation at 1d following LFPI and in fact shows a trend towards increased neuronal degeneration. (NS30308. UCLA Brain Injury Research Center).

P365.

OXYGEN, GLUCOSE AND LACTATE METABOLISM AS PREDICTORS OF OUTCOME AFTER TRAUMATIC BRAIN INJURY

Daniel F. Kelly, Thomas C. Glenn, W. John Boscardin, David L. McArthur, Paul Vespa, David A Hovda, Neil A. Martin. (UCLA Brain Injury Research Center, Los Angeles, CA US).*

Object: Although acute metabolic dysfunction is presumed to play a fundamental role in the pathophysiology of TBI, the impact of such metabolic derangements on long-term outcome has not been well delineated. The purpose of this prospective study was to determine if the degree of abnormal oxygen, glucose and lactate metabolism were predictive of 6 month global outcome after moderate or severe TBI, relative to other prognostic factors.

Methods: Serial measurements of the cerebral metabolic rates for glucose (AVDglu and CMRglu), oxygen (AVDO2 and CMRO2) and lactate (AVDlac and CMRlac) were performed using a modified Kety-Schmidt method, with bedside 133Xenon CBF. Forty-two patients, (mean age 37 ± 17 years, median GCS 6, 71% male), were studied from post-injury days 0 to 5.

Results: Six month post-injury Glasgow Outcome Scale was most strongly associated with cerebral metabolic rate of oxygen (CMRO2, $p < 0.0001$) and CBF ($p < 0.005$) and post-resuscitation pupillary status ($p < 0.01$); other important factors were patient age ($p < 0.05$) and % time CPP < 60 mmHg ($p < 0.05$). Patient survival versus death was most strongly associated with CMRO2 ($p < 0.0005$), post-resuscitation GCS ($p < 0.02$), pupillary status ($p < 0.02$), mean ICP ($p < 0.01$), mean CPP ($p < 0.005$) and % time CPP < 60 mmHg ($p < 0.001$). The associations of the metabolic predictors to outcome remained strong even after controlling for known prognostic factors such as GCS, pupillary status, CT score, CPP and ICP.

Conclusions: During the first 5 days after moderate or severe head injury, reduced CMRO2 and CBF are strongly associated with poor long-term outcome. Overall, CMRO2 appears to be a key predictor of global neurological outcome. Whether manipulations to decrease metabolic demands or alternatively to increase metabolic capacity will ultimately improve neurological recovery warrants further study. Support: NIH/NINDS, Grant # NS30308

P366.

PERICONTUSIONAL TISSUE DISPLAYS VULNERABILITY TO REDUCTION IN CEREBRAL PERFUSION PRESSURE WITHOUT MICRODIALYSIS EVIDENCE OF ISCHEMIA

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Intracerebral hematoma may induce a state of metabolic dysfunction in surrounding brain tissue. Spontaneous reduction in cerebral perfusion pressure (CPP), may induce further damage in vulnerable tissue. We Hypothesize that spontaneous changes in CPP elicit ischemic neurochemical changes in both perihematomal tissue and in minimally injured white matter (MIWM). Intracerebral microdialysis was performed in 13 consecutive patients with traumatic intracerebral hematoma (ICH). MD catheters were placed into MIWM adjacent to the ventriculostomy in all patients and a second MD catheter adjacent to perihematomal tissue in 7 patients. Hourly values of glucose, glutamate, lactate and pyruvate concentrations were measured and correlated with hourly CPP, ICP and SJVO2 values. In comparison with MIWM, perihematomal values of glucose were lower and glutamate, lactate and lactate/pyruvate (L/P) ratio were higher ($p < 0.01$) during normal CPP. In MIWM mean glucose concentrations were lower with CPP < 70 mm Hg (0.78 vs 0.93 mM, $p < 0.05$). However, mean glucose concentrations in perihematomal tissue decreased to a greater extent during reduced CPP (0.63 vs. 0.40 mM, $p < 0.001$). In both MIWM and pericontusional tissue mean lactate concentrations were higher with CPP < 70 mm Hg (1.2 vs 0.8 mM $p < 0.01$ and 1.3 vs 0.9 mM, $p < 0.01$). However, the L/P ratio did not increase when CPP < 70 mm Hg in either tissue type ($p < 0.4$). Despite this, pericontusional tissue displayed increase in glutamate (8.5 vs 6.8 uM, $p < 0.03$) with a reduction in CPP, whereas MIWM did not. We conclude that under conditions of normal CPP baseline differences in brain neurochemistry exist between pericontusional and MIWM tissue. However, during reduction in CPP < 70 mmHg, perihematomal tissue demonstrates a more dramatic reduction in glucose and increase in glutamate without an "ischemic" increase in L/P ratio. (Support: NINDS 30306; NS02089).

P367.

SIMULTANEOUS QUANTITATIVE MEASUREMENTS OF GLUCOSE METABOLISM, CEREBRAL BLOOD FLOW AND ADENOSINE TRIPHOSPHATE (ATP) LEVELS FOLLOWING TRAUMATIC BRAIN INJURY.

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Traumatic brain injury (TBI) causes an acute uncoupling of glucose metabolism and CBF, which can profoundly affect neuronal cell viability. We have developed a quantifiable technique to simultaneously measure regional cerebral glucose metabolism (CMRglc), CBF and the resulting tissue ATP levels in rats. Four male Sprague-Dawley rats were urethane-anesthetized (1.6g/kg) and cannulated for double-label autoradiography using 18F-fluoro-deoxyglucose (FDG) and 14C-iodoantipyrine (IAP) and secured in a 5 kW microwave (Thermex). FDG bolus (1mCi) was injected intravenously and timed samples were collected through the catheterized femoral artery for 30 min. Immediately after the FDG study, IAP (33μCi) was infused over 60 s, and timed arterial blood droplets were collected onto filter paper and assessed for radioactivity. Animals were sacrificed by microwave irradiation (6 sec, 1.8kW). Brains were frozen and coronal sections (20μm) were processed immediately for autoradiography and ATP quantification. Autoradiographic sections were dried onto coverslips and exposed to BioMax film for 75 min to obtain the FDG image. Two days later, sections were re-exposed to film for 1 day with 14C-standards (Amersham) to obtain the CBF image. For ATP analysis, 20μm tissue sections were mounted onto subbed slides and reacted to 80μm sections of frozen luciferin-luciferase enzymatic solution. The slides were reacted for 30 s and bioluminescence images were captured with Fluor-S MultiImager (Bio-Rad) and quantified using the ATP standard curve ($R2 = 0.99$). Regional ATP levels were obtained from the standard curve and expressed as μmol/g ATP. FDG-based CMRglc and IAP-based CBF were determined by the operational equations of Sokoloff (1977) and Sakurada (1978), respectively. The mean (±SEM) rates for control cortical cases are 1.43 ± 0.19 μmol/g ATP, 69.83 ± 2.68 μmol/100g/min CMRglc and 77.03 ± 2.54 mL/100g/min rCBF. This method of quantifying ATP, CMRglc and rCBF rates from the same region is a potentially powerful tool in understanding the events following TBI.

P368.

DO NEURONS UTILIZE ALTERNATIVE FUELS ACUTELY FOLLOWING HUMAN TRAUMATIC BRAIN INJURY?

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We studied gray-white (GM-WM) metabolic differences of glucose and oxygen utilization in acutely-injured TBI patients using positron emission tomography (PET). The objective was to determine whether there was evidence of abnormal cellular compartmentalization of energy metabolism and/or alternative fuel utilization.

Methods: 8 adult TBI patients and 11 age-matched healthy volunteers were studied. Each subject underwent quantitative FDG, [O-15]H2O, and [O-15]O2 PET studies and a coincident 3-D SPGR MRI. Parametric images of cerebral metabolic rate of glucose (CMRglc, μM/100g/min) and CMRO2 (μM/100g/min) were generated. Restricted masks delineating cortical GM and WM were extracted using a MRI-based segmentation technique (JCBFM 2001:21;S572) and applied to the parametric PET images. The area-weighted-average values of CMRglc and CMRO2 for the cortical GM and WM regions were calculated. The metabolic ratio (oxygen to glucose use ratio, OGR, in μM O2/μM glc) was calculated for GM and WM. A two-tailed t test was used for statistical analysis.

Results: Statistical analysis confirmed the following findings: (1) TBI patients show a selective depression of CMRglc in GM compared to controls (16.8 ± 3.0 vs. 23.7 ± 5.0; $p < 0.005$). GM-to-WM ratio of CMRglc was significantly lower following TBI (1.74 ± 0.24 vs. 2.36 ± 0.29; $p < 0.0001$); (2) the percent reduction in oxygen utilization was similar in GM (110 ± 35 vs. 140 ± 33; $p > 0.05$) and WM (42 ± 6 vs 55 ± 15; $p > 0.01$) following TBI. Compared to normals, OGR in GM (6.6 vs. 5.9) and WM (4.4 vs. 5.4) yielded a significantly higher GM-to-WM ratio in TBI patients (1.47 ± 0.26 vs. 1.12 ± 0.12, $p < 0.01$).

Conclusion: TBI causes discordant changes of CMRO2 and CMRglc in GM but not in WM. The selective increase of OGR in GM, where most neuronal cell bodies are, suggests an altered metabolic state specifically in neurons following TBI. The high GM OGR value (>6.0) further suggests that alternative substrates are used in GM after TBI. Supported by NINDS 30308 and DE FC0387-ER60615.

P369.

MICROARRAY GENE EXPRESSION ANALYSIS OF POSTNATAL DAY 19 RAT CORTEX AFTER LATERAL FLUID PERCUSSION INJURY

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Traumatic brain injury (TBI) triggers an interconnected cascade of pathophysiological changes. Following TBI to the immature brain, these effects are superimposed upon a growing and developing substrate. To better understand the complex patterns of gene expression after developmental brain trauma, we used microarray analysis of total parietal cortex RNA collected 4 hours (n = 4) and 24 hours (n = 4) after mild-moderate lateral fluid percussion injury (FPI) in the postnatal day 19 rat pup. Injury severities were similar in 4h and 24h animals (apnea 22.5 ± 5.4s vs 22.3 ± 2.6s, respectively; unresponsiveness to toe pinch 46 ± 16.7s vs 37.3 ± 4.7s, respectively). Comparisons were made with age and time-matched shams (n = 4 per time point). RNA was labeled and hybridized to Rat Genome Arrays (Affymetrix). Gene expression changes occurring in 75% of the comparisons between sham and injured were considered significant if the average fold-induction was >/=2. Using these criteria, 11 genes were significantly altered at 4h post-injury and 25 genes at 24h. At each time point, approximately 70% of these genes were upregulated and 30% downregulated. Several transcription factors (NAC-1, NGFI-B) and signal transduction molecules (tyrosine phosphatase, CaM kinase) were reduced after developmental FPI. Genes induced after FPI included those coding for metabolic enzymes (acyl CoA hydrolase, UDP-glucoronosyltransferase), neurotransmitter receptors (mGluR6), transcription factors (immediate-early serum responsive JE) and glial proteins (GFAP, vimentin, s100). These results demonstrate involvement of diverse molecular pathways following traumatic brain injury. This work provides a starting point for better understanding the unique vulnerability of the developing brain to traumatic injury. Support: NS30308, NS37365, NS27544 and UCLA Brain Injury Research Center.

P370.

AGE-RELATED MORPHOLOGIC CHANGES FOLLOWING TRAUMATIC BRAIN INJURY

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Abundant clinical data indicates increased morbidity and mortality following traumatic brain injury (TBI) in aged individuals. Little is currently known about the cellular substrates underlying this adverse response in the aged nervous system. The present study was designed to help characterize possible age-related morphologic response to TBI using a rodent model. Male Fisher 344 rats (3, 12, and 24 mos) were subjected to either a mild, moderate, or severe cortical injury using a lateral controlled cortical impact model of TBI. The animals were anesthetized with isoflurane and killed at 7 days post injury for assessment of cortical tissue sparing using unbiased stereology. We found an age-related and injury severity related difference in the magnitude of cortical sparing. The greatest sparing at each level of injury severity was observed in young animals while the aged animals showed the least amount of tissue sparing. Following a mild injury, young animals show almost no adverse consequence to the injury while aged rats have significantly less tissue sparing. At the moderate and severe injury levels, clear differences could be observed even in the 12 mos subjects compared to 3 mos subjects. Within each age group, there was a marked decline in tissue sparing dependent upon injury severity, the least amount of sparing observed with the most severe injury. The mortality rates among the different age groups were injury sensitive. The aged rats demonstrated the highest mortality rates similar to that observed in the clinical data. These results support the feasibility of using aged F344 rats to study age-related changes following TBI. Supported by NIH NS39828 and KSCHIRT #9-20.

P371.

IN VIVO APPLICATION OF INOS ANTISENSE OLIGONUCLEOTIDES EXACERBATES HYPOPERFUSION AND UPREGULATES ENDOTHELIN-1 EXPRESSION FOLLOWING TRAUMATIC BRAIN INJURY.

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Nitric oxide (NO, a vasodilator) and endothelin 1(ET-1, a powerful vasoconstrictor) participate in the regulation of brain s microcirculation influencing each other s expression and synthesis. Following injury to the brain, NO is derived largely from the inducible form of nitric oxide synthase (iNOS). We used the Marmarou s model of traumatic brain injury (TBI) to study the cerebral blood flow and expression (mRNA) of ET-1 in rats that were pretreated with antisense iNOS oligodeoxynucleotides (ODNs). Sprague-Dawley male rats were sacrificed 4, 24 and 48 h after TBI. Intracerebroventricular application of iNOS ODNs resulted in reduced synthesis of iNOS as detected by Western analysis. The cerebral blood flow (measured by laser Doppler flowmetry), generally decreased after TBI, was further reduced in the treated animals and remained at low levels up to 48 h post TBI. The expression of ET-1 (detected by in situ hybridization in cortex and hippocampus) was increased 2-3 fold following TBI alone and this increase reached 5-6 fold in animals pretreated with antisense iNOS ODNs. The results suggest that NO generated by iNOS, but not by other isoforms of the enzyme, suppresses ET-1 production and that a decrease of NO results in upregulation of ET-1 via transcriptional and translational mechanisms. Increased availability of ET-1 at the vascular bed and the neuropil may contribute to the altered microvascular reactivity and reduced perfusion of the brain following TBI. Supported by: NIH Grant NS39860

P372.

MICROARRAY ANALYSIS OF MICROGLIA ACTIVATED BY SOLUBLE FACTORS FROM TRAUMATICALLY INJURED ASTROCYTES.

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The emerging field of genomics provides potential for new insight into the pathophysiology of traumatic brain injury, by determining differences in gene expression in normal vs. injured brain. However, examination of expression differences in brain tissue does not provide a profile of genetic changes in the many individual cell types in the brain. Using an in vitro model for traumatic brain injury, we have previously identified several alterations in microglial calcium signaling and chemotaxis. Here, we examine selected gene expression profiles in resting and activated microglia. MG were isolated from 7–10 day old mixed brain cell cultures from neonatal rats. Resting MG were maintained in astrocyte-conditioned medium. Activated MG were prepared by a 24 hr exposure to medium conditioned by traumatically injured astrocytes for 3 hr, using an in vitro model for traumatic injury. cDNA was prepared from resting and activated microglia, followed by hybridization to selected expression arrays. Activated microglia displayed a dramatic increase in osteonectin (SPARC, secreted protein acidic & rich in cysteine), an important component of the extracellular matrix that regulates cell motility. HNK-1 sulfotransferase was also increased, as well as mitochondrial enoyl CoA hydratase and the endoplasmic reticulum protein ERp29. Interestingly, expression of lysozyme and the ferritin-H subunit were decreased. Alterations in these genes represent, in part, the early response of microglia to soluble factors released by injured astrocytes through the 3 hr post-injury period, and may be important in initiation of the inflammatory component of trauma. Supported by NS40490.

P373.

APOLIPOPROTEIN E EPSILON 4 IN PEDIATRIC TRAUMATIC BRAIN INJURY: PHASE I—DIFFICULTIES IN OBTAINING APPROVALS AND PATIENT ENROLLMENT

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Identification of children with high risk of neurologic sequelae following traumatic brain injury (TBI) would allow more focused treatment. We hypothesized that children with an allele of apolipoprotein E epsilon 4 (apoE 4) are predisposed to poor neurological and functional outcome after TBI, indicated by a higher pediatric cerebral performance category (PCPC) score. Our goal was to conduct a retrospective cohort study of children admitted to Children's Hospital of Wisconsin from 1/1/95 to 5/31/01 with a diagnosis of TBI.

Obtaining IRB and informed consent approvals caused a 1-year delay, illustrating the difficulties associated in dealing with pediatric patients. The IRB had initial concerns about the adequacy of consent, the propriety of genetic testing in children and confidentiality.

Following IRB approval, a chart review obtained demographic and clinical variables associated with outcome after TBI. Discharge and follow up pediatric overall performance category (POPC) and PCPC scores were obtained. An interview with the family was conducted to assess current status at follow-up. DNA was isolated from blood or buccal swab samples for standardized apoE4 genotyping. We found that either blood or buccal samples were sufficient to provide DNA for genotyping and hypothesized that patient enrollment is more easily accomplished in children when there is no blood draw. A total of 37 children were enrolled, ages 1mo to 16 yrs. Median Glasgow coma scale score (GCS) was 12 (range 4–15) and median pediatric risk of mortality (PRISM) score was 4 (0–25). Chi square analysis showed significant associations between admit GCS and PRISM score with both discharge and follow-up PCPC scores.

P374.

THE ROLE OF CEREBRAL INFLAMMATION AFTER TRAUMATIC BRAIN INJURY—A CONCEPT REVISITED

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Cerebral inflammation begins immediately after traumatic brain injury (TBI) and is orchestrated by a large variety of inflammatory mediators secreted by activated cells of the immune and the nervous system. Propagation of immunoactivation through the brain parenchyma also affects the healthy tissue surrounding the lesion, possibly causing alteration of the homeostasis of larger regions of the brain. Despite the extensive knowledge acquired in recent years on the role of cerebral inflammation after TBI, it still remains to clarify to what extent inflammation contributes to the progressive loss of neuronal cells, a process which persists for long time after the traumatic event.

Although inflammation has been considered as a potentially harmful cascade due to its ability to induce several neurotoxic molecules, increased blood-brain barrier permeability, cerebral accumulation of leukocytes, and neuronal cell death, evidence gained in the last decade has spurred a new concept of neuroinflammation, making it essential for the mechanisms of tissue repair. This dual function is suggested by both in vitro studies and animal models of TBI, which provide evidence for the induction of neurotrophic factors by cells stimulated with cytokines, as well as by specifically blocking cytokine action in animals resulting in improved neurological outcome after TBI. However, experiments with cytokine gene knockout mice have added further controversial significance to neuroinflammation rendering it as a temporally bifunctional event. The distinction between the acute and the delayed phase of inflammation seems to be crucial for its beneficial or deleterious effects. Despite the controversial action displayed by inflammation, it appears clearly that the experimental setting chosen is crucial to address any scientific issue related to inflammation as often distinct approaches - molecular cytokine blockade versus cytokine-gene knockout mice - can lead to apparently opposite results. Therefore, caution is required when interpreting such data.

P375.

THE POTENTIAL ROLE OF THE CHEMOKINES MCP-1 AND IL-8 AS WELL AS ICAM-1 IN TRAUMATIC BRAIN INJURY

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Chemokines and adhesion molecules are required to orchestrate cerebral accumulation of leukocytes after traumatic brain injury (TBI) in particular within focal lesions. Monocyte chemoattractant protein (MCP)-1 and Interleukin (IL)-8 as well as the intercellular adhesion molecule (ICAM)-1 were measured by ELISA in ventricular cerebrospinal fluid (CSF) of patients with severe TBI for up to 14 days after trauma. For comparison, the same factors were detected either in brain homogenates by ELISA or in tissue sections by immunohistochemistry of rats subjected to diffuse impact-acceleration TBI.

In all TBI patients, increased levels of MCP-1, IL-8 and ICAM-1 were detected as compared to control CSF. The mean MCP-1 CSF levels were highest at the day of admission and declined rapidly thereafter, whereas IL-8 and ICAM-1 concentrations remained elevated during the whole study period. Interestingly, IL-8 levels in patients presenting with focal brain injuries (NEML, NEML according to the Marshall Score) were significantly higher than in patients with diffuse injury (DI II, DI III) ($p < 0.05$; Student's t-test). Impact-acceleration brain injury in rats resulted in early upregulation of MCP-1 concentrations between 4 and 16 hours and a relatively late ICAM-1 overexpression between 1 and 4 days post-injury as compared to sham operated control animals. In contrary, concentrations of the chemotactic factor macrophage inflammatory protein (MIP)-2 (the rodent analogue of human IL-8) did not significantly exceed the constitutive levels detected in control brains, corroborating the findings of absence of neutrophil infiltration in this model as well as the similarity of low IL-8 levels in patients with diffuse vs. patients with focal brain injury.

These data provide evidence that in the impact-acceleration model, the ongoing inflammatory response is comparable to that seen in patients with diffuse axonal injury. The selective induction of inflammatory mediators present in focal vs. diffuse TBI suggests the existence of distinct immunoactivation pathways for each type of TBI.

P376.

INTRACRANIAL PRESSSURE DYNAMICS: CHANGES OF BANDWIDTH AS AN INDICATOR OF CEREBROVASCULAR TENSION

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The transmission bandwidth (BW) of arterial blood pressure (ABP) to intracranial pressure (ICP) was examined as a means of bedside monitoring of the state of cerebrovascular tension of patients with severe head-injury. Changes of experimental values of BW, relative arteriolar resistance and intracranial compliance were obtained from a piglet model equipped with a cranial window during induction of asphyxia, hypercapnia, and hypoxia. Comparisons of experimental BW values to simulated changes of BW produced by a mathematical model of ICP dynamics were used to evaluate the hypothesis that during active cerebrovascular tension changes of BW are inversely related to cerebral perfusion pressure (CPP) and during passive cerebrovascular tension, changes of BW are directly related to changes of CPP.

Induction of asphyxia produces BW changes characterized by an initial active cerebrovascular tension phase followed by a passive cerebrovascular tension phase. During the active tension phase the correlation between BW and CPP was inversely correlated to 1.41*10-5 x^(-3.21) ($r = 0.98$, $p < 0.005$, $n = 45$). During passive tension the relationship was correlated to 10^(-12) x^(9.22) ($r = 0.94$, $p < 0.005$, $n = 18$). One hour later during reventilation both BW and CPP increased. Furthermore, the observed 2 Hz increase of BW was predicted by regression relationship between BW and CPP for passive tension. Hypercapnic and hypoxic challenges produced changes of experimental BW that were matched with BW simulations of the mathematical model designed to depict active tension. Relationships between values of BW and relative average cerebral arteriolar resistance and intracranial compliance were inverse and strongly correlated ($r > 0.80$) to a regression of x^(-a), where the parameter ?a? ranged from 0.832 to 1.145. These preliminary experimental and theoretical results support the stated hypothesis.

P377.

THE ABBREVIATED INJURY SCALE IS A NEGLECTED TOOL IN TRAUMATIC BRAIN INJURY RESEACH

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Background: The Abbreviated Injury Scale (AIS) is an anatomically based, consensus derived, global severity scoring system that classifies each injury in every body region according to its relative importance on a six point ordinal scale. It has gone through several revisions since it's origin in 1976 and over the course of time, it has become the most widely used injury severity scoring system in the world. Despite its uniform acceptance as the gold standard for the determination of severity of injury by most trauma centers and national transportation agencies worldwide, the AIS has been little used in neurotrauma circles.

Methods: The AIS dictionary was reviewed, evaluated and the reasons that the AIS has not become popular in neurotrauma circles were documented after interviewing more than 100 investigators. The structure and utility of the AIS are demonstrated. The AIS and the GCS were then individually and correlationally evaluated from a data set of 56,000 head injured patients.

Results and Discussion: There are several sections of the AIS involving head injury that describe injuries to the scalp, cranial nerves, skull and brain. Brain injuries are described in three redundant schemes involving anatomic injury, length of unconsciousness and level of consciousness. The AIS differs from physiological injury severity tools such as the Glasgow Coma Score and is complementary to the GCS so that the use of both systems will increase the precision of injury severity and injury outcome predictions. Correlations between the AIS and the GCS are demonstrated to be significant ($p < 0.01$) and the cross-product of GCS and AIS show a stronger relation to early death than does either tool individually.

Conclusion: This is an opportune time for the neurotrauma community to evaluate the AIS since it is now being revised and input from the neurotrauma community would be welcome.

P378.

CORTICAL COMPACTION INJURY IN TRANSGENIC MICE TO STUDY THE ROLES OF REACTIVE ASTROCYTES

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Astrocytes respond to CNS injury by hypertrophy, altered gene expression and proliferation, a process commonly referred to as 'reactive astrocytosis'. Both beneficial and detrimental effects in the response to injury have been attributed to reactive astrocytes; their roles are incompletely understood. Transgenic technology provides various means of manipulating specific cell types and dissecting the functions of specific molecules. We are developing various transgenic models to study the roles of reactive astrocytes and the specific molecules that they produce in traumatic brain injury (TBI). These transgenic models include (i) the selective ablation of reactive astrocytes in adult mice expressing GFAP-HSV-TK, and (ii) the knockout of genes encoding specific molecules synthesized by reactive astrocytes in adult mice using the Cre-loxP and tetracycline regulatable systems. As a model of TBI in mice we have chosen cortical compaction injury (CCI). Here we will report on the characterization of CCI and initial studies in non-transgenic and transgenic mice of the C57Bl6 background strain. Quantitative morphometric analyses will include lesion size and responses of specific cell types identified by immunohistochemistry after transgenically targeted ablation of reactive astrocytes and TBI. (Supported by UCLA BIRC and NIH NS42039)

P379.

THE EFFECT OF POST-INJURY IRRADIATION ON NEURAL STEM CELL PROLIFERATION AND RECOVERY OF FUNCTION

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Neural stem cells are considered by many to be the 'magic bullet' for neurological disorders. Several studies with Parkinson's disease and dysmyelinating disorders have demonstrated a functional benefit with exogenously supplied neural stem cells. It has also been shown that treatments such as enriched environment. running. estrogen and anti-depressants cause increased neurogenesis. while stress. glucocorticoids and opiates cause a reduction. Furthermore. many brain insults such as ischemia. status epilepticus and traumatic brain injury (TBI) have been shown to result in an increased proliferation and neuronal differentiation of endogenous neural stem cells. However. no functional benefit of the increased neurogenesis following traumatic brain injury has been demonstrated. To examine the role of neural stem cells. we used irradiation. to kill the proliferating cells (putative neural stem/progenitor cells). Half of the animals were irradiated at 12 Gy 24 hrs following TBI. Animals were then evaluated both histologically and for cognitive deficits in the Morris water maze at varying times after TBI. We observed a decrease in number of proliferating cells due to the irradiation. as expected. At 15 and 30 days after TBI. there was not a significant difference in cognitive deficits between injured irradiated and injured non-irradiated animals. By 60 days the TBI non-irradiated animals displayed some recovery of cognitive deficits. however the TBI-irradiated animals were still impaired compared to sham- irradiated animals. We conclude that the increased neurogenesis observed following TBI plays a role in long-term cognitive recovery. Supported by NS-12587-26 and the Reynolds and Lind Lawrence Foundations.

P380.

EFFECTIVENESS OF SEATBELTS TO PREVENT HEAD INJURY IN LATERAL VERSUS FRONTAL MOTOR VEHICLE IMPACTS

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Motor vehicle crashes remain one of the highest sources of traumatic brain injury. The object of this study was to evaluate the efficacy of seatbelt restraints to prevent head injury after frontal versus side impact motor vehicle accidents. The US Dept of Transportation National Automotive Sampling System (NASS) database files from 1993–2000 were evaluated for drivers and right front seat occupants in frontal [principal direction of force (PDOF) 11–1 o'clock] and near side (PDOF 8–10 o'clock or 2–4 o'clock) impacts. Head injury was graded using the Abbreviated Injury Scale (AIS).

From the weighted data set there were 1,296,336 near side impact occupants (88.7% were belted) and 10,803,453 frontal impact occupants (86.0% were belted). Frontal impact resulted in a head injury (AIS 1–6) in 3.0% of occupants (9.6% of unbelted and 2.0% of belted occupants). Near side impact resulted in head injury in 6.8% of occupants (11.5% of unbelted and 6.2% of belted occupants). The pattern of injury was similar for moderate/severe head injury (AIS 3–6) with near side impact resulting in 2.4% injuries when unbelted and 0.3% when belted versus frontal impact causing 2.7% injuries unbelted and 0.9% belted. Of all occupants with a head injury, 17.4% of side impact occupants suffered a moderate/severe head injury compared to 19.3% of frontal impact occupants.

These data suggest that belted occupants have a 3-fold increased risk of suffering a head injury from a near side impact compared to a frontal impact. This trend is seen at all severities of head injury. While unbelted occupants have a higher rate of head injury independent of the PDOF the variance is less than that seen in belted occupants.

P381.

REGIONAL AND TEMPORAL PROFILE OF MITOTICALLY ACTIVE CELLS THROUGHOUT THE TRAUMATIZED BRAIN FOLLOWING BRAIN INJURY

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Following a variety of acute CNS injuries, a massive proliferation of stem/progenitor cells occurs. This study characterized cellular response of moderate traumatic brain injury (TBI) using indicators of cellular proliferation and mitotic division. Male Sprague-Dawley rats underwent fluid-percussion brain injury. The population of dividing cells was identified with bromodeoxyuridine (BrdU). Animals were sacrificed at 3 hr (n = 3), 1(n = 5), 2(n = 3), 3(n = 5), 7(n = 5), and 14 days (n = 5) after injury. Quantitative analyses of dividing cells were performed by non-biased cell counting in the ipsilateral and contralateral cortices and hippocampus. BrdU+ cells were observed in all sections ipsilateral and contralateral to the injured hemisphere at various times after TBI. In the cerebral cortex, the number of BrdU+ stained cells (mean x104 ± SD x104) increased in a time-dependent fashion between 3 hrs (8.1 ± 2.9) and 3 days (146.6 ± 17.3, $p < 0.001$), after which, the number of cells remained elevated up to 14 days (144.5 ± 55.4). Contralaterally, a gradual increase in BrdU+ cells between 3 hours (6.3 ± 2.9) and 14 days (77.3 ± 53.7, $p < 0.001$) was observed. In the hippocampus, there was an increase in the number of BrdU+ cells between 3 hours (6.3 ± 4.0) and 3 days (54.1 ± 11.3, $p < 0.001$), after which it remained elevated up to 14 days (41.6 ± 16.7). On the contralateral side, a gradual increase in BrdU+ cells, between 3 hours (5.5 ± 1.8) and 14 days (28.5 ± 17.2, $p = 0.008$) was observed. There was a statistically significant difference ($p < 0.001$) between ipsilateral and contralateral sides at all time points except 3 hours. Within the subventricular zone, there was a significant increase in BrdU+ cells at all time periods investigated. A large number of double-labeled cells stained positively for GFAP, with smaller numbers being positive for neuN. These results demonstrate a time-dependent increase in the number of proliferating cells following moderate TBI and provide baseline data for future studies assessing the effects of growth factor treatment on cellular proliferation and differentiation. NS30291.

P382.

THERAPEUTIC HYPOTHERMIA PRESERVES ANTIOXIDANT DEFENSES AFTER TRAUMATIC BRAIN INJURY IN INFANTS AND CHILDREN

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A wealth of experimental and clinical data support a contribution of oxidative stress to secondary damage after traumatic brain injury (TBI)(1). Hypothermia has been shown to decrease endogenous antioxidant consumption and lipid peroxidation after experimental brain injury (2,3). We hypothesized that therapeutic hypothermia attenuates oxidative damage as assessed by markers of lipid peroxidation, protein oxidation, and antioxidant status (glutathione and total antioxidant reserve [AOR]) in cerebrospinal fluid (CSF) after severe TBI in infants and children. We compared the effects of moderate hypothermia (32–33°C) vs normothermia in 19 patients with severe TBI (GCS score <8). Patients were treated in a single center involved in a multi-center randomized controlled trial of hypothermia in pediatric TBI. The general paradigm for patients treated with hypothermia (n = 9) involved cooling to target within ~6h for 48h and then re-warming. Protein thiols and glutathione (fluorescence assay), AOR (chemiluminescence assay), and F2-isoprostane (ELISA) were assessed in ventricular CSF samples (n = 49) on d1-3 after injury. Protein oxidation was attenuated by hypothermia vs normothermia ($p < 0.05$, d1-2). CSF levels of glutathione were higher on d3 in hypothermic vs normothermic patients ($p < 0.05$). AOR was ~25–30% higher in hypothermic vs normothermic patients ($p < 0.05$, d1-3). F2-isoprostane levels were ~4-fold higher in normothermic vs hypothermic patients ($p < 0.06$, d1). To our knowledge this is the first study assessing the effect of hypothermia on oxidative stress after severe TBI in infants and children. We report dramatic protection by hypothermia across a broad spectrum of markers of oxidative stress. Our data also demonstrate that CSF represents a valuable tool for monitoring treatment effects on oxidative stress after TBI. (1) Bayir et al, 2002 (2) Karibe et al, 1994 (3) Lei et al, 1994. SUPPORT: Eric Bundy Memorial Fund, Charles Schertz Fellowship grant, NS 34884, Laerdal Foundation, Children's Hospital of Pittsburgh GCRC.

P383.

SHORT-TERM EFFICACY IN THE TREATMENT OF BRAIN TRAUMA MAY NOT TRANSLATE INTO LONG-TERM IMPROVEMENTS

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We have previously found that treatment of brain-injured rats with MgSO4 or the non-competitive NMDA-receptor antagonist, NPS-1506, improved cognitive and histopathologic outcome by one week following trauma. To determine the persistence of this efficacy, we evaluated the effects of both treatments at 4 months following injury. Male Sprague-Dawley rats (350–400g) were subjected to parasagittal fluid percussion injury (2.6–2.8 atm) and were given injections of NPS-1506 (1.15 mg/kg, i.p., n = 12) 5 min and 4 hr post-injury, MgSO4 (125 mmol, i.v., n = 12) 15 min post-injury, or vehicle (saline, 2 ml/kg, n = 9). Sham animals received identical surgery without injury, and received saline (2 ml/kg, n = 10). Four months following injury, animals were evaluated for learning ability using a water maze paradigm. Briefly, animals were trained over 3d (6 trials/day) to locate a submerged platform. Escape times (latency) were averaged over the final 6 trials. Following behavior testing, animals were sacrificed and perfused with 4% paraformaldehyde. To evaluate the extent of cortical tissue loss, coronal sections between -5.8 and -6.04 bregma were stained with hemotoxylin and eosin and analyzed using NIH-imaging software. Using the contralateral hemisphere as a control, the percentage of tissue loss in the ipsilateral hemisphere was calculated. We found long-term learning dysfunction (increased latency times) in the vehicle-treated injured compared to sham animals ($p < 0.001$). However, no improvements in latency were found for the MgSO4-or NPS-1506-treated injured animals. Likewise, while injury in vehicle treated animals resulted in a 30% loss of tissue in the ipsilateral hemisphere, this atrophy was not reduced in the drug-treated animals. These results suggest that acute treatment with MgSO4 and NPS-1506 may not convey long-lasting efficacy following injury. Long-term supplementary therapies may have to be considered to combat progressive neurodegeneration induced by brain trauma. Supported by NIH grants AG 21527, NS38104, and NS08803.

P384.

CALPAIN MEDIATED SPECTRIN BREAKDOWN PRODUCTS IN THE CEREBROSPINAL FLUID OF SEVERELY HEAD INJURED PATIENTS

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Calcium-induced, calpain-mediated proteolytic processes are considered key players in brain and spinal cord injury. Recent observations have suggested that therapeutic interventions, limiting the formation of calpain-mediated spectrin breakdown products (SBDP), are associated with improved outcome in experimental TBI.

The present study was initiated to determine if SBDP accumulation can be detected in the cerebrospinal fluid (CSF) of severely head injured patients, providing a potential diagnostic and perhaps, prognostic tool for monitoring the course and severity of TBI.

Ventricular CSF was obtained from 9 severely head injured patients (Glasgow Coma Scale<9) as part of an established protocol for raised intracranial pressure (>20mmHg). CSF samples of 4 patients treated for acute hydrocephalus associated with subarachnoid hemorrhage constituted the control group. The presence of SBDP were evaluated via Western blots using a monoclonal antibody capable of detecting intact non-erythroid alphaII-spectrin (280 kD) as well as its 150, 145 and 120kD cleavage fragments.

All severely head-injured patients displayed calpain-mediated SBDP 150/145 accumulation. Elevated protein levels were most striking in those samples taken at 2-5 days post-injury, with the most elevated levels associated with less favorable outcomes. In three of the controls, a less pronounced SBDP-accumulation was also detected, a finding most likely associated with periventricular white matter injury due to hydrocephalus.

These results indicate that high SBDP-levels are detectable in ventricular CSF following severe TBI, and in some cases of acute hydrocephalus. Our observations suggest that detection of SBDP-levels may provide useful information on the severity of proteolytic processes set in motion by brain/white matter injury of various origins. Further, they may serve as a useful tool in the assessment of injury severity and the prediction of outcome.

Grants of the Hungarian Science Foundation (OTKA T 035195, ETT), Bólyai Scholarship of the Hungarian Academy of Sciences and the Fogarty International Research Collaboration Award (1-RO3-TW0131302A1) supported this work.

P385.

TOPIRAMATE ATTENUATES TRAUMATIC BRAIN INJURY-INDUCED NEUROMOTOR DEFICITS IN RATS

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The present study evaluated the ability of topiramate, an antiepileptic drug, to reduce edema formation and cognitive and motor deficits following lateral fluid percussion (FP) brain injury in rats. Anesthetized adult rats were subjected to lateral FP brain injury (n = 60) or left uninjured (n = 47, sham surgery). At 30 min. post-injury, animals were randomized to receive either topiramate (30 mg/kg, i.p.; injured n = 35, sham n = 21) or sterile water (injured n = 25, sham n = 26) followed by administration of topiramate (30 mg/kg, p.o.) or vehicle at 8, 22 and 32 hrs postinjury. Following cognitive evaluation in the Morris water maze at 48h, a subset of animals was sacrificed for brain water content evaluation. All other animals received motor function testing at 48h, 1, 2, 3, and 4 wks post-injury using a 28-point neuroscore and the rotating pole test, followed by cognitive testing at 1 month. Injured animals showed significant edema formation, cognitive and motor deficits when compared to uninjured animals. However, no differences in brain water content or cognitive function were detectable between drug and vehicle treated animals. Topiramate significantly attenuated motor dysfunction in injured animals in the neuroscore at 4 weeks postinjury ($p < 0.05$) and the rotating pole test at 1 and 4 weeks postinjury ($p < 0.05$) when compared to vehicle treated brain injured rats, suggesting a potentially beneficial effect of topiramate in improving motor function following TBI. Supported by J & J Pharm. R & D, LLC, NIH NS 08803, NS 40978, & GM 34690.

P386.

ADENOSINE 2a RECEPTOR KNOCKOUT MICE ARE NEUROPROTECTED AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY.

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The adenosine 2a (A2a) receptor exhibits multiple roles in the CNS -both detrimental (direct and indirect excitotoxic actions) and beneficial (blood flow promotion and anti-inflammatory effects). In models of stroke and Parkinson's disease, A2a knockout (ko) mice and animals treated with A2a receptor antagonists are neuroprotected.1,2 We hypothesized that A2a receptor ko mice would be neuroprotected after experimental traumatic brain injury (TBI) produced by controlled cortical impact (CCI). A2a ko and wild-type (wt) littermates (n = 13), genotypes confirmed by southern blot and immunohistochemistry, were injured at 15 wks of age. CCI was performed at a velocity of 5 m/s, a depth of 1.2 mm. Brain temperature was controlled at 37°C. At 24 h, mice were perfused with 4% paraformaldehyde and brain tissue was paraffin embedded for sectioning. Sections were stained with H&E and hippocampal neuronal counts (40X) were performed in CA1, CA2 and CA3 regions. In CA1, the A2a ko was markedly protected vs wt (91.75 ± 17.53 vs 33.20 ± 16.72 cells, respectively, mean ± SEM, $p < 0.05$). A 2-fold trend towards neuroprotection was also seen in CA3 (but not in CA2) for ko vs wt. We conclude that neuroprotection in A2a ko mice in the CCI model is consistent with recent reports in stroke and Parkinson's disease, suggesting a key pro-excitotoxic regulatory role for the A2a receptor. Our findings support the need to evaluate A2a receptor antagonists as a possible therapy in experimental and clinical TBI. 1Chen et al, 1999, 2Chen et al, 2001. SUPPORT: NS38037, NS 30381, HD 40686

P387.

THE THERAPEUTIC EFFICACY OF THE 5-HT1A RECEPTOR AGONIST 8-OH-DPAT IN TRAUMATICALLY BRAIN-INJURED RATS IS NOT MEDIATED BY CONCOMITANT HYPOTHERMIA.

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We recently demonstrated beneficial effects on cognitive and histological outcome after a single acute dose (0.5 mg/kg, i.p.) of the serotonin (5-HT)1A receptor agonist 8 hydroxy-2-di-n-(propylamino)tetralin (8-OH-DPAT), which induces mild hypothermia transiently (Dixon et al., J Neurotrauma, 18:1172, 2001). Thus, to determine if the beneficial effects observed were mediated by hypothermia, we conducted an experiment identical to the previous, but included a group of 8-OH-DPAT-treated rats that were actively kept normothermic (37 ± 0.5°C) with a heating lamp. Briefly, thirty-nine isoflurane-anesthetized rats underwent a controlled cortical impact (2.7 mm deformation) or sham injury and then were randomly assigned to one of five groups (Sham/VEH n = 5, Sham/0.5 mg/kg 8-OH-DPAT n = 5, TBI/VEH n = 9, TBI/8-OH-DPAT Normothermic n = 10, TBI/8-OH-DPAT Hypothermic n = 10). 8-OH-DPAT or VEH was administered (i.p.) 15 min after TBI or sham injury. Cognitive performance was assessed in the Morris water maze on post-operative days 14–18. Both 8-OH-DPAT-treated groups attenuated cognitive impairment after TBI vs. VEH ($p < 0.05$). No significant differences were observed between the normothermic and hypothermic DPAT-treated groups, despite a rapid (15 min after injection), mild (34.4–34.9°C), and transient hypothermic effect (1 hr) in the latter group. These data confirm that systemic administration of the 5-HT1A receptor agonist 8-OH-DPAT provides cognitive protection after moderate TBI. The data also suggest that the beneficial effect is not mediated by concomitant hypothermia. 5-HT1A receptor agonists may be a novel alternative therapeutic strategy after TBI in humans.

Supported by the Walter L. Copeland Fund (D 2001-0288) of The Pittsburgh Foundation (AEK) and NS33150 and NS40125 (CED).

P388.

TEMPORAL AND REGIONAL ALTERATIONS IN ENDOGENOUS GDNF EXPRESSION AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

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Glial cell line-derived neurotrophic factor (GDNF) is an important regulator of neuronal development and exhibits neuroprotective activity in several models of CNS injury. However, to date, there has been no assessment of endogenous GDNF expression following traumatic brain injury (TBI). In the current experiment, the temporal and regional endogenous expression of GDNF was examined following lateral fluid percussion (FP) brain injury (2.7–2.8 atm, n = 4) in anesthetized adult rats using enzyme-linked immunosorbent assay (ELISA). Control rats received neither surgery nor injury (naÔve animals, n = 3). Twenty-four hours following injury, the animals were sacrificed and the injured brain hemispheres were dissected into cortical region. Cortical regions from brain-injured animals demonstrated a significant increase in GDNF protein expression at 24 h postinjury (23.9 ± 6.4 pg/mg protein) compared to naïve cortical regions (9.3 ± 3.6 pg/mg protein) ($p < 0.05$). These alterations in GDNF protein expression may be involved in modulating the neuronal response after brain injury. Supported by, in part, by NIH NS 40478, NS 08803, GM3640 and a Veteran Administration-DOD Consortium Merit Review Grant.

P389.

DOWNREGULATION OF MATRIX METALLOPROTEINASE-9 AND ATTENUATION OF EDEMA VIA INHIBITION OF ERK MAP KINASE

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As a family of extracellular proteases, matrix metalloproteases (MMPs) are capable of degrading or modifying almost all components of the extracellular matrix and it may be involved in the pathophysiology of acute brain injury. However, the regulatory mechanisms involved in vivo remain unclear. We previously reported mitogen activated protein kinase (MAPK) are upregulated after traumatic brain injury (TBI) and intraventriclar administration of MAPK/ERK kinase (MEK) inhibitor decreased lesion volume after injury. In this study, we focus on a MAPK pathway that may trigger MMP-9 after TBI. We examined whether inhibition of the extracellular signal regulated kinase (ERK) would attenuate MMP-9 levels, reduce blood-brain barrier damage, and attenuate edema after trauma induced by controlled cortical impact in mouse brain. A rapid upregulation of phospho-ERK occurred within minutes after trauma. Double-labelled immunohistochemistry showed that ERK activation occurred primarily in neuronal cells in traumatized cortex. Treatment with U0126, MEK inhibitor effectively prevented the activation of ERK and reduced the trauma-induced upregulation in MMP-9. Correspondingly, U0126 ameliorated the degradation of the tight junction protein ZO-1, which is an MMP-9 substrate, and significantly attenuated tissue edema. At 7 days after trauma, traumatic lesion volumes were significantly reduced by U0126 compared with saline-treated controls. These data indicate that the ERK MAPK pathway triggers the upregulation in MMP-9 after trauma, and further suggest that examination of signaling mechanisms that regulate deleterious MMP-9 activity may reveal new therapeutic opportunities for traumatic brain injury.

P390.

TREATMENT WITH THE IRREVERSIBLE, CELL PERMEABLE CASPASE-9 INHIBITOR III, PROVIDES PROTECTION AGAINST CA1 TRAUMATIC NEURONAL INJURY IN THE HIPPOCAMPAL SLICE

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Caspase-9 holds a key position in the initiation of programmed cell death. Caspase-9 is also released from mitochondria with brain injury. and is activated with binding to Apaf-1 and dATP. Activated Caspase-9 then cleaves other Caspases further downstream. including Caspase-3. -6 and -7. Due to the critical role of Caspase 9 in programmed cell death pathways. we hypothesized that inhibitors of Caspase 9 would be neuroprotective against CA1 traumatic neuronal injury. Therefore. we investigated whether treatment with the cell permeable. irreversible Caspase-9 inhibitor III. Ac-LEHD-CMK. would preserve CA1 evoked response in hippocampal slices following fluid percussion trauma. Recovery was assessed one hour after trauma. Trauma induced rapid loss of CA1 orthodromic and antidromic PS response. After recovery for 60 min. CA1 orthodromic and antidromic population spike (PS) response regained only a mean 11% ± 2 and 15% ± 2 of initial amplitude. In contrast. treatment with 5 _M Ac-LEHD-CMK initiated within one min after trauma. improved recovery of CA1 orthodromic and antidromic PS response to 93% ± 5 and 94% ± 4 of initial amplitude ($p < 0.05$). Long-term-potentiation (LTP) was completely lost following trauma. while treatment with Ac-LEHD-CMK preserved LTP. With this inhibitor. tetanus produced a mean increase in CA1 orthodromic PS amplitude to 127% ± 4. similar to LTP induced in sham slices. which showed an increase of 126% ± 3. These findings suggest that caspase-9 mediated-effects play an important role in injury to CA1 neurons from trauma. Supported by the VA Research Service and the UCLA Brain Injury Research Center.

P391.

THE EFFECTS OF MEK INHIBITOR U0126 FOLLOWING TRAUMATIC BRAIN INJURY IN RATS

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(Introduction) Biochemical cascades underlying posttraumatic neuronal degeneration have not been fully elucidated. We recently demonstrated that traumatic brain injury (TBI) induces ERK-phosphorylation in the rat brain. In this study, we investigate the role of ERK-phosphorylation using MEK1/2 inhibitors U0126 in histopathological changes and motor function after TBI.

(Material & Methods) Adult male Sprague-Dawley rats (300–400 g) were subjected to lateral fluid percussion injury of moderate severity (3.5–4.0 atm). U0126 (100, 200, or 400 μg/kg) or vehicle (DMSO) was injected into the femoral vein 15 min before injury. Sham control animals were subjected to all the same procedures except for actual insult. Serial coronal sections (5-μm-thick) were counterstained with hematoxylin and eosin. Severity of neuronal damage in CA3 subfield was evaluated by the number of survived or damaged neurons 72 hrs after TBI. Contusional brain volume 72 hrs after TBI was measured using an image analysis system by summing the contusional brain areas. Evaluation of brain atrophy 3 wks after TBI was calculated using the same formula. In addition, we assessed the motor function using a beam-balance task and a beam-walking task.

(Results) The present results indicate that intravenous administration of the U0126 promote the CA3 neuronal survival and reduce the contusional volume 72 hrs after TBI. U0126 also improve the brain atrophic changes 3 wks after TBI. In addition, U0126 make a significant recovery of the motor function 3, 4, 5 days after TBI compared to that in vehicle groups.

(Conclusion) Inhibition of the ERK-phosphorylation ameliorates the neurological and histopathological outcome following TBI in rats. These findings suggest that the activation of the ERK cascades has deleterious effects on the damaged neurons following TBI.

P392.

CHANGES IN DARPP-32 PROTEIN EXPRESSION FOLLOWING CONTROLLED CORTICAL IMPACT

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DARPP-32, a dopamine- and cAMP-regulated phosphoprotein, is a cytosolic protein abundant in medium-sized spiny neurons in the neostriatum. When phosphorylated by cAMP-dependent protein kinase (PKA), DARPP-32 is a potent inhibitor of protein phosphatase-1, thereby regulating a large array of downstream effectors. DARPP-32 plays an integral role in signal transduction of dopaminoceptive neurons; regulators of its phosphorylation include dopamine, glutamate, GABA, and adenosine. To determine whether levels of DARPP-32 are altered following traumatic brain injury (TBI), Western blot analysis and immunohistochemistry were performed to compare DARPP-32 protein expression in the striatum of rats that received controlled cortical impact (CCI) injury versus sham-injured controls.

Twenty-four hours after CCI (2.7 mm impact at 4 m/s), adult male rats (n = 4 injured, 4 sham) were anesthetized and decapitated, and the ipsilateral and contralateral striatum processed for Western blot analysis. Protein expression was measured using a primary antibody against DARPP-32, generously donated by Dr. Paul Greengard. Semi-quantitative densitometry revealed an injury-induced decrease in DARPP-32 protein expression in both ipsilateral (28%) and contralateral (43%) hemispheres. Immunohistochemistry for DARPP-32 suggests the decrease persists up to 7 days post-injury. Qualitative analysis indicates a lack of apparent change in the number of DARPP-32 immunopositive cells, suggesting the decrease in protein expression is not due to neuronal cell death in the striatum.

The wide range of signaling cascades that include DARPP-32 suggests a reduction may influence multiple facets of the injury process. Behaviors that are mediated by DARPP-32 include motor function, learning and memory, and motivation. Further insight into injury-induced changes in DARPP-32 protein expression and phosphorylation may further the development of novel treatments for TBI patients.

(Supported by NS-33150)

P393.

SYNAPTOSOMAL DOPAMINE UPTAKE IN RAT STRIATUM FOLLOWING CONTROLLED CORTICAL IMPACT

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Functional deficits following traumatic brain injury (TBI) are associated with alterations in markers of dopaminergic neurotransmission. Using the controlled cortical impact (CCI) model of TBI in rats, our lab previously demonstrated decreased dopamine (DA) D2 receptor protein in striatum (Yan et al., 1999), increased tyrosine hydroxylase protein in frontal cortex (Yan et al., 2001), and decreased dopamine transporter (DAT) protein in the striatum (preliminary data) 4, 4, and 2 weeks after injury, respectively. DAT plays a critical role in maintaining DA homeostasis. To assess the effects of TBI on the functional integrity of DAT, we investigated synaptosomal DA uptake in the striatum, where levels of DAT expression are highest.

Fifteen days after lateral CCI (2.7 mm impact at 4 m/s) or sham injury, adult male rats (n = 4 injured, 4 sham) were decapitated and striatal tissue rapidly dissected. Synaptosomal preparations from the ipsilateral and contralateral hemispheres were incubated at 37°C with 20 nM [3H]DA and varying concentrations of unlabeled DA in the presence or absence of 5μM uptake inhibitor, mazindol. DA uptake in injured-ipsilateral, injured-contralateral, sham-ipsilateral and sham-contralateral tissue was compared using a repeated-measures ANOVA. No significant difference in synaptosomal uptake was found between groups 15 days after CCI. Our data suggest that striatal DAT is capable of normal function 15 days after CCI. However, it is unclear whether neurons in the injured striatum can properly regulate the activity of DAT. The lack of change in DAT kinetic activity in the injured striatum is surprising in light of our previous finding of decreased DAT protein by western blotting. Future studies will examine DA uptake at additional timepoints in the striatum and other brain regions, and assess other aspects of DA neurotransmission after TBI. (Supported by NS-33150)

P394.

MORPHOLOGICAL, DYNAMIC AND CYTOSKELETAL PROPERTIES UNDERLYING NEURITE DEVELOPMENT AND AXONAL SPROUTING FOLLOWING LOCALISED TRANSECTION OF CORTICAL AXONS IN VITRO

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Accumulating evidence demonstrates that mature central neurons retain the capacity to mount a regenerative attempt in response to physical injury when provided with a facilitative environment. Cytoskeletal recovery and reorganisation may be crucial to this regenerative capacity. We utilised an in vitro model of neuronal trauma to explore whether the cytoskeletal alterations associated with post-injury axonal sprouting correlate with the sequence of cytoskeletal changes underlying neurite development. Neocortical cultures were generated using E18 rats. Neurons were dissociated and grown in Neurobasal medium containing B27 supplement. Under these conditions neuronal cell bodies formed aggregates that became progressively interconnected by neurite bundles and lattices, which were transected under microscope guidance. Neurons were examined during development at 3 days in vitro (DIV) and at various time points following axotomy at 21DIV. Time-lapse imaging demonstrated that the post-injury response is highly dynamic, progressing through an initial phase of axonal retraction, followed by substantial axonal sprouting into injury sites within 4–6 hours following transection. Analogous to developing neurites, post-injury sprouts were highly motile and consisted of a slender shaft and an expanded growth cone-like end structure. AlexaFluor™488 Phalloidin and immuno-labelling (utilising antibodies specific for cytoskeletal and other proteins) demonstrated that filamentous-actin was the predominant cytoskeletal constituent in the extreme distal tips of sprouts, whereas betaIII-tubulin and tau were localised to sprout shafts and proximal regions of putative growth cones. The neurofilament triplet was, however, restricted to sprout shafts. A similar distribution of these cytoskeletal proteins was present in developing neurites at 3DIV. Interestingly, a greater proportion of sprouts were immunoreactive for betaIII-tubulin and tau than for neurofilament triplet proteins. The morphology, cytoskeletal constitution and dynamic properties of sprouts emerging from transected axons at 21DIV closely resembled those of developing neurites, indicating that post-injury axonal sprouting recapitulates these aspects of initial neurite development.

This research was funded by the NHMRC and RHH Research Foundation.

P395.

CLINICAL BIOMECHANICS OF PENETRATING BRAIN TRAUMA

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INTRODUCTION: Since 1992 gunshot wounds are the leading cause of brain injury death in the US. To investigate primary and secondary wound profiles in the brain due to penetrating head trauma, prospective clinical study, experiments, and computer-driven finite element model (FEM) were used.

METHODS: Patient CT/MRI scans were evaluated. Psychosocial measures and neurological status determined outcome. Images were transferred to software to create three-dimensional surface models of the primary and secondary wound tracks from which volumetric data were determined. A three-dimensional FEM of the brain and skull was constructed to reproduce penetrating trauma. It was validated with human tissue simulant experiments. Projectiles were discharged at 700 meters/second into 50-cm gelatin blocks (embedded nylon strings). Projectile paths were recorded using a digital camera at 9,000–18,000 frames/second. Wounding deformations quantified as a function of time validated the FEM. The computer model was exercised with varying projectile geometries (flat-face, pointed-face) and velocities to reproduce the damage profile seen in the CT/MRI.

RESULTS AND DISCUSSION: Time-varying intracranial deformations demonstrated the progression of the projectile into the skull and brain. Similar skull penetrations occurred with both projectiles. However, the extent of brain damage was greater with the flat-face projectile that produced transient, secondary waves with larger area/volume. Furthermore, the brain tissue sustained these waves for 1–2 milliseconds, approximately twice the duration of the pointed projectile. A significant loss of penetrating velocity occurred with the flat-face projectile. The wounding capacity of the other projectile was low. These results indicate that kinetic energy from the flat-face projectile is transmitted to brain tissue not only in the region immediately surrounding the path of the projectile but also in regions away from its path. The geometry of the projectile is an important determinant in the extent of brain trauma in regions away from the primary penetration path.

ACKNOWLEDGMENTS: CDC R49CCR519614, VA Medical Research.

P396.

LOCALISATION OF ALPHA-SYNUCLEIN FOLLOWING AXONAL TRANSECTION: IMPLICATIONS FOR REGROWTH AND REPAIR

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Alpha-synuclein is highly enriched in presynaptic terminals, and abnormal processing of alpha-synuclein has been demonstrated in several human neurodegenerative diseases. Following head trauma, alpha-synuclein also accumulates in axonal swellings, but its relationship with the plastic changes associated with axonal regrowth and repair has not been examined. We investigated the localisation of alpha-synuclein in an in vitro model of axonal transection that results in regenerative sprouting. Primary rat neocortical neuronal cultures were established using E18 embryos from Hooded Wistar rats. Neurons were grown on coverslips in Neurobasal medium (GIBCO) containing B27 supplement. At 21 days in vitro, axonal bundles were transected using a goniotomy knife. Cells were fixed 4 and 24 hours post-injury (PI) and immunolabelled for alpha-synuclein in combination with markers for phosphorylated neurofilaments, synaptophysin, microtubule- and growth-associated proteins. At 4 hours PI, alpha-synuclein accumulated diffusely as enlarged puncta in transected axons. In axons that reacted to injury by forming neurofilament ring- and bulb-like structures, high concentrations of alpha-synuclein were observed in the centre of these structures. By 24 hours PI, there was a decrease in alpha-synuclein accumulation at transection sites, but ring- and bulb-like structures remained positive for alpha-synuclein. In post-traumatic sprouting axons at 24 hours PI, alpha-synuclein immunoreactive puncta were concentrated in the most proximal region of growth cone-like structures, with little labelling in the distal tips as revealed by GAP-43. Our findings suggest that alpha-synuclein may be actively involved in axonal injury and repair.

This research was funded by grants from the NH&MRC and RHH Research Foundation.

P397.

REACTIVE AND REGENERATIVE NEURONAL CYTOSKELETAL ALTERATIONS FOLLOWING ACUTE LOCALIZED INJURY TO THE RAT NEOCORTEX.

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We investigated the alterations in neuronal cytoskeletal proteins associated with localized brain injury. The neocortex of anaesthetized adult Wistar rats was injured by insertion of a 25 gauge blunt needle for 10 minutes. Animals were re-anaesthetized and perfused transcardially with 4% paraformaldehyde at 1, 4, 7 and 14 days post-injury (PI). Multiple immunohistochemical labelling with antibodies to the neurofilament (NF) triplet, alpha-internexin, neuron-specific beta-tubulin, cytochrome C, GFAP and ferritin was performed. Needle injury resulted in a cavity surrounded by necrotic tissue (1 day PI) followed by significant wound healing (4–7 days PI) characterised by the development of central microglial/macrophage mass and peripherally located reactive astrocytes. By 14 days PI, a neuropil of generally normal appearance surrounded a narrow microglial remnant. At 1 day PI, the needle tract was surrounded by abnormal neurites variably immunoreactive for beta-tubulin, cytochrome C, NF triplet and alpha-internexin, the latter intermediate filaments also localized to bulb- or ring-like structures. Surrounding nerve cell bodies had fragmented NF labelling as well as a high degree of cytochrome C labelling. By 4-7 days PI, substantial neurite sprouting beyond the zone of astrocyte proliferation and into the central mass of ferritin labelled cells had occurred, with more sprouting neurites labelled for beta-tubulin labelling than the NF proteins. Real-time PCR also demonstrated variable regulation of cytoskeletal gene expression following injury. In addition, beta-tubulin labelled cells were present within the central cellular mass at 7 days PI. Thus, cytoskeletal proteins are variably involved in the reactive and regenerative response of cortical neurons to injury. Furthermore, some neurons have motile properties that may contribute to remodelling of brain architecture.

Funded by the NHMRC.

P398.

LONG-TERM ACCUMULATION OF AMYLOID-BETA, BETA-SECRETASE AND PRESENILIN-1, AND CASPASE-3 IN DAMAGED AXONS FOLLOWING BRAIN TRAUMA.

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Plaques composed of amyloid-beta (A-beta) have been found within days following a single incident of brain trauma in humans, similar to the hallmark plaque pathology of Alzheimer's disease (AD). We have recently found that a potential source of this amyloid-beta is extensive accumulations in damaged axons following inertial brain injury in the pig. Here, we used the same model to evaluate long-term changes in A-beta accumulation and potential mechanisms that could lead to its production. Brain injury was induced via nonimpact head rotational acceleration of 110? over 20 ms in the coronal plane. Injured pigs were sacrificed at 3 days (n = 3), 7 days (n = 3) and 6 months (n = 3) postinjury. Two non-injured animals served as controls. Immunohistochemistry and Western blot analysis were performed on brain sections and tissues using antibodies specific for amyloid precursor protein (APP), A-beta, BACE, presenilin-1 (PS-1), caspase-3, and caspase-mediated cleavage of APP. Surprisingly, substantial co-accumulation for all of these factors was found in swollen axons at all timepoints, including up to 6 months following injury. Western blot analysis of tissue from injured brains confirmed a substantial increase in the protein levels of these factors, particularly in the white matter. Although in AD, A-beta is thought to be primarily produced via transmembrane cleavage of APP by BACE and PS-1 our data suggests that following trauma these factors, as well as caspase activity may produce A-beta within the axonal membrane compartment. Supported by NIH grants AG21527, NS38104 and NS08803.

P399.

DELAYED DISRUPTION IN AXONAL TRANSPORT FOLLOWING LATERAL FLUID PERCUSSION BRAIN INJURY IN RATS

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Retrograde degeneration may contribute to delayed cell death in areas remote from the cortical contusion following lateral fluid percussion(FP) brain injury. We examined the integrity of axonal projections to the injured cortex using the retrogradely transported neuronal tracer, Fluoro-Gold. Adult male Sprague-Dawley rats were anesthetized (sodium pentobarbital, 60 mg/kg, i.p.) and subjected to lateral FP brain injury(2.8 ± 0.1 atm) or sham injury. Rats were then injected with 1mL of 2% Fluoro-Gold into the ipsilateral parietal cortex(AP -4.3, ML 5.5, DV 2.0) at 6 hours (n = 6) or 24 hours (n = 5) post-injury or immediately after sham injury(n = 6). At 1 week post-injection, frozen brain sections(40mm) were cut coronally from 1.6 to -6.3 mm bregma. In uninjured animals, numerous Fluoro-Gold positive neuronal cell bodies were observed in the bilateral parietal cortex and ipsilateral thalamus. In the group of animals injected at 6 hours post-injury, a modest reduction in the number of labeled neurons was observed in these areas, particularly in the contralateral cortex and ipsilateral thalamus. In contrast, Fluoro-Gold labeling was nearly absent in the contralateral cortex and ipsilateral thalamus in the group of animals injected 24 hours post-injury. These results suggest that there is a delayed, progressive impairment of retrograde axonal transport from the injured parietal cortex to the contralateral cortex and ipsilateral thalamus. Reduced axonal transport may be due to synaptic damage, cytoskeletal alterations, secondary axotomy, or cell death of the projecting neuron.

Supported by NIH NS08803.

P400.

INCREASED APPARENT DIFFUSION COEFFICIENT IN NORMAL APPEARING WHITE MATTER

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Introduction: Diffuse brain injury (axonal, ischaemic, swelling) occurs following traumatic insult, causing persistent functional or psychological deficits, but may go undetected in conventional imaging. There is evidence of diffuse metabolic/structural abnormalities in normal appearing white matter (NAMW) of traumatic brain injury (TBI) patients, demonstrated by proton magnetic resonance spectroscopy (1). Laboratory models (percussion injury) have demonstrated focal biphasic changes in apparent diffusion coefficient (ADC) with initial low converting to high values after 1 week (2). We propose that the ADC of NAWM is altered in humans following traumatic brain injury.

Methods: A common EPI sequence was used to obtain co-registered quantitative T1, T2 and ADC maps. An unsupervised clustering technique based on absolute T1 and T2 relaxation times was used to obtain reliable white matter regions of interest. ADC values for supra-ventricular NAWM were obtained.

Subjects: 18 patients (mean age 36, range 17–63) admitted with a diagnosis of head injury (mean admission GCS 7.5, 9 mild, 6 moderate and 3 severe) were scanned an average of 7.5 days after injury (range 1–40). Results were compared with a control group (n = 12, av. age 35, 18–59).

Results: The patient group showed a trend towards an increase in ADC in the NAWM reaching significance (p = 0.039 Mann-Whitney U) in superior slices corresponding to centrum semiovale (645 ± 42 x10-6 mm2s-1, mean ± SD; controls 621 ± 30; p = 0.039 Mann-Whitney U), with no significant changes in absolute T1 or T2 relaxation times.

Conclusions: TBI patients at this time point have a diffuse increase in ADC in NAWM with a lack of concomitant rise in absolute T1 and T2 values suggesting a mechanism other than an increase in tissue water. Neuronal damage/loss or a disruption in tissue architecture may cause an increase in water mobility leading to a rise in ADC.

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P401.

TEMPORAL VULNERABITY TO REPETITIVE EXPERIMENTAL BRAIN INJURY: LONG TERM SEQUELAE OF MULTIPLE CONCUSSIONS.

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We evaluated the effects of the interval between two repetitive concussive brain injuries (CBI) on cognitive function and histological damage. Mice (n = 131) were anesthetized using isoflurane and subjected to sham-injury (n = 22), single CBI (n = 32) or repetitive CBI (n = 77) using a modified controlled cortical impact model. In the repetitive CBI group, the interval between the first and second concussion was either 3 days (n = 53), 5 days (n = 12) or 7 days (n = 12). Cognitive function was tested using the Morris Water Maze. Mice subjected to repetitive CBI 3 days apart exhibited greater dysfunction than either sham animals ($p < 0.05$) or than mice receiving a single injury ($p < 0.01$). These deficits were retained when the injuries were 5 days apart ($p < 0.05$ compared to single CBI), but not 7 days apart, suggesting that there is a transient vulnerability of the brain during the first 7 days following a concussion. Contusions were not appreciated with the routine histological analysis, but scattered degenerating neurons, evidence of cytoskeletal damage and axonal injury were detected in the cortex, hippocampus, thalamus and hypothalamus from 72 hours to 4 weeks postinjury in all the brain-injured mice. These data suggest that CBI is associated with subcellular alterations and cell death as well as a transient vulnerability during which a second injury leads to behavioral dysfunction.

Supported by the National Football League Charities, Grant 536661, NIH NS 08803, NS 40978, GM 34690, RO1-NS 41561 and a Veteran Administration Merit Review Grant.

P402.

NET CONTRACTILE FORCES OF THE ACTOMYOSIN NETWORK POWER THE DELAYED ELASTIC RESPONSE OF THE AXONAL CYTOSKELETON FOLLOWING STRETCH INJURY

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Immediately following stretch injury in vitro, initially straight axons immediately exhibit several undulated regions along their length, slowly retracting to their initial shape over a period of 20 to 45 minutes. We have termed this response 'delayed elasticity'. We hypothesize the initial undulated appearance of the axons is due to local cytoskeletal damage, and the subsequent imbalance of motor protein forces acting upon the actin and microtubule networks in the local areas of disarray lead to a restoring force to power the retraction.

In this study, cultured rat cortical axons were exposed to a single, dynamic stretch of 65–110% that simulated mechanical injury. The mechanisms of the ensuing morphological recovery were examined by exposing the axons to actin depolymerizing agent latrunculin A, myosin inhibitor butanedione monoxime (BDM), microtubule depolymerizing agent colchicine, and microtubule polymerizing agent paclitaxel. The delayed elastic response was significantly inhibited in axons exposed latrunculin A, BDM, and paclitaxel, suggesting the contractile forces provided by the actomyosin network are important regulators for axonal shape following stretch injury. Delayed elasticity was not inhibited by colchicine, suggesting the microtubule network is not necessary to drive rapid axonal re-alignment after mechanical injury. Funding provided by NIH HD 41699 and P01 NS 08803.

P403.

THE INCREASED INTRACRANIAL PRESSURE AS AN IMPOTRANT NEGATIVE INDICATOR OF SEVERE HEAD INJURY OUTCOME

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The main consequence of cerebral blood flow disturbances following severe head injury is the development of secondary mechanism of traumatic brain lesion There is direct correlation between post injury cerebral blood flow impairment resulting in the increase of intracranial pressure (ICP), and brain tissue ischemia.

The aim of this study is to point out the importance of the increased ICP as main negative indicator of severe head injury outcome, as well as to stress the value of ICP monitoring in the management of such an injury.

During one-year period (January 2001–January 2002), 36 patients suffering severe head injury whose presenting Glasgow Coma Scale Score (GCS) was less then 9, were treated at Division of Neurosurgery, Osijek University Hospital, Osijek, Croatia. External ventriculostomy and ICP monitoring was performed in 24 (66.7%) patients. Duration of ICP monitoring was between 2 and 8 days after admission, mean 5.3 days. Control group consisted 12 patients in whom ICP monitoring was not applied. All were admitted in Intensive Care Unit (ICU) and mechanically ventilated. Intracranial pressure was maintained below 25 mm Hg by moderate hyperventilation ($pCO2 > 30$ mm Hg), intermittent Mannitol infusion, and in the group of ICP monitorized by ventricular drainage. Early mortality in the group of monitorized patients was 29.1% (7/24), while it was 58.3% (7/12) in the control group. The data were statistically analyzed using chi-square test and nonparametric correlation tests. The significance was set at $p < 0.05$.

Correlation between the duration of the increased ICP in hours per day and low GCS at ICU discharge was noticed ($p = 0.04$).

Considering the results of this study, raised ICP is a strong negative indicator of outcome. Since early mortality in ICP monitorized patients was significantly lower ($p < 0.05$) then in control group, ICP monitoring is well justified in the management of severe head injury.

P404.

TRAUMATIC BRAIN INJURY IN HUMANS CAN INDUCE PROGRESSIVE CEREBRAL ATROPHY

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Atrophy of the brain is one of the most common radiologic findings in survivors of brain trauma. It is generally believed that this atrophy is a passive process, reflecting loss of nonviable tissue within a few months following injury. However, using animal models of brain trauma, others and we have found long-term neruodegenerative changes associated with remarkably progressive brain atrophy. Here, we examined serial images of brain trauma patients to evaluate potential progressive atrophic changes. To determine delayed atrophy, we limited our analysis to images taken no earlier than 3–4 months following injury to be compared with ones taken months to years later. We also limited our examination to easily seen changes. We excluded patients due to age (>50 years old at time of injury), history of alcoholism, evacuated hematomas, and multiple head injuries. In all, we evaluated 24 moderate to severe brain-injured patients (Glasgow Coma Scale score of 3–12), comparing serial cross-sectional imaging (computerized tomography and magnetic resonance). Ten of the patients were noted to have moderate-to-severe progressive enlargement of the subaracnoid and ventricular spaces, indicative of diffuse parenchymal volume loss. An additional three patients developed mild diffuse sulcal dilatation. Focal, progressive malacic cavitations were noted in seven of the patients. Some of these changes were found to progress even years following injury. These data suggest that chronically progressive degenerative processes may be initiated by a single brain trauma event in humans. However, the incidence of progressive atrophy following trauma remains to be determined. Nonetheless, we may have to consider developing therapeutic strategies to ameliorate long-term neurodegeneration in brain-injured patients. Supported by NIH grants, AG12527, NS38104, and NS08803

P405.

CEREBRAL PERFUSION PRESSURE MANAGEMENT AS AN IMPORTANT FACTOR INFLUENCING THE OUTCOME OF SEVERE BRAIN INJURY

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Following severe brain injury, cerebral blood flow impairment occurs as a result of secondary mechanism of traumatic brain lesion. Cerebral perfusion pressure (CPP) decrease is the main cause of brain tissue ischemia after such an injury. Therefore, maintaining CPP values above critical level, as well as reducing intracranial pressure (ICP), is necessary to avoid ischemic brain lesion.

The purpose of this report is to stress the value of CPP management as an important factor that influence the outcome of brain injury.

Between January 2001 and January 2002, 36 patients who suffered severe brain injury were treated at Division of Neurosurgery, Osijek University Hospital, Osijek, Croatia. In all the admission Glasgow Coma Scale Score (GCS) was less then 9. Intracranial pressure monitoring was performed in 24 (66.7%) patients. Mean duration of ICP monitoring was 5.3 days. Cerebral perfusion pressure was individually calculated from the difference between mean arterial blood pressure and ICP. Control group consisted of 12 patients in whom ICP monitoring was not performed. Following admission and neuroradiologic diagnostics, as well as early surgery if necessary, all patients were admitted in Intensive Care Unit (ICU) and mechanically ventilated. Cerebral perfusion pressure was maintained above 70 mm Hg by moderate hyperventilation ($pCO2 > 30$ mm Hg), intermittent 20% Mannitol infusion, and in the group of ICP monitorized by external ventricular drainage. Chi-square test and nonparametric correlation tests to get Spearman's coefficient of correlation were used in statistical analysis with the significance set at $p < 0.05$.

The relationship between duration of the decreased CPP in hours per day and low GCS at ICU discharge was observed ($p < 0.05$). Early mortality rate in ICP monitorized patients was significantly lower then in control group ($p < 0.05$).

Concerning the results of this report, CPP management is proved important factor influencing the outcome of severe brain injury.

P406.

ASSOCIATION BETWEEN INTRAVASCULAR MICROTHROMBOSIS AND CEREBRAL ISCHEMIA IN TRAUMATIC BRAIN INJURY

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Ischemia is a prominent finding in traumatic brain injury (TBI). Some cases result from cerebral herniations or brain compression by hematoma, others from hypotension or hypoxemia. However, over half of fatal TBI cases exhibit varying degrees of selective neuronal necrosis (SNN), the cause of which is unclear.

We reviewed samples of frontal and hippocampal cortical tissue from a cohort of 90 cases with fatal TBI. Tissue stained with hematoxylin and eosin (H&E) was reviewed and rated for severity of SNN. Since intravascular fibrin microthombi may lyse within a few days of TBI, we restricted our analysis to subjects dying within 48 hours of injury. Medical records in all cases were reviewed to rule out severe or prolonged hypotension or hypoxemia. Eleven cases with severe or global SNN were compared to eleven cases in whom SNN was mild or absent. Slides adjacent to H&E sections were were stained with immunoflurescent antibody to antithrombin III and reviewed for intravascular coagulation (IC). The number of microthrombi on each slide was counted by an investigator blinded to the H&E findings, and IC density calculated.

Intravascular microthombi were noted in every section, excluding control (non-TBI) brain tissue. However, the density of IC varied with the degree of SNN. We found a highly significant difference in the mean IC density between cases with little or no SNN ($2.58 + 0.29$/cm2), and cases of severe SNN ($7.74 + 1.10$). These data support a strong link between IC and neuron death following brain trauma in humans and may have important implications for new therapeutic approaches. Supported by NIH grants, AG12527, NS38104 and NS08803.

P407.

TRANSIENT HEMORRHAGIC HYPOTENSION DOES NOT AGGRAVATE BEHAVIORAL AND COGNITIVE DEFICITS IN BRAIN-INJURED RATS

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Traumatic brain injury is commonly associated with hemorrhage known to aggravate evolving brain damage. Blood loss exceeding 20% of total blood volume may compromise organ perfusion, oxygenation, and initiate a plethora of systemic and cerebral autodestructive cascades. We sought to determine the impact of a transient superimposed hemorrhagic hypotension (HH) on behavioral and cognitive deficits following fluid percussion injury (FPI) in rats.

After arterial and venous cannulation, pentobarbital-anesthetized rats were subjected to moderate-severe FPI (3.0 atm). Five minutes later, rats were randomized to controlled arterial HH (30% total blood volume; 30 minutes; $n = 10$) or unhemorrhaged ($n = 8$) groups. Following HH, rats were fluid resuscitated with Lactated Ringer's solution (3x shed blood volume) for 30 minutes. During HH rats were kept normothermic. MABP was recorded continuously and neurobehavioral (motor and cognitive) changes were evaluated up to 8 weeks.

The 50% reduction in MABP to 50–60 mmHg coincided with significant decreases in hemoglobin levels (15 ± 1 to 10 ± 1 g/dl, $p < 0.05$). Fluid resuscitation significantly increased MABP to pre-injury levels without, however, improving the hemoglobin count.

Significant motor and cognitive deficits following FPI (decreased composite neuroscore, beam balance score, and increased latencies in the Morris water maze) were not aggravated by the superimposed HH.

These preliminary data suggest that the chosen level of HH did not exacerbate FPI-induced motor and cognitive changes. Further studies are warranted to characterize the different independent variables (severity of brain injury, HH, and fluid resuscitation) more closely.

Supported, in part, by NIH NIGMS Ro1-34690, NS Ro1- and a VA Merit Review Grant.

P408.

RISK FACTORS FOR DEVELOPMENT OF NEUROGENIC FEVER FOLLOWING TRAUMATIC BRAIN INJURY

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Neurogenic fever is a sequela of traumatic brain injury (TBI) and may be associated with poorer outcome. The purpose of this study was to identify factors associated with the development of neurogenic fever following severe TBI in adults. Charts of patients admitted from 1996–1999 with severe TBI at a large, urban Mid-Atlantic teaching hospital were retrospectively evaluated based on diagnostic criteria for each episode of hyperthermia to determine the diagnosis of neurogenic fever ($N = 76$). The incidence of neurogenic fever in this population was 11.8%. Data was collected regarding mechanism and area of injury, severity of injury, and demographic factors to determine potential predictors of neurogenic fever using logistic regression modeling. Diffuse axonal injury (Odds Ratio (OR) 9.06, 95% Confidence Interval (CI) .99, 82.7) and frontal lobe injury of any type (OR 6.68, 95% CI 1.1, 39.3) are independently predictive of an increased risk of development of neurogenic fever following severe TBI. The presence of a skull fracture and lower initial Glasgow Coma Score were individual predictors of development of neurogenic fever, but did not contribute to the final model. A set of predictor variables was identified to help clinicians target patients at high risk for development of neurogenic fever following severe TBI. Clinicians evaluating unexplained fever in TBI patients need to consider these factors during their diagnostic assessments.

This work was supported by NIH F31 NR07694-01and the Neuroscience Nursing Foundation (HJT).

P409.

REPEATED MILD INJURY CAUSES CUMULATIVE DAMAGE TO HIPPOCAMPAL CELLS

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Recent studies testing cognitive deficits in athletes and child abuse victims and in animals injured experimentally in vivo support the hypothesis that repeated mild traumatic brain injury (rMTBI) may result in cumulative damage to cells of the brain. However, post-injury sequelae are difficult to address at the cellular level in vivo. Therefore, it is necessary to complement these studies with experiments conducted in vitro. In this report, the effects of single and repeated mild traumatic injury in vitro were investigated in cultured mouse hippocampal cells using a well-characterized model of stretch-induced injury. Cell damage was assessed using fluorescein diacetate (FDA) and propidium iodide (PrI), and by the release of neuron specific enolase (NSE) and S-100β protein, two common clinical markers of CNS damage. Cultures received a second injury one hour after the initial insult. Repeated injury caused a slight increase in PrI uptake versus single injury, primarily evident in the underlying glial layer. A reduction of neuronal phenotypes was also apparent 24 hr post-injury. In addition, the neurites of neurons that received repeated insults showed signs of damage not evident after single injury. Six hours post-injury, both NSE and S-100β levels were elevated after repeated injuries when compared to the single injury group. These results suggest that cells of the hippocampus may be susceptible to cumulative damage following repeated mild traumatic insults. Both glial cells and neurons appear to exhibit increased signs of damage after repetitive injury. The biochemical pathways of cellular degradation following repeated mild injuries may differ considerably from those that are activated by a single mild insult. Therefore, we hope to use this model in order to investigate secondary pathways of cellular damage after repeated mild traumatic injury and as a rapid and economical means of screening possibilities for treatment strategies for rMTBI. Supported by NWO-MW,-ALW,-PIONIER.

P410.

ACTIVATION OF GROUP II MGLUR'S DO NOT PREVENT TRAUMATIC OR ISCHEMIC INJURY OF WHITE MATTER

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Recent studies indicate that activation of group II mGluRs attenuates excitotoxicity. Here we examined the effects of selective agonists on traumatic as well as ischemic injury on spinal cord white matter in vitro. At concentrations shown to be selective for group II mGluR's.

A 30mm length of dorsal column was isolated from the spinal cord of adult rats, pinned in an in vitro recording chamber (37°C) and injured with 95% N2 and 5% CO2 or modified clip (2g closing force) for 15 sec. The functional integrity of the dorsal column was monitored electrophysiologically by quantitatively measuring the compound action potential (CAP).

The mean CAP decreased to 49.4 ± 2.6 % and 49.5 ± 5.7 % of control ($p < 0.05$) after trauma and hypoxia/ischemia injury, respectively. The selective group II agonist APDC agonist did not attenuate the posttraumatic and ischemic reduction of CAP amplitude. Whereas, blockade of group II mGluR receptors with LY 341495 and Eglu resulted in significant improved recovery of compound action potentials (CAP) amplitude of control ($p < 0.05$) after hypoxic/ischemic and traumatic injury to dorsal column white matter. Western blot analysis also identified the presence of mGluR II ([2/3]) positive immunolabeling of ~152 kD proteins in the spinal cord dorsal columns. The expression of protein is decreased after acute traumatic or hypoxic/ischemic injury of white matter. In conclusion, these data indicate that traumatic and ischemic injury induced acute activation of mGluRs II does not contributes to cellular pathophysiology of spinal cord dorsal columns in vitro.

P411.

MEDIATORS OF PRECONDITIONING EFFECT IDENTIFIED BY MICROARRAY ANALYSIS OF RAT SPINAL CORD AFTER BRIEF ISCHEMIA

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A brief period of spinal cord ischemia protects neurons and preserves locomotor function after subsequent ischemic insult. Gene expression changes after preconditioning are likely to alter the response of the spinal cord to further injury. Using microarrays, we sought to identify gene expression patterns and neuroprotection-associated genes (NAGs) that mediate the preconditioning effect. Rats were exposed to 3 minutes of spinal ischemia by balloon occlusion of the descending aorta followed by 30 minutes to 48 hours of reperfusion. The mRNA levels of preconditioned lumbar spinal cords were compared to sham-injured controls in four replicates using microarrays containing 5,000 oligonucleotide probes. Cluster analysis reveals two discrete groups of significantly induced genes. The two most prominent K-means clusters each contain a stress-induced gene that has been associated with ischemic preconditioning in the heart and brain. The first group peaks at 30 minutes and includes several transcripts for inducible heat shock protein, hsp70. We validated mRNA levels by real-time PCR, with hsp70 levels reaching 40 times those of sham-injured controls. Immunostaining for HSP70 protein was observed by 6 hours and returned to baseline at 24 hours. A second group of genes shows increased expression at 6 and 12 hours after preconditioning and includes metallothionein-1 and -2. These results suggest a role for hsp70 and metallothionein, along with several other NAGs, in spinal cord neuroprotection.

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Supported by: The SCI Project of the W M Keck Center, NJ Commision on Spinal Cord Research, NS32794

P412.

ETHYL PYRUVATE IS PROTECTIVE AGAINST MILD AND SEVERE TRANSIENT GLOBAL ISCHEMIA IN GERBILS

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Background and Objective: Pyruvate has been shown to have protective effects in several ischemia models in different organs in both in vitro and in vivo. However, the mechanism(s) action need to be further elucidated. The purpose of this study is to test whether ethyl pyruvate, a derivate form of pyruvate, has protective effects against mild and severe transient forebrain ischemia.

Materials and Methods: Transient forebrain ischemia in adult male Mongolian gerbils was induced by the bilateral occlusion of common carotid arteries for 5 min (mild) or 10 min (severe). Ethyl pyruvate (500 mg/kg) or sodium chloride (104.5mg/kg) (isoosmolar) were injected at the time of occlusion. Seventy-two hours after occlusion, the animals were euthanized and their brains removed and frozen. The brains were sectioned at 10 um thickness using cryostat and stained with hematoxylin and eosin. The eosinophilic cells were counted and the data were analyzed using Statview v.5.

Results: The data show that ethyl pyruvate has neuroprotective effect against both mild ($p = 0.0131$) and severe global ischemia ($p = 0.0131$), respectively.

Conclusions: The data show that ethyl pyruvate is neuroprotective in global ischemia and could replace sodium pyruvate, which was abrogated as clinical therapeutic agent due to its instability.

P413.

MILD HYPOTHERMIA REDUCES ZINC TRANSLOCATION, NEURONAL CELL DEATH, AND MORTALITY AFTER TRANSIENT GLOBAL ISCHEMIA IN MICE

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The purpose of this study is to determine whether Zn2+ translocation associated with neuronal cell death occurs after transient global ischemia (TGI) in mice and to determine the effect of mild hypothermia on this reaction.

To validate the TGI model, carbon black injection and laser-Doppler flowmetry were compared in three strains of mice (C57BL/6, SV129 and HSP70 transgenic mice) to assess posterior communicating artery (PcomA) development and cortical perfusion. These results were then used to determine the optimal occlusion time (20 minutes) for C57BL/6 in TGI. Brain and rectal temperature measurements were compared to monitor hypothermia. Results of TGI were compared in normothermia (NT: 37°C) (n = 14) and mild hypothermia groups (HT: 33°C) (n = 14) by staining with Zn2+-specific fluorescent dye, N-(6-methoxy-8-quinolyl)-para-toluenesulfonamide (TSQ) and hematoxylin-eosin after 72 hours reperfusion.

Zn2+ translocation observed in hippocampus CA1, CA2 and Hilus 72 hours after 20 minutes of TGI was significantly reduced by mild hypothermia. The number of degenerating neurons in the HT group was significantly less than in the NT group. Mild hypothermia reduced mortality significantly (7.1% in HT, 42.9% in NT).

Results suggest that mild hypothermia may reduce presynaptic Zn2+ release, which protects vulnerable hippocampal neurons from ischemic necrosis. Future studies may further elucidate mechanisms of Zn 2+ induced ischemic injury.

P414.

MLN519 AND NEUROPROTECTION: CONTINUING THERAPEUTIC WINDOW STUDIES IN EXPERIMENTAL BRAIN ISCHEMIA INJURY AND COMPLETION OF A PHASE I SAFETY TRIALS IN HUMAN VOLUNTEERS

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At the 2000 INTS meeting, we reported our preclinical rat and early Phase I clinical results with the MLN519 (formerly PS519). As documented in published reports, MLN519 is a novel small molecule inhibitor of the 20S proteasome exhibiting neuroprotective properties in multiple rat models of cerebral ischemia, with efficacy established over a range of i.v. doses and distinguished by a substantial therapeutic window (TW). Since the failure of neuroprotection drug trials has been attributed, in part, to poorly established TWs, we have continued to evaluate the TW for MLN519 in the rat MCAo model, as well as having now completed a Phase I i.v. safety trial in 67 individuals.

Using the rat MCAo/72 h recovery model, significant reductions in brain infarction can be achieved with treatment delays of 10 h. MLN519 (1.0 mg/kg, i.v.) neuroprotection correlated with 1) improved neurological function, 2) significant decreases in the number of neutrophils and macrophages present in ischemic brain regions, and 3) reductions in fragmented astrocytes throughout the core area of injury.

A Phase I double-blind, randomized, placebo-controlled trial investigating the safety, tolerability and pharmacodynamics of MLN519 has now been completed in healthy, young male volunteers. MLN519 was administered i.v. where 39 subjects received single doses of 0.012 mg/m2–1.6 mg/m2, and 28 subjects received doses of 0.5 mg/m2–1.6 mg/m2 on three consecutive days. The drug was well tolerated; there was no clear treatment-emergent symptoms or abnormality of laboratory tests. Also, 20S proteasome activity in blood achieved the intended maximum target level of 70–80% inhibition, and was reproducible with repeated dosing. At Walter Reed, rat MCAo studies are continuing to further elucidate the cellular mechanism(s) of action of MLN519, while Millennium and PAION Pharmaceuticals have recently joined forces to collaboratively advance the clinical development of MLN519 to Phase II stroke trials.

P415.

NEUROPROTECTIVE TREATMENT WITH THE SNAIL PEPTIDE CGX-1007 EFFECTIVELY REDUCES BOTH C-FOS GENE EXPRESSION AND APOPTOSIS IN A RAT MODEL OF FOCAL ISCHEMIA

Anthony J Williams and Frank C Tortella. (Walter Reed Army Institute of Research, Silver Spring, MD US).*

The role of the immediate early gene (IEG) c-fos as either a neuroprotective or neurodegenerative promotor following cerebral ischemia has not been definitively determined to date. In this study we have evaluated the upregualtion of c-fos mRNA and protein levels during the initial 24 h of transient middle cerebral artery occlusion (MCAo) in the rat and the effects of treatment with the novel NMDA receptor antagonist and neuroprotective agent CGX-1007. Treatment with CGX-1007 (0.5 nmol, i.c.v.) was given at 30 min post-MCAo. Brain tissue was collected at 1, 4, and 24 h post injury and processed for quantitative RT-PCR and histological staining including TTC, TUNEL, and c-fos conjugation. CGX-1007 effectively reduced the presence of TUNEL positive cells at 24 h, predominately in cortical brain tissues. C-fos mRNA levels peaked at 1 h post-injury in both cortical and subcortical ischemic brain regions (30 fold increase), remained elevated at 4 h and returned to normal, pre-injury levels by 24 h post-injury. The increase in mRNA levels correlated to increased c-fos protein expression in the entire ipsilateral hemisphere at 1 h. Regions of necrosis at 4 h were void of c-fos immunoreactivity with continued upregulation in surrounding regions. At 24 h, no upregulation of c-fos protein was observed in the injured hemisphere except for sustained increases in the cingulate and piriform cortex of vehicle treated rats. Post-injury treatment with CGX-1007 effectively reduced both mRNA and protein levels of c-fos at all time points examined. CGX-1007 treatment also reduced c-fos immunostaining in the cingulate cortex, a brain region which has previously been reported to be resistant to treatment with NMDA antagonists. This supports the hypothesis that c-fos may have neurodegenerative properties and that treatment with CGX-1007 is able to reduce the upregulation of c-fos and provide neuroprotective relief of cerebral ischemia.

P416.

ETHYL PYRUVATE IS PROTECTIVE IN TRANSIENT FOCAL ISCHEMIA IN RATS

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Background and purpose: Sodium Pyruvate has proved to be neuroprotective against global ischemia in rats. Ethyl Pyruvate, with better stability than sodium pyruvate, is protective for the intestinal ischemia-reperfusion injury. However, whether ethyl pyruvate is protective in transient focal ischemia is not known.

Methods: Male Sprague-Dawley rats (275–300g) received 90 min middle cerebral artery occlusion (MCAO) by an intraluminal suture followed by 23 hrs reperfusion and were treated with Ethyl pyruvate (1000mg/kg, n = 5) or iso-osmolar NaCl (n = 4) infused intravenously at the beginning of ischemia, during ischemia and during reperfusion. TTC (2,3,5-triphenyl tetrazolium chloride) staining was used for measuring infarct size. Neurological deficits were measured 1h after surgery and before sacrifice.

Results: Neurological deficits were decreased 24hrs after surgery in the ethyl pyruvate group when compared to the NaCl group. The body weight loss in the ethyl pyruvate group was also less than that in the NaCl group. The total infarct volume in cortex and striatum following ethyl pyruvate therapy decreased almost 40% less than that in the NaCl-treated group.

Conclusion: Continuously infusion of ethyl pyruvate has the potential to protect against transient focal ischemia in rats.

P417.

QUANTIFICATION OF NEURONAL DEGENERATION IN IN VIVO CLIP COMPRESSION MODELS OF SPINAL CORD INJURY USING FLUORO-JADE B

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Fluoro-Jade B has been used as a fluorescent marker for neuronal degeneration in rat brain tissue. However, its effectiveness in spinal cord tissue has not yet been documented. The simple staining procedure makes it an attractive tool for examining neurodegeneration in central nervous system injury models. In this study we examine the effectiveness of Fluoro-Jade B as a marker of neuronal degeneration after acute spinal cord injury (SCI). In vivo spinal cord injuries in rat and mice were performed using a clip compression model of SCI at closing forces of 20g and 8.3g respectively. Rat spinal cord sections were stained with Fluoro-Jade B and double labeled with NeuN to determine cellular colocalization. Specificity of staining for dead or dying cells was determined using double labeling with Fluoro-Jade B and TUNEL. Results demonstrate that Fluoro-Jade B co-localizes with NeuN as well as TUNEL. Neuronal degeneration was quantified using Fluoro-Jade B staining in 1, 2, and 3 day injured mice (n = 3/group). Data indicated a significantly larger number of Fluoro-Jade B labeled neurons 1 day (113.8 ± 18), 2 days (74.3 ± 4.2) and 3 days (31 ± 8.9) following injury compared with uninjured mice (3.3 ± 2.1). Spatial distribution of neuronal degeneration indicated a larger number of neurons labeled at the epicentre compared to the periphery of the injury. Fluoro-Jade B labeled neurons in mice with varying degrees of injury were also examined. Mice were injured at the T7 level using clips calibrated at 3.1g (mild injury), 8.3g (moderate injury) and 24.1g (severe injury). Preliminary results indicate a larger number of Fluoro-Jade B labeled neurons are present with a 24.1g injury. Fluoro-Jade B is a quantifiable marker for neuronal degeneration in the in vivo mouse and rat SCI compression models that can be used as an efficient and reliable histological tool.

P418.

INFLAMMATORY MECHANISMS REVEALED BY EXPRESSION PROFILING OF ACUTE SPINAL CORD INJURY

Jonathan Z. Pan; Patricia Soteropoulos; Peter Tolias; Ronald P. Hart. (Rutgers University, Newark, NJ US).

Inflammation likely contributes to secondary damage after spinal cord injury (SCI). An anti-inflammatory glucocorticoid, methylprednisolone (MP) is the standard therapy for acute SCI. Since other compounds are not as effective, we compared several drugs for their inhibition of inflammatory mechanisms following SCI. Using quantitative real-time PCR (Q-RT-PCR) and custom rat microarrays, we compared expression profiles following treatment with MP, acetaminophen, indomethacin, NS-398, IL-1ra and soluble TNFR:Fc. We also correlated the established MASCIS impactor model with a cultured slice model of SCI. Adult spinal cord was dissected into 1 mm segments and incubated with or without drugs in serum-free medium for 4 hrs, then total cellular RNA was prepared . By Q-RT-PCR, IL-6 and TNFa mRNAs were reduced by acetaminophen and indomethacin, respectively, while IL-1b, TNFa, IL-6 and MIP-1a were all decreased following MP or combined IL-1ra and sTNFR:Fc. There was no effect of NS-398 on these cytokine mRNAs. Clustering analysis of microarray results identified genes regulated by NS-398 differing from those affected by other drugs, which indicated distinct targets of cyclooxygenase II. Comparing data from slice cultures to the MASCIS model showed good correlation for most genes, but some differences were also noted. In summary, using microarray analysis, anti-inflammatory drugs with different mechanisms were distinguished by their gene expression profiles. Understanding inflammatory cascades initiated by SCI should allow design of improved therapies.

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Supported by: SCI Project of the Keck Center, New Jersey Commission on Spinal Cord Research

P419.

TREATMENT OF SPINAL CORD INJURY USING AN ANTIBODY TO THE LEUKOCYTE INTEGRIN ALPHA D/BETA 2 EFFECTIVELY PREVENTS THE EARLY INFILTRATION OF PHAGOCYTES AND MONOCYTES/MACROPHAGES INTO THE LESION.

L.R. Saville, F.C. Simedria, L.C. Weaver, and G.A. Dekaban. (BioTherapeutics Research Group, Robarts Research Institute, London, ON, Canada).*

The inflammatory response following spinal cord injury (SCI) extends the damage caused by the initial physical insult, reducing the functional outcome. The alphaD/beta2 integrin, located primarily on the surface of neutrophils and monocytes/macrophages, plays a role in the migration of these cells to sites of tissue damage. Monoclonal antibodies (mAb) to the alphaD subunit of the alphaD/beta2 integrin inhibit the infiltration of monocytes/macrophages into the spinal cord following clip compression injury (CCI) in the rat. We determined the most effective course of treatment and characterized its effect on the inflammatory response using immunocytochemistry to identify myeloperoxidase positive (MPO+) phagocytes (monocytes/macrophages and neutrophils) and ED-1+ monocytes/macrophages within the SCI at 8hr, 18hr, 72hr and 7 days after severe CCI. Wistar rats were administered i.v. anti-alphaD or control mAb in one of three treatment regimes: 24hr, 2hr/24hr, or 2hr/24hr/48hr post-CCI, with the tissue processed at 72hr post-CCI. Treatment with anti-alphaD mAb at 2hr/24hr/48hr post-CCI was the only regimen that effectively reduced the infiltration of MPO+ phagocytes (by 86%) and ED-1+ monocytes/macrophages (by 41%) into the lesion at 72hr when compared to untreated SCI controls. The 24hr regimen equally reduced ED-1+ monocytes/macrophages infiltration (by 42%); however, the 24hr and 2hr/24hr regimes did not significantly reduce MPO+ phagocyte accumulation. Using the 2hr/24hr/48hr treatment regime, anti-alphaD inhibited the infiltration of MPO+ phagocytes as early as 8hr (by 31%) and continued to reduce them at 18hr (by 57%), whereas a reduction in ED-1+ monocytes/macrophages at the SCI was not evident until 18hr post-CCI (by 28%). By 7 days post-CCI, the accumulation of monocytes/macrophages in anti-alphaD and untreated SCI rats was qualitatively indistinguishable. Quantification is underway. Neurological outcomes using BBB open field locomotor skills are being assessed. The 2hr/24hr/48hr treatment regime may therefore have more therapeutic potential, inhibiting only the early infiltration of phagocytes and monocytes/macrophages after SCI.

Supported by the Ontario Neurotrauma Foundation and ICOS Corporation.

P420.

CHARACTERIZATION OF THE LYS-EGFP-KI TRANSGENIC MOUSE: A NOVEL SPINAL CORD INJURY MODEL

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The inflammatory response produced following spinal cord injury (SCI) has been shown to promote either neuroprotection/regeneration or to extend the primary tissue damage. Two of the major inflammatory effectors involved are CNS-derived microglia and infiltrating systemically-derived monocyte/macrophages. These cell populations are distinct in the non-activated state but when activated become indistinguishable, making it difficult to determine their temporal roles in SCI. This prevents the accurate assessment of the role of each cell type in the inflammatory response to SCI. The Lys-EGFP-ki transgenic mouse, created by Faust et al (Blood, 96: 719, 2000), has the enhanced GFP gene inserted into its genome under the control of the lysozyme M promoter that is specifically expressed in myelomonocytic cells including the monocyte/macrophages. This study examines the microglia in the Lys-EGFP-ki mouse at 3, 7, and 14 days post-SCI to determine if the microglia express GFP. If the microglia remain GFP- than this would allow for their distinction from the GFP+ monocyte/macrophages at the SCI. We confirmed that no GFP+ cells were present in uninjured spinal tissue and that the inflammatory response produced in the transgenic animals was normal in magnitude and location of infiltrating cells compared to the wild type parent strain. The presence of ramified microglia was confirmed in both the white and gray matter in the Lys-EGFP-ki mice by identification with an anti-CD11b antibody; however, no GFP+ ramified microglia were identified at 72hr post-SCI. This finding supports the hypothesis that the microglia will initially be GFP- allowing for their distinction from systemically derived GFP+ M/F. Further confirmation using an anti-GFP antibody is currently underway. We are also examining the spinal tissue at 7 and 14 days post-SCI to determine if the activated microglia gain GFP expression when they lose their ramified structure.

Supported by Ontario Neurotrauma Foundation and ICOS Corporation.

P421.

LOCOMOTOR BEHAVIOR AFTER SPINAL CANAL NARROWING IN RAT

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The objective of this study was to examine the effects of progressive spinal canal narrowing on locomotor function in rats. Thirty male adult Wistar rats were equally divided into three experimental groups: sham, with 35% and 50% spacers placed in spinal cord canal. Weekly, 1 to 5 weeks after procedure, neurological recovery was monitored by evaluation trough Basso, Beattie, Bresnahan (BBB) Locomotor Rating Scale, inclined plane and open field test

Spacer placement resulted in important neurological impairment. The BBB scale and inclined plane test scores were progressively lower as spacer size increased in all times after injury analyzed (ANOVA, $p < 0{,}0001$). Results from both tests were highly correlated ($r = 0.934$, $p < 0.001$). In open field test the results were similar. However the difference between the groups 35% and 50% was less pronounced. The injured groups presented significant functional recovery on time (MANOVA, $p < 0{,}0001$). The recovery was more evident at the first and second weeks.

This result suggests first that the prognosis for neurological recovery is affected by percentage of canal narrowing. Second that BBB scale and inclined plane test produced equivalent analyzes for functional recovery after spinal cord injury. Financial support FAPESP, CNPq.

P422.

T CELL-MEDIATED PROTECTIVE AUTOIMMUNITY AFTER SPINAL CORD INJURY AFFECTS IMMUNE-RELATED ACTIVITY OF MICROGLIA AND LEADS TO NEUROPROTECTION AND SPROUTING

Oleg Butovsky, Ehud Hauben, and Michal Schwartz. (Department of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel).*

Studies in our laboratory have shown that the spread of damage after central nervous system (CNS) injury can be reduced by post-traumatic passive transfer of T cells specific to myelin self-antigens, or by active vaccination. This phenomenon was defined by our group as "protective autoimmunity." Here we show that active or passive vaccination after spinal cord injury not only leads to protection of tissue but also results in extensive sprouting and limits cavitation. Electron microscopy and fluorescence immunohistochemistry, using antibodies to calcitonin gene-related peptide, 5-hydroxytryptamine, and GAP-43, revealed that post-traumatic vaccination not only rescues neurons and thus limits degeneration but also promotes massive growth of regenerating axons into the lesion site of the injured rat spinal cord. These findings suggest that T cells mediate both neuroprotection and sprouting by engaging in cross-talk with activated resident microglia/macrophages, thereby coordinating the cellular effects with the needs of the tissue. When spinal cord injury in rats was followed by passive T-cell vaccination, accumulation of T cells in the vicinity of the lesion site was accompanied by a dramatic increase in MHC-II and B7-2 expression on macrophages/microglia, possibly augmenting the ability of the microglia to support antigen-dependent T cell activation. Our in vitro studies showed that co-culturing of microglia with autoimmune T cells resulted in up-regulation of the expression of MHC-II and B7-2 (both reminiscent of the activity of antigen-presenting cells) as well as increased expression of TNF- α and TNF-RI and decreased expression of TNF-RII (possibly associated with termination of the microglial activity). We suggest that autoimmune T cells exert their protective effect through dialog with local microglia and invading macrophages, enabling these cells to express their beneficial activity, while at the same time imposing strict regulation on them to avoid their potentially detrimental long-term effects.

P423.

PROTEOMICS PROFILING AFTER SPINAL CORD INJURY: EARLY DETECTION OF A NEUROPROTECTION SIGNATURE

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The recent development of new proteomics tools allowing a rapid analysis of protein regulation led us to assess CNS disorders by a novel strategy. The present study reports the elaboration of proteomics fingerprints from our model of spinal cord injury (SCI)(US 5,724,995) and their use as an index of neuroprotection. Several groups of female SD rats were submitted, after SCI, to a neuroprotective treatment (NMDA-antagonist) with differences in effectiveness and compared to a non-treated group. Body fluids and spinal lysats were sampled on a daily basis or according to the stage of behavioral recovery. The protein expression profiles were generated by SELDI-TOF mass spectroscopy, using several proteinchips arrays (SAX2, WCX2, IMAC3) to target different classes of proteins. SAX2, a strong anion exchange array, provided different spectra depending on the pH and allowed the identification of a number of peaks of interest. The evolution of these protein expression profiles correlated to the behavioral recovery revealed several classes of relevant proteins. A first class demonstrated an upregulation in the TCP-treated group (2mg/kg) during the first week post-SCI. At least 4 proteins with a mass ranging from 11 to 46 kDa were expressed at greater levels than in the control group, in relation to the neuroprotection. A second class of proteins ranging from 8 to 16 kDa showed an early and transitory upregulation in absence of neuroprotection, specifically, suggesting that these protein species might reflect several early drug targets. Correlated to the functional recovery, these kinetics protein expression profiles are providing effective prognostic tools as well as giving insights into the drug action mechanisms.

Supported by: NEUROLAB

P424.

FK506 AND CYCLOSPORIN IMPROVE HYPOXIC INJURY TO WHITE MATTER

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INTRODUCTION: Calcium influx into cells is responsible for initiating the "final pathway" to cell death in neuronal tissue after traumatic or hypoxic injury. In the present study we examined the role of cyclosporin A (CsA), FK506, and rapamycin in modulating the effects of Ca2+ influx through their interactions with immunophilins and specifically the end result of calcineurin modulation.

MATERIALS AND METHODS: Dorsal columns were isolated from the spinal cord of adult rats and injured by exposure to hypoxic conditions for 60 minutes while perfused with Ringers solution alone or with various immunosuppressants. The samples were monitored and the compound action potential (CAP) was measured with glass microelectrodes. Functional recovery of the dorsal column was then assessed by recovery of the CAP.

RESULTS: The mean CAP decreased to about 20% of baseline control levels during hypoxia, and returned $53.8 \pm 7.6\%$ of baseline ($p < 0.05$) after reoxygenation. CsA promoted a significantly greater recovery of CAP amplitude to $76.8 \pm 5.2\%$ of control ($p < 0.05$) after hypoxic injury and reoxygenation of dorsal columns. FK506 promoted CAP amplitude recovery to $82.6 \pm 5.0\%$ of control after hypoxic injury and reoxygenation of dorsal columns. The addition of rapamycin, which binds to the same immunophilin as FK506, to the FK506 solution during hypoxic injury only showed recovery of CAP amplitudes to $56.9 \pm 6.7\%$ of control. Electron microscopy revealed remarkable protection of axons and prevention of organelle disruption in segments treated with CsA and FK506 during hypoxia when compared to hypoxic controls.

DISCUSSION: In conclusion, both CsA and FK506 confer in vitro protection to dorsal columns during hypoxic injury at physiological temperatures, and rapamycin blocks the protective effect of FK506. Calcineurin may play an important role in the physiology of neuronal injury.

P425.

GLUTAMATE KILLS OLIGODENDROCYTES IN THE RAT SPINAL CORD IN VIVO

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Glutamic acid reaches toxic levels in the gray matter of the spinal cord following spinal cord injury (SCI).1 Whether it also does so in the white matter is also important to determine because damage to white matter causes much of the crippling that results from SCI and because oligodendrocytes bear AMPA glutamate receptors.2,3 Therefore we analyzed the level of glutamate released into the white matter of the spinal cord of the rat following SCI and then established whether that level of glutamate is toxic to oligodendrocytes in vivo in the spinal cord. Microdialysis sampling followed by HPLC analysis yielded a maximum estimated release of glutamate of 700 mM in the white matter following SCI. Glutamate was administered into the cord, oligodendrocytes were labeled with antibody CC-1, photographed under a confocal/image analysis system and counted in defined areas. Administration by microdialysis of the estimated concentration of glutamate released into the cord resulted in a 48 ± 4.5% (s.d.) decrease in the number of oligodendrocytes at 24 h versus an 11 ± 4% decrease when artificial cerebral spinal fluid was administered in the same fashion. Thus glutamate release following SCI appears to be toxic to white as well as gray matter, and likely contributes significantly to the crippling that follows SCI.

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Supported by Mission Connect at the TIRR Foundation and NS 39161.

P426.

REGIONAL ENERGY METABOLISM FOLLOWING SHORT-TERM NEURAL STEM CELL TRANSPLANTATION INTO THE INJURED SPINAL CORD

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Stem cells are shown to partly restore CNS function after transplantation into the injured CNS. Nothing is known about their influence on acute energy metabolism after spinal cord injury. The present study was designed to analyse regional changes in energy metabolites. Young adult mice were subjected to laminectomy with subsequent hemisection at the L3/4 vertebral level. Immediately thereafter 2 μl of a suspension of the C17.2 neural stem cell line in phosphate buffered saline, pH 7.3 (PBS) were injected into the lesion site. PBS served as a vehicle control. After 4 and 24 h spinal cords were removed and ATP and glucose were analysed by a bioluminescence approach in serial sections and compared to a laminectomized or hemisectioned vehicle control groups. The area of ATP decline was also determined morphometrically.

At both time points ATP content of the hemisected group in the tissue segments adjacent to the lesion was increased and glucose content decreased when compared to the laminectomized control. At 24 h the area of ATP decline at the lesion site was significantly lower in the PBS group as compared to the hemisected or transplanted group. The decrease in glucose combined with an increased ATP in the adjacent segments may indicate that the tissue adjacent to the lesion responds with an increased use of glucose to support the tissue with sufficient ATP. The lower area of ATP decline 24 h after PBS administration suggests that PBS washes out toxic mediators, thus ameliorating hemisection-dependent secondary tissue damage.

Supported by Deutsche Forschungsgemeinschaft.

P427.

VACCINATION THERAPY IN RAT SPINAL CORD INJURY

Crista L. Adamson, Rimini Varghese and Wise Young. (Rutgers University, Piscataway, New Jersey US).*

Huang et al., demonstrated that vaccination therapy with homogenates of spinal cord in adult BALB/c mice results in extensive regeneration of large numbers of axons in the corticospinal tract (CST) following hemisection. The present study was carried out to determine whether therapeutic vaccinations promote axonal regeneration and functional recovery in a rat spinal cord contusion model.

Adult Long-Evan's rats (77 ± 3days) were injured using the MASCIS standard weight drop impactor (10g, 25mm). One group (n = 20) was vaccinated twice-weekly beginning 3 weeks prior to contusion as well as 3 weeks post injury with spinal cord homogenate (SCH, n = 10) or liver homogenate (LH, n = 10). Second group, (n = 20) vaccination started the day of injury and continued for 3 weeks with SCH (n = 10) or LH (n = 10). The BBB scale was used to track the recovery of the rats for 3 months following injury. Blood was collected the day of injury, 6 weeks post, and at the time of sacrifice to determine the plasma IgG and IgM. Two weeks prior to sacrifice, biotinylated dextran amine (BDA) was injected bilaterally into the motor cortex. Upon sacrifice, perfused spinal cords were embedded in paraffin and processed to visualize BDA-labeled axons in the CST and sparred fibers.

Both BBB and sparred tissue analysis show no significant difference between LH and SCH groups irrespective of vaccination protocol. Histological examination of spinal cords did not show CST regeneration across the injury site; however there appears to be an increase in the density of axon sprouting in animals vaccinated before injury, (SCH and LH). Although our data thus far indicates that vaccination with SCH has no effect on axonal regeneration in rats, preliminary findings show no change in serum IgGs suggesting a different vaccination protocol may be more effective in rats.

P428.

EFFECTS OF INOSINE ON RAT SPINAL CORD INJURY.

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The purine nucleoside inosine has been shown to have neuroprotective and neuroregenerative effects in cell culture. Benowitz et al (1999) reported inosine mediated sprouting after unilateral lesion of the corticospinal tract (CST) in rats. We therefore assessed the effects of inosine on the well-standardized rat contusion spinal cord injury model. Adult Long-Evan's rats (77 ± 3days) were injured using the MASCIS Impactor by dropping a 10g weight 25mm onto exposed T13 spinal cord. To assess possible neuroprotective effects, we gave half of the rats 10μl of saline or 10μl of 10mM inosine intrathecally at 30 minutes after injury. Rats were euthanized 6 hours or 24 hours after injury and we calculated lesion volumes from potassium concentration of spinal cord samples. To assess chronic effects of inosine we applied either PBS (n = 10) or 10mM inosine (n = 10) intrathecally at 0.5μl/hour for 2 weeks using an osmotic minipump. After evaluating the animals weekly for locomotor recovery using BBB score, we traced the CST by injecting biotinylated dextran amine (BDA) into the motor cortex at 6 weeks after injury. Two weeks later, we perfused the rats and examined the spinal cord for BDA labeled axons. Inosine did not significantly alter 6 or 24 hours spinal cord lesion volumes. However, rats treated for two weeks with inosine showed a slight but statistically significant ($p < 0.05$) improvement in BBB scores. Histological examination of spinal cords did not show CST regeneration across the injury site but suggested more CST sprouting in cord proximal to the impact site. We conclude that inosine is not neuroprotective but improves locomoter recovery and promotes axonal sprouting in the proximal cord.

P429.

DEPLETION OF NORADRENERGIC FIBERS ATTENUATES HINDLIMB LOCOMOTOR RECOVERY FOLLOWING THORACIC CONTUSION INJURY

M. Rachael Lovett, Darlene A. Burke, Y. Ping Zhang, Christine Nunn, Kim Fentress and David S. K. Magnuson. (University of Louisville, Louisville, KY US).

Descending noradrenergic (NA) axons are involved in the modulation of spinal cord circuitry associated with locomotion. N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) has been shown to deplete central nonadrenaline levels inducing degeneration of certain NA axons (Fritschy et al., 1989). At 50mg/Kg, DSP-4 is selectively toxic to neurons of the locus coeruleus and easily crosses the blood brain barrier. Furthermore, it can easily be administered by i.p injection and does not affect hindlimb locomotion in uninjured rats. Although the effects of DSP-4 on behavior, electrophysiology, and even ischemic lesions have been examined, the effect of DSP-4 treatment on hindlimb locomotor recovery after a spinal cord contusion injury has not been documented. This study tests the hypothesis that DPS-4 treatment resulting in a 60% loss of spinal cord NA fibers (Fritschy et al., 1989) alters the recovery of hindlimb locomotor activity after a T9 12.5g-cm contusion injury.

Three groups of three animals were used; T9 12.5g-cm contusion injury, T9 12.5g-cm contusion injury treated with DSP-4 and uninjured controls treated with DSP-4. The animals were assessed weekly for 4 weeks using the BBB open-field locomotor score, a grid-walking task and SSEPs. Injured animals treated with DSP-4 had a mean BBB score of 12.3 ± 0.40 compared to 15.2 ± 1.5 for untreated animals (mean $\pm$ s.d.; $p < 0.05$, t-test). Uninjured, DSP-4 treated control animals had a mean BBB score of 20 ± 0.0. We conclude that DSP-4 treatment attenuates hindlimb locomotor recovery following thoracic contusion injury suggesting that the additional loss of noradrenergic fibers induced by DSP-4 treatment (beyond loss due to the contusion injury) restricts hindlimb locomotor recovery.

Supported by the Kentucky Spinal Cord and Head Injury Research Trust.

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P430.

TRANSPLANTATION OF OLFACTORY ENSHEATHING GLIA CELLS GENETICALLY MODIFIED TO SECRETE THE NEUROTROPHINS BDNF AND NT-3 MEDIATES ENHANCED RECOVERY OF HIND LIMB FUNCTION IN RUBROSPINAL TRACT LESIONED RATS

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Transplantation of olfactory ensheathing glia (OEG) is a promising strategy to augment long-distance regeneration in spinal cord following injury. Genetic engineering of OEG to express additional neurotrophic genes may improve the growth-promoting properties of these cells yielding optimal 'bridging'-substrates for CNS regeneration. In the present study we have investigated the relevance of ex vivo adenoviral vector-mediated gene transfer to OEG in order to overexpress the neurotrophins BDNF and NT-3. Primary cultures of rat OEG infected with adenoviral vectors encoding BDNF and NT-3 express high levels of neurotrophin mRNA as detected by in situ hybridisation and ELISA techniques. Biological activity of transgenic BDNF and NT-3 was tested in a dorsal root ganglion (DRG) bioassay. Conditioned medium from Ad-BDNF or NT-3 infected OEG cultures induced a robust neurite outgrowth from embryonic DRG explants indicating transgenic proteins were biologically active. Following transplantation of transduced OEG into intact or C4 unilaterally hemisected dorsal spinal cord, high levels of transgene expression were observed. Transgene expression gradually declined between 7 and 30 days post implantation in lesioned spinal cord. Locomotion analysis during rope-walking, a test specifically developed for rubrospinal tract lesions, showed enhanced functional recovery of hind limb function in rats that received an implant of BDNF and NT-3 secreting OEG starting at 9 weeks post transplantation. Histological analysis of rubrospinal tract axons regeneration is currently in progress. Supported by: (NOW-GMW), NHMRC (Australia), NRP and ASRT.

P431.

PROLONGED SPINAL CORD EDEMA IN ACUTE CERVICAL CORD INJURY

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It has been known that spinal cord edema is one of major mechanisms causing secondary injuries of acutely traumatized spinal cord. Experimental studies and clinical investigations using MRI indicate that spinal cord edema occurs several hours after injury and lasts for several days to weeks. However, spinal cord edema more than several months is quite unusual in acute spinal cord injury. This paper describes two cases of acute cervical cord injury showing prolonged spinal cord edema after acute trauma. Case 1: This 68 year-old man became tetraplegic after fall from bicycle, and he eventually developed respiratory paralysis. The patient was referred to our hospital 84 days after trauma. MRI demonstrated marked cervical canal stenosis with OPLL and an extensive intramedullary edema from the lower medulla to the upper thoracic level. Slight improvement of the level of sensory loss and decreased intramedullary edema were obtained after decompressive surgery, but there was no recovery of motor function. Case 2: This 31-year-old man became mildly tetraparetic after falling down on the floor during Judo exercise. Three days after trauma, he visited our out patient clinic. MRI showed spinal cord edema and spinal canal stenosis at C3-4 and T1-3 levels. He was treated conservatively. Although his symptoms had improved several weeks after injury, MRI at 1 year revealed that spinal cord edema still existed. Disturbed circulation of cerebrospinal fluid (CSF) around the traumatized spinal cord and venous congestion will explain such a prolonged spinal cord edema. It is likely that alteration of intramedullary blood and CSF circulation underly several types of posttraumatic progressive cystic myelopathy which should be intensively treated.

P432.

HP184 IMPROVES LOCOMOTOR PERFORMANCE IN RATS WITH MILD ESTABLISHED SPINAL CORD COMPRESSION INJURY

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Demyelination of surviving axons after spinal injury causes axonal conduction block. potassium channel blocking ameliorates this. Clinically, potassium channel blockade improves symptoms in spinal cord injury. HP184 is a voltage-dependent blocker of potassium currents in PC12 cells and a use- and frequency dependent blocker of sodium channels. This combination of activities allows high levels of HP184 to be administered without danger of convulsion in animals. To determine whether HP184 could improve motor function in rats with an established mild spinal cord compression injury (Gruner et al., Brain Res., 729:90–101, 1996). HP184 (3, 10, 20 mg/kg) was administrated daily by gavage from day 25–28 post-compression. All three groups of HP184 treated animals showed similar improvements in the open field walking task.

P433.

SRC FAMILY KINASE INHIBITOR PP1 IMPROVES MOTOR FUNCTION AFTER SPINAL CORD CONTUSION IN RATS.

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Following spinal cord injury, vascular permeability advances around the area of the injury and this causes secondary injury. The activation of Src participates in this phenomenon. We previously reported that Src family kinase inhibitor PP1 reduced inflammatory response after spinal cord contusion. In the present study, we examine the effect of PP1 in motor function with the slight spinal cord contusion model. Twenty-five female Wistar rats (body weight = 220g) were used in this study. Under general anesthesia the spinal cord was compressed for 5 seconds over the dura with Biemer vessel clip, which has a squeezing power of 25g. The Src inhibitor PP1 (1.5 mg/kg) was administrated intraperitoneally 10 minutes after compression (PP1 group). The vehicle only was administrated to the control rats using the same method (control group). The motor function of hind limbs after surgery was evaluated in 7 scales (S0-6). At 3 days, 7 days and 14 days after surgery, the spinal cords were removed and the sagittal sections were obtained. The ranges of edema formation and inflammation in both groups were examined by using immunohistochemistry of anti-rat IgG and anti-ED-1 antibody. The motor function immediately after surgery was flaccid in both groups (S6). On 3 days after surgery, the gait on the knuckles was observed in PP1 group (S3), but spasticity was observed in control group (S5). On 7 days after surgery, the ataxic gait was observed in PP1 group (S2), although only the slight motion of knee was observed in control group (S4). The immunohistochemical analysis revealed that the ranges of edema formation and inflammation were also remarkably reduced in the PP1 group. The motor function was significantly improved by administration of PP1. In the near future, we believe that Src family kinase inhibitor will be applicable to the treatment of spinal cord injury. The adverse effect should be investigated.

P434.

EXPERIENCE OF ANTERIOR RECONSTRUCTION WITH KANEDA SR IN THE TREATMENT OF THORACOLUMBAR BURST FRACTURE

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Study design: A retrospective clinical study was performed in twenty-two patients with thoracolumbar burst fractures who underwent anterior decompression and reconstruction using Kaneda SR.

Objectives: To determine the effectiveness and safety of anterior decompression and reconstruction using Kaneda SR in the patients with thoracolumbar burst fractures.

Background of Data: The treatment of the thoracolumbar burst fractures using Kaneda SR has been reported with some variety in results and complications. Additional data were needed. This report includes the result of our initial experience using Kaneda SR in the treatment of the thoracolumbar burst fractures.

Materials and Methods: Twenty-two consecutive patients with 23 thoracolumbar burst fractures who underwent anterior decompression and reconstruction using Kaneda SR were included in this study. The surgery was done with single-stage anterior decompression, strut grafting or Harms cage insertion, and Kaneda SR spinal instrumentation. Average follow-up period was 24 months (range, 14 months to 30 months)

Results: Mean age of the patients was 39.3 years old. The fractures were in the thoracolumbar junction (between T11 and L2) in 18 out of 23 fracture levels. Eighteen cases except 4 were associated with neurological deficit from cord and/or cauda equina injury. The majority of the patients with neurological deficit (17 out of 18 patients) were improved by at least one grade after the surgery, as measured with a modification of the grading scale of Frankel.

Average canal compromise was 42.2% preoperatively, and improved to 1.4% postoperatively. Average preoperative kyphotic angle was 18.5 degree and it was improved to 6.7 degree postoperatively, and this correction was lost approximately 2.3 degree in the follow up period. There was not screw fracture or pseudoarthrosis.

Conclusion: The authors suggest that anterior decompression and reconstruction using Kaneda SR is an effective and safe method in the treatment of thoracolumbar burst fractures. Long term follow up study may be necessary.

P435.

S-100BETA LEVELS AND MYELOPEROXIDASE ACTIVITY AFTER SPINAL CORD INJURY IN THE RAT.

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Previously, we were able to demonstrate that administration of (Christopher Reeve quercetin dihydrate contributed to recovery of motor function after spinal cord compression injury in an animal model. Here, we report on biochemical, histological and immunocytochemical changes observed within the first 24 hr after spinal cord injury in the same model.

Twenty-nine male and 20 female adult Wistar rats, 22 male and 16 female animals were submitted to mid-thoracic spinal cord compression injury (50g calibrated aneurysm clip closed for 5 seconds). Animals received either 2 doses (12 hr survival) or 3 doses (24 hr survival) of 0.025 mmol/kg quercetin dihydrate intra-peritoneally, or weight adjusted doses of normal saline treatment, starting 1hr after injury. Spectrophotometric analysis for myeloperoxidase (MPO) activity was performed for 20 male and 20 female animals. Analysis for S-100beta protein was performed in the compressed spinal cord segment of 2 animals per group using an immunoluminometric assay (LIAmat® Sangtec® 100). Nine male animals were used for histological and immunocytochemical analysis.

MPO activity at the site of injury was significantly lower in female rats treated with quercetin as compared to saline controls ($p < 0.0001$). While a trend to lower MPO activity was also observed in male animals, there was no statistically significant difference between treated animals and saline controls. S-100beta levels, however, were higher in quercetin treated males compared to saline controls, with statistical significance at 12 hr after injury ($p < 0.05$). No increased S-100beta levels were seen in quercetin-treated females within 24 hr after injury. Supported by HSURC Saskatchewan and the Christopher Reeve Paralysis Foundation.

P436.

EFFECTS OF HP184 ON C-FIBER MEDIATED HYPERREFLEXIVE BLADDER CONTRACTIONS INDUCED BY EITHER ACUTE SCI OR IRRITATION

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Normally, the bladder stores urine while the external urethral sphincter (EUS) is contracted. When the bladder switches to elimination, the EUS relaxes via an inhibitory supraspinal reflex. Following SCI and loss of supraspinal input, the EUS does not relax during bladder contractions and voiding does not occur. Moreover, normally silent C-fiber afferents become dominant in triggering reflex bladder contractions resulting in hyperactive bladder contractions, a condition that also occurs following bladder irritation. HP184 is a dual K+ and use-dependent Na+ channel blocker. We have tested HP184 in rats using a novel acute mild spinal crush (to 75% diameter for 15 sec at T9) model and following bladder irritation in normals (intravesical delivery of 10 mg/ml protamine sulfate followed by physiological urinary KCl (300mM) or Heloderma venom (100nM). Preliminary studies using mild acute SCI reveal that non-voiding, high frequency and high-pressure contractions of rat bladder are ameliorated by a single bolus dose of 10 mg/kg HP184 (iv). Furthermore, this same dose reverses the effects of bladder irritation caused by either Heloderma venom or 300mM KCl. These data strongly support the notion that HP184 can selectively restore descending spinal inhibitory input after acute injury or inhibit the runaway activity of C-fibers due to inflammatory agents.

P437.

MITOCHONDRIAL FUNCTION AS MEASURED BY REDOX POTENTIAL IS REDUCED FOLLOWING LATERAL FLUID PERCUSSION BRAIN INJURY.

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Strong evidence suggests that aerobic respiration may be compromised following traumatic brain injury (TBI), and this may be in part due to mitochondrial dysfunction. Mitochondria effect aerobic respiration through oxidative phosphorylation which is coupled to the electron transport chain and uses the oxidation-reduction potential (redox) to generate ATP.

In order to test this hypothesis, we utilized Alamar Blue dye as an indicator of the redox potential to examine the effects of lateral fluid percussion injury (FPI) and oxygen treatments on mitochondrial function. Rat cerebral cortex was taken and homogenized from animals treated with 30% O2, 100% O2, or hyperbaric O2 (100% O2 at 1.5 ATA) at 1 or 4 hours after lateral FPI (~2.11 Atm) or sham injury. A synaptosomal fraction was prepared to enrich for mitochondria.

Synaptosomes were incubated with Alamar Blue dye and relative fluorescence was measured as an indicator of redox potential. In animals treated with 30% O2, the injured hemisphere showed a significant reduction in redox potential when compared to shams at both 1 and 4 hours after treatment. In animals on 100% O2 there was a significant reduction when comparing the injured hemispheres to sham animals at 1 hr but not at 4 hrs. Hyperbaric oxygen for 1 hr, significantly reduced redox potential in the injured hemisphere, compared to shams. Thus TBI appears to cause significant mitochondrial impairment, as measured through reductions in redox potential. Supported by NS-12587-26 and the R.W. Johnson Foundation.

P438.

GLYCOGEN LEVELS IN CORTEX AND HIPPOCAMPUS INCREASE 24 HOURS AFTER LATERAL FLUID-PERCUSSION BRAIN INJURY.

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Previous studies have demonstrated that traumatic brain injury (TBI) increases the vulnerability of the brain to an acute episode of hypoxia/ischemia. Recently, however, we demonstrated decreased vulnerability to forebrain ischemia 24 hr after TBI, similar to the phenomenon of ischemic tolerance. Glycogen is an important energy substrate, which, if elevated after TBI, may protect the brain against ischemia. The objective of the present study was to determine whether TBI elevates glycogen. TBI was evoked in Sprague-Dawley rats using lateral fluid-percussion. After recovery for 24 hr, the brain was frozen in situ with liquid nitrogen, and the brain was sampled bilaterally in 6 predetermined regions of cerebral cortex and in 2 regions of hippocampus for measurement of glycogen, using enzymatic, fluorometric methods.

The results indicated that in 5 of the 6 cortical regions analyzed, glycogen in the hemisphere ipsilateral to the KCl application was elevated 2.3-fold relative to that in the contralateral hemisphere ($p < 0.05$). In the 2 hippocampal regions, ipsilateral glycogen levels were 1.6-fold higher than those in the contralateral hippocampus ($p < 0.05$). Thus, the elevation in glycogen after TBI may contribute to the induction of tolerance to forebrain ischemia. Supported by NIH grant NS-08803 (FAW).

P439.

THE EFFECTS OF DELAYED BUT PROLONGED HYPOTHERMIA ON THE PIAL VASCULAR RESPONSE AFTER TRAUMATIC BRAIN INJURY IN RATS

Yuji Ueda, Enoch P. Wei, Eiichi Suehiro and John T. Povlishock. (Medical College of Virginia Campus/VCU, Richmond, VA US).*

Recently, in both the clinical and laboratory setting, the potential vascular protective effects of hypothermia have received attention in both stroke and traumatic brain injury (TBI). In laboratory studies of TBI, most have focused on the use of early hypothermic intervention, with little consideration of the potential efficacy of delayed but prolonged hypothermia, which would constitute a more clinically relevant paradigm. In the current investigation, we evaluated whether delayed but pronged hypothermia after TBI protected the cerebral microcirculation. Male Sprague-Dawley rats were equipped with cranial windows for direct visualization of the pial arterial circulation and then subjected to impact acceleration brain injury, with the delayed (1h) induction of either 1h or 2h of hypothermia (32 degrees C) followed by the slow rewarming (32–37 degrees C/90 min). Nonhypothermic animals served as controls. The pial arteriolar responses to acetylcholine (ACh) or hypercapnia were measured. Through this approach we found that both delayed hypothermia groups maintained normal arteriolar vascular responses in terms of ACh-dependent dilation and carbon dioxide reactivity, however, the prolonged hypothermic group showed more significant recovery. In contrast, arterioles subjected to TBI followed by normothermia demonstrated severely impaired vasoreactivity, with arteriolar dilation maintained through the duration of the study. The results of this study show that delayed but prolonged hypothermia attenuates the impaired vascular responsiveness seen after TBI, suggesting its potential clinical usefulness. This work was supported by NIH grants NS-20193 and T32 NS7288.

P440.

CHANGES IN CEREBRAL PERFUSION AND HIGH ENERGY-RELATED METABOLITES IN RESPONSE TO FLUID PERCUSSION INJURY

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Introduction: The purpose of this study was to document changes in cerebral perfusion and energy-related metabolites before, during and after a fluid percussion injury. It is becoming clear that energy/metabolic state changes in the first few hours following injury may play a role in long term outcome.

Methods: Following induction of anesthesia Sprague-Dawley rats were intubated and artificially ventilated. After placement of femoral arterial and venous catheters, burr holes were made in the skull for positioning of the fluid percussion mount, bilateral laser Doppler fibers, and a microdialysis probe. Arterial blood pressure, cerebral perfusion, and microdialysis samples were collected over a four hour period, from two hours pre- to two hours post-injury. Fluid percussion was administered at 2 atmospheres, a moderate injury.

Results: Immediately in response to the fluid percussion injury, cerebral perfusion decreased by 40% and slowly returned toward pre-injury levels over the two hour post period. All three metabolites increased in response to injury: hypoxanthine 4 fold ($p > .001$), inosine 2 fold ($p > .02$), and adenosine 7 fold ($p > .01$).

Conclusions: Early changes occur in both cerebral perfusion and energy metabolites in response to a moderate cerebral injury. These changes may be long lasting and contribute to the overall long-term outcome. Recognition of this early derangement of perfusion and energy state may be important in the overall therapeutic regimen.

P441.

PERIVASCULAR NERVE DAMAGE IN THE CEREBRAL CIRCULATION FOLLOWING TRAUMATIC BRAIN INJURY

Yuji Ueda Susan A. Walker, Christina R. Marmarou, Richard H. Singleton and John T. Povlishock. (Medical College of Virginia Campus/VCU, Richmond, VA US).*

It is well recognized that traumatic brain injury (TBI) causes alterations in the cerebral microcirculation ranging from abnormalities in cerebral dilation to impaired reactivity to challenges such as altered carbon dioxide or acetylcholine application. Most have assumed that these impaired vascular responses were the result of endothelial and /or smooth muscle alteration, triggered by the traumatic event. No consideration, however, has been given to the possibility that the forces of injury may also damage the perivascular nerve network, thereby contributing to the observed abnormalities. To test this premise, we subjected rats to impact acceleration and sham injury. At 6hr, 24 hr or 7days post injury, the rats were re-anesthetized and transcardially perfused. Portions of the vertebrobasilar and internal carotid system were removed and processed with antibodies targeting 5-hydroxytryptamine (5-HT) and the neuropeptide PGP-9.5. Lastly, the Fluoro-Jade procedure was employed to detect primary nerve fiber damage. Selected vessels were analyzed to determine the distribution, of these markers and their overall density. Using the Fluoro-Jade marker for axonal degeneration, the perivascular nerve network showed no reactivity in either the sham or 6 hr animals; however, by 24 hr postinjury, Fluoro-Jade reactivity was noted in the perivascular regions. In concert with this marker of damage, antibodies targeting 5-HT accumulation and normal neuropeptide (PGP-9.5) distribution demonstrated intact and unaltered fiber populations in the sham and 6hr animals. By 24 hr postinjury, however, a significant reduction in the perivascular 5-HT accumulation occurred, together with a reduction in PGP-9.5 fiber staining. Collectively, these studies illustrate that within the cerebral circulation perivascular nerve fiber damage is a consistent feature of TBI. These studies suggest that neurogenic damage may be a contributor to some of the vascular abnormalities associated with TBI and obviously, this issue merits further consideration. Supported by NIH grants NS-20193 and T32 NS7288.

P442.

HYPOTHERMIC CEREBROVASCULAR PROTECTION IS RELATED TO THE RATE OF POST HYPOTHERMIC REWARMING

Enoch P. Wei, Yuji Ueda, Eiichi Suehiro and John T. Povlishock. (Medical College of Virginia Campus/VCU, Richmond, VA US).*

Recently, our labs, and others, have focused on the potential neuroprotective and cerebrovascular protective effects of hypothermia following traumatic brain injury. We have observed that the efficacy of posttraumatic hypothermia was related to the rate of rewarming after hypothermic intervention, with the finding that rapid rewarming exacerbated traumatically induced axonal injury and cerebrovascular dysfunction (J. Neurosurg 94:93–498, 2001). In the current communication, we revisit the use of hypothermia with varying degrees of rewarming to ascertain if, in the normal cerebral vasculature, varying rates of rewarming could differentially affect cerebrovascular responsiveness. To this end, we examined the effects of rewarming on the cerebral microcirculation in non-traumatized rats equipped with closed cranial windows. All animals were exposed to hypothermia of 32 degrees C for 1 hr duration, followed by either slow rewarming over a 90 min. period or rapid rewarming over a 20 min. period. Vasoreactivity to hypercapnia and ACh were assessed. Animals receiving hypothermia, followed by slow rewarming showed a restoration of normal vascular responsivity following rewarming. In contrast, the use of hypothermia followed by rapid rewarming elicited impaired cerebrovascular responses to ACh and arterial hypercapnia. Furthermore, hypothermia followed by fast rewarming impaired the dilator responses of sodium nitroprusside, a NO donor, and pinacidil, a KATP channel opener. These findings support the use of hypothermia followed by slow rewarming. They demonstrate that fast rewarming can trigger vascular abnormalities most likely through primary endothelial as well as vascular smooth muscle damage. Supported by NS 20193.

P443.

STUDY OF MILD HYPOTHERMIA ON PBTO2 AND BT PATIENTS WITH SEVERE HEAD INJURY

Professor Shuyuan Yang. (Huanhu Hospital, Tianjin, Tianjin CN).

Objective To study the changes of partial pressure of brain tissue oxygen (PbtO2) and brain temperature (BT) in acute phase of patients with severe head injury, and effect of mild hypothermia on PbtO2 and BT.

Methods PbtO2 and BT of 33 patients with severe head injury were monitored, and hypothermia was induced within 20 hours of injury. Rewarming was begun on 1–7 days (average 57.7 ± 28.4 hours) after the rectal temperature reached 31.5–34.9. Monitorings of PbtO2 and BT were lasted for 1–5 days (average 54.8 ± 27.0 hours). According to Glasgow Outcome Scale (GOS), the prognosis of the patients was evaluated at 6 months after injury.

Results Within 24 hours after severe head injury, PbtO2 was significantly lower (9.6 ± 6.8mmHg) than the normal value (16–40mmHg). After treatment in mild hypothermia, mean PbtO2 raised to 28.7 ± 8.8 mmHg during the first 24 hours , and the PbtO2 was maintained within the range of normal value at 3days postinjury. BT was higher than RT in acute phase of patients with severe head injury. The difference between BT and RT significantly increased in mild hypothermia. Hyperventilation ($PaCO_2 \approx$ 25mmHg) induced low PbtO2 since high ICP had been decreased.

Conclusion This study demonstrates that monitoring of PptO2 and BT is a safe, reliable and sensitive diagnostic method to follow cerebral oxygenation. It might become an important tool in our treatment regime for acute patients of severe head injury requiring hypothermia and hyperventilation.

P444.

APOPTOTIC CELL DEATH FOLLOWING IN VITRO TRAUMATIC INJURY IS INDEPENDENT OF CALCIUM INFLUX

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Neuronal apoptotis is a common feature of traumatic brain injury in vivo. In this study we used an in vitro model to study the role of calcium in the induction of apoptosis following mechanical injury. Hippocampal neurons were plated onto a silastic membrane then injured with either mild strain (12–17%) or severe strain (>50%) at 10 days in vitro. Viability was assessed at 24 hours following injury. Fluorescence microscopy was used to detect live (Fura-2 positive), dead (propidium iodide positive, PI), and/or apoptotic cells. Apoptosis was detected using a fluorogenic substrate (FAM-DEVD-FMK) for activated caspase-3.

In physiologic salt solution (PSS), both severe stretch and NMDA application resulted in significant cell death (PI positive, $p < 0.001$ compared to sham injured cultures). The extent of cell death after severe stretch injury was unaffected when either extracellular calcium was removed or when cultures were treated with 100 uM MK-801. As expected, treatments attenuated cell death following NMDA exposure ($p < 0.001$).

No significant increase in the number of apoptotic cells was observed following either stretch or NMDA in PSS when compared to uninjured cultures. Removal of calcium from the media resulted in increased apoptosis in both stretch injured and NMDA treated cultures ($p < 0.001$). Treatment with MK-801 did not affect the extent of apoptotic cell death after either mild stretch or NMDA application but did increase the number of apoptotic cells following severe stretch ($p < 0.001$, compared to uninjured cultures).

These data suggest that total cell death following mechanical injury is independent of NMDA activation. Using treatments to blunt the acute calcium transient resulted in an unmasking of a stretch activated apoptotic pathway that does not appear to depend on elevated cytosolic calcium.

Funding provided by NIH RO1 HD 41699, NS 35712, R01 NS41561, and P01 NS08803

P445.

REPEATED RAPID ACCELERATIONS PRODUCE INCREASED AXONAL INJURY IN THE IMMATURE BRAIN

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Inflicted brain injury associated with widespread traumatic axonal injury (TAI) and subdural hematoma (SDH) is a leading cause of death in infants and children. A pediatric (3–5 day old, representing an infant < 3mo) anesthetized porcine model was used to study single (n = 5) and double (n = 6, injured approximately 20min apart) rapid (<20msec), nonimpact axial rotations of the head. Load level (peak velocity of 172 ± 17 for single, 135 ± 8 rad/s for double) was selected to induce a brief period of unconsciousness. Pinch reflex was absent for 1–20 min in all injured piglets. At 6h post-injury, animals were sacrificed and their brains perfusion fixed.

Gross inspection showed SDH in frontal lobes and brainstem of 3 single injury brains, all double injured brains, and absent in uninjured controls (N = 3). Under light microscopy no subarachnoid hematoma (SAH) was observed in any brain. All double injured and three single injured brains demonstrated TAI, defined as an accumulation of the 200kDa neurofilament protein in either continguous axons or terminal bulbs. Nearly all TAI was observed in peripheral and central white matter tracts. Double injured piglets had significantly more injured foci (5.5 regions/brain) compared with single injury (0.8 regions/brain, p*0.05), but the density of injured axons was not significantly different (2.2 ± 1.2 injured axons/mm2 in the double injured piglets versus 1.3 ± 1.3 axons/mm2 in the single).

The data demonstrate that repeated, mild, nonimpact brain injuries have more widespread acute axonal injury compared with those experiencing a single event. Since inflicted head injuries may be single or multi-load events, these results have implications for the development of appropriate animal models to study inflicted brain injuries in children.

Support provided by NIH R01 NS 39679 and NIH R01 NS 541561.

P446.

NEURONAL LOSS FROM BRAIN NUCLEI AFTER HUMAN BLUNT HEAD-INJURY

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Quantitative evidence for loss of neurons after human blunt head-injury is lacking. The hypothesis that an unbiased quantitative analysis might provide such evidence was tested.

Material was obtained from the archive of the Department of Neuropathology, Southern General Hospital, Glasgow. That material (age range 18–64) consisted of 9 age and sex matched control patients with no history of head-injury in life, 4 severely head-injured (SHI) (GCS on admission less than 8) with survivals of 3–7 days, and 9 (SHI) patients with survivals between 8 and 395 days. Paraffin, coronal sections of the left and right thalami and the left hippocampus were cut and stained using cresyl violet. The point counting technique was used to estimate the area of each brain nucleus and these were compared between patient groups. The size of pyramidal neuronal cell bodies in sub-fields of the hippocampus, dorsomedial, lateral and ventral thalamic nuclei was measured and used to determine the size of the counting boxes used for determination of the number of neurons within a defined volume of brain tissue. Numbers of neurons within the brain regions of interest (vide supra) were counted in either non-head injured control (n = 9) or severely head-injured patients (n = 13) (age range 18–64). The Student's t test was used for statistical analysis.

At 1 week survival there was loss of pyramidal neurons from hippocampal sub-fields CA1 (p = 0.012), CA3 (p = 0.003) and CA4 (p = 0.002) but not CA2. Further loss occurred at 6 months but only in sub-fields CA1 (p = 0.033) and CA4 (p = 0.013). No quantitative evidence for loss of neurons from any thalamic nucleus was obtained.

Loss of neurons after blunt head-injury occurred both with a different time scale and to a different extent in different brain nuclei. Stereology provided evidence for different levels of loss of neurons from different subfields of the hippocampus at a week after injury. But, in addition, provided novel data for loss of neurons from hippocampal sub-fields at longer post-traumatic survivals. However, stereology did not provide support for loss of neurons from thalamic nuclei after head-injury.

P447.

STEREOLOGICAL COMPARISON OF REGIONAL HIPPOCAMPAL CELL LOSS IN INBRED MOUSE STRAINS FOLLOWING FLUID PERCUSSION INJURY

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Fluid percussion injury (FPI) causes hippocampus-dependent memory dysfunction in mice. Standard post-mortem histological analysis have reported hippocampal CA3 and hilar neuronal loss. We have employed design-based stereology combined with the optical volume fractionator to estimate quantitative neuronal loss within specific subregions of the hippocampus after lateral FPI in the mouse. Stereology is an unbiased, systematic random sampling procedure which combines cell numerical density estimates (from the optical dissector) with volume estimates (generated by point counting and the fractionator stereology method) to estimate the absolute cell number for hippocampal subregions: CA1, CA3, dentate gyrus, and hilus.

Anesthetized adult male C57BL/6 and C57BL/10 mice were randomly selected and subjected to either lateral FPI (1.1–1.4 atm) or surgery without injury. Mice were transcardially perfused 7–10 days post-injury, the brains removed, and post-fixed for 24 hours. Paraffin-processed mouse brains were embedded, sectioned exhaustively at 50 μm in the horizontal plane, and wet mounted on gelatin-subbed slides. Using the Olympus CAST system, optical dissectors were systematically placed across every 3rd section containing the hippocampus and the volumes of hippocampal subregions were estimated by point counting on these same sections.

Our preliminary data indicate that (1) the number of CA1 pyramidal neurons in sham animals are similar between mouse strains, and (2) the ~17% reduction in the number of CA1 neurons one week following FPI in C57BL/6 mice is exacerbated in the hyperexcitable C57BL/10 strain (~40% reduction). The loss of CA1 neurons and the inbred strain differences indicate cellular pathology throughout the hippocampus that may underlie hippocampus-dependent memory deficits. With these promising results, our attention is now focused on the CA3, dentate gyrus, and hilar subregions of the hippocampus.

Supported by NIH grant NS08803 (M.S.G.) and the Sharpe Trust

P448.

AGE-ASSOCIATED MITOCHONDRIAL DNA DELETIONS AND OXIDATION ARE NOT EVIDENT CHRONICALLY FOLLOWING EXPERIMENTAL BRAIN INJURY IN THE RAT

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The enduring cognitive and sensory-motor deficits that result from traumatic brain injury (TBI) are associated with metabolic stress and free radical cascades, which establish conditions that may promote mitochondrial DNA (mtDNA) deletion and oxidation, often observed as a consequence of normal aging. Without substantial mtDNA repair mechanisms, permanent alterations to essential mitochondrial enzymes could perpetuate post-injury pathologic cascades. To determine whether mitochondria from the injured cortex and hippocampus sustain mtDNA damage after TBI, we evaluated both deletion and oxidation of mtDNA following lateral fluid percussion TBI in the anesthetized adult Sprague-Dawley rat (4 mo) compared with uninjured adult and aged rats (n = 4/group). The presence of the 4.8 KB common deletion in mtDNA was assessed by conventional PCR to generate products representing total, non-deleted wild-type, and deleted mtDNA in homogenized tissue and isolated mitochondria 3 and 14 days following TBI. Total and wild type mtDNA amplification products were obtained from cortical and hippocampal tissue and mitochondria for all conditions. Although no mtDNA deletions were observed following experimental TBI, mtDNA deletion was detected in cortical tissue, but not isolated mitochondria, of naive, aged (24 mo) Sprague-Dawley rats, suggesting that the isolation may exclude mitochondria harboring mtDNA damage. Oxidative mtDNA damage in isolated mitochondria assayed by ELISA for 8-hydroxy-2′-deoxyguanosine (8-OHdG) from cortical (0.50 ± 0.08 pg 8-OHdG/μg mitochondria) and hippocampal (0.35 ± 0.02) regions were unaffected by TBI. However, mitochondrial protein yields from injured and aged brains were comparable, and significantly smaller than uninjured brain, suggesting some similar underlying pathology between TBI and aging.

Supported by NIH NS08803, NIA Dissertation Research Support, and a Veterans Administration Merit Review grant.

P449.

LONG-TERM PRION PROTEIN ACCUMULATION IN DAMAGED AXONS FOLLOWING INERTIAL BRAIN INJURY IN THE PIG.

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Observing that trauma elicits the accumulation of pathologic aggregates found in neruodegenerative diseases, we evaluated the possible accumulation of prion protein (PrP) in pig model of diffuse axonal injury (DAI). This investigation was initiated by the assumption that PrP transport in axons would be interrupted following brain trauma. Nine miniature young adult (6 months age) swine, weighting 20–26 kg, were used for this study. Six were subjected to head coronal plane rotational acceleration and the brains evaluated at 3 hours, 3 days, 7 days, and 6 months postinjury. Three animals were used as controls. Immunohistochemistry and western blot analysis was performed using anti-PrP monoclonal antibodies 3F4, F99/97.6.1. and polyclonal antibody CD230 To determine potential accumulation of an abnormal PrP isoform, PrPsc, sections and tissues were pretreated with 0.1% proteinaseK. At all post-injury timepoints, we found extensive accumulation of prion protein in damaged axons in the brain injured pigs, including in the proteinaseK treated sections. Additionally, we found a limited number of plaque-like profiles in the brain sections. No overt spongiform changes were found in the brains. On western blot, strongly immunoreactive bands were found with a mass of less than 30 kDa in proteinaseK treated tissue at 3 hours, 3 days and 7 days following injury, which were slightly shifted down from faint bands found in the control animals. These data demonstrate a long-term process of PrP accumulation following brain trauma. Furthermore, these data suggest that some of the accumulated PrP is the abnormal isoform, PrPsc, thought to be the key pathologic agent in transmissible spongiform encephalopathies. However, it remains to be determined whether PrP accumulation plays a role in the progressive neurodegenerative changes observed following brain trauma. Supported by NIH grants, AG12527, NS 38104, and NS08803

P450.

TRANSCRIPTIONALLY PROFILING THE EFFECTS OF CHRONIC METHYLPHENIDATE TREATMENT IN RATS AFTER TRAUMATIC BRAIN INJURY

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Dopamine (DA) pathways have been implicated in cognitive deficits after traumatic brain injury (TBI). Clinical and laboratory studies have shown that posttraumatic cognitive deficits can be attenuated with DA agonists, including methylphenidate (MPD). While the beneficial effects of DA agonists have been attributed to increasing DA tone, we hypothesized that MPD's effects were also associated with a unique profile of chronic gene expression. We examined the effects of daily MPD treatment on gene expression using microarray technology following TBI produced by controlled cortical impact injury (4 m/sec, 2.8 mm tissue deformation). Beginning one day after injury, rats were daily injected with either MPD (5 mg/kg, i.p., n = 3) or saline (n = 3). Sham rats (n = 3) were surgically prepared, but not injured. After 21 days, the rats were sacrificed and total RNA was extracted from a tissue region containing DAergic cell bodies (bilateral substantia nigra and ventral tegmental areas). Oligonucleotide expression arrays (Affymetrix neurobiology array) containing 1,322 mRNA sequences were used to determine the transcriptional profiles. Only mean expression level changes of 2-fold or more relative to sham controls are reported. The TBI+saline group produced 38 mRNA sequences that were upregulated and 22 sequences that were downregulated. The TBI+MPD group produced 34 mRNA sequences that were upregulated and 28 sequences that were downregulated. Of particular interest were treatment-associated changes in mRNA sequences involved in regulating neurotransmitter function. Approximately half of the mRNA expression changes in the TBI+MPD group were unique from the TBI+saline group. These changes in transcriptional profiles in the substantia nigra and VTM regions support the conclusion that there may be a transcriptional basis for the neuroprotective effects of chronic posttraumatic MPD therapy. Further gene profiling of experimentally effective treatments may help differentiate and refine specific therapies targeting clinical recovery of function after TBI.

Support: NS 30318, NS 40125, NS 33150.

P451.

ASSOCIATIONS BETWEEN DOPAMINE TRANSPORTER GENOTYPE AND CEREBRAL SPINAL FLUID DOPAMINE LEVELS AFTER SEVERE TRAUMATIC BRAIN INJURY

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Dopamine pathways have been implicated in cognitive deficits after traumatic brain injury (TBI). While not associated with alterations in protein structure, the dopamine transporter (DAT) 3'-VNTR polymorphism has been associated with differences in DAT protein density and development of DA mediated pathophysiological conditions. Differential DAT expression presumably affects both presynaptic DA release, via reverse transport, and DA reuptake. Catacholamines, including DA and its metabolites, are subject to auto-oxidation, resulting in the formation of reactive oxygen species that can contribute to oxidative stress associated with secondary brain injury. Therefore, the purpose of this study was to determine the relationship between DAT genotype and cerebral spinal fluid (CSF) DA levels after severe TBI. We hypothesized that the DAT 10/10 genotype would be associated with higher CSF DA/DA metabolite concentrations after TBI. We evaluated 30 patients with severe TBI (GCS<8) admitted between 1995–1998 and determined DAT genotype for patients using previously banked samples of CSF. High performance liquid chromatography was used to determine post injury CSF levels of DA/DA metabolites. Mann Whitney-U analyses were used to determine differences between DAT genotype groups with post-injury average and maximum levels of DA and DA metabolites, including 3,4-dihydroxyphenylacetic acid (DOPAC) and homovaneillic acid (HVA). Seventeen patients had the DAT 10/10 genotype, and 13 patients had either the DAT 9/9 or 9/10 genotype. Results showed no differences between genotype groups for post-injury average or maximum CSF DA levels. However there were significant increases in maximum DOPAC ($p = 0.023$), HVA ($p = 0.041$), and DOPAC/DA ratios ($p = 0.038$) for the DAT 10/10 genotype group compared to the 9/9–9/10 group. Average DOPAC ($p = 0.044$) and DOPAC/DA ratios ($p = 0.048$) were also significantly higher for the DAT 10/10 group. These results indicate higher DA metabolism in patients with DAT 10/10 genotype, which may increase susceptibility to DA mediated oxidative injury after TBI. K08HD40833, R01NS40125, Pittsburgh Foundation

P452.

DOPAMINE TRANSPORTER GENOTYPE IS ASSOCIATED WITH FUNCTIONAL AND NEUROPSYCHOLOGICAL OUTCOME FOLLOWING TRAUMATIC BRAIN INJURY

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Alterations in dopamine (DA) pathways appear to impact cognition after traumatic brain injury (TBI). The dopamine transporter (DAT) 3'-VNTR polymorphism has been associated with DA system function and DA mediated cognitive disorders, with the DAT 10/10 genotype being implicated in attention deficit disorder. DAT regulation may have a role in DA mediated neurotoxicity acutely after TBI and play a compensatory role in improving DA neurotransmission chronically after TBI. Therefore, the objective of this study was to evaluate the relationship of DAT genotype to functional and neuropsychological outcome after severe TBI. DAT genotype was determined using previously banked cerebral spinal fluid samples from 36 patients with severe TBI. DAT 10/10 genotype was considered to be the risk genotype for poor outcome and neuropsychological function. DAT genotype was compared to Disability Rating Scale (DRS), Glasgow Outcome Scale (GOS), Wisconsin Card Sort Test (WCST), Trail Making Test, and WAIS-R Digit Span six months after injury. Fifty percent of the population had 10/10 DAT genotype. There were no significant differences with gender, age or injury severity between comparison groups. Results showed that people with the DAT 10/10 genotype had worse six month DRS scores ($p = 0.024$) and showed a trend to have worse GOS scores ($p = 0.112$). Patients with the DAT 10/10 genotype had fewer correct responses for digit span forward testing ($p = 0.035$), showed a trend to do worse with forward digit span ($p = 0.085$), and tended to be too cognitively impaired to count perseverative errors with WCST ($p = 0.088$). DAT genotype is associated with measures of functional and neuropsychological outcome after TBI. The results of this study suggest a role for DAT genotype in affecting cognition and outcome after TBI, and future work should focus on the role that DAT genotype may play in individual response to pharmacological and therapeutic interventions. K08HD40833, R01NS40125, NIDRR#H133P970013-00

P453.

MICROGLIAL CHEMOTAXIS IS REGULATED BY ATP AND ADP RELEASED BY TRAUMATICALLY INJURED ASTROCYTES.

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Microglia (MG), the immune effector cells of the brain, are rapidly activated and recruited to the site of traumatic brain injury within hours of the initial insult, via chemotaxis. Recruitment of MG may contribute to neuronal damage through release of inflammatory mediators and secondary damage to uninjured cells. Therefore, control of chemotaxis may be a target for pharmacological intervention. Using Boyden-like chemotaxis chambers, we examined 2 types of MG, resting and activated. MG were isolated from 7–10 day old mixed brain cell cultures obtained from neonatal rats. Resting MG were maintained in astrocyte-conditioned medium. Activated MG were prepared by a 24 hr exposure to medium conditioned by traumatically injured astrocytes, using an in vitro model for traumatic injury. Adherence to collagen was decreased in activated MG, consistent with the enhanced mobility of these cells. Medium conditioned by injured astrocytes was chemotactic for both resting and activated MG, suggesting that astrocytes release a soluble factor that induces chemotaxis. Glutamate (5–200 mM) was not chemotactic for resting or activated MG. Histamine was chemotactic for resting, but not activated MG. The purinergic nucleotides ATP and ADP, but not UTP, were chemotactic for both resting and activated MG, suggesting that purinergic receptors are involved in chemotaxis. The chemotactic effects of medium conditioned by injured astrocytes was decreased by the purinergic receptor antagonists suramin, and pyridoxalphosphate-6-azophenyl-2′-4-disulphonic acid (PPADS). These results suggest that ATP and ADP released by injured astrocytes are involved in microglial recruitment to the site of traumatic injury. Supported by NS40490.

P454.

MILD OR MODERATE TRAUMATIC BRAIN INJURY: BEHAVIORAL AND HISTOPATHOLOGICAL OUTCOMES IN MICE

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Traumatic injury may initiate specific cell death pathways dependent upon injury severity, thereby resulting in graded histopathological and behavioral deficits. The present study examined cognitive and motor function and histological damage associated with mild (0.5mm deformation; 5.0 m/sec) ($n = 20$) or moderate (1.0 mm deformation; 5.0m/sec) ($n = 20$) CCI injury in anesthetized C57BL/6 mice. Memory function was assessed using the Morris water maze and gross motor function was assessed using a standardized battery of tests. Both mild and moderate injury produced significant memory impairment ($p < 0.001$) and motor deficits ($p < 0.0005$) compared to sham injury. In addition, animals subjected to moderate brain injury exhibited greater cognitive ($p < 0.02$) and motor ($p < 0.01$) dysfunction than mice subjected to mild injury. Brain-injured and sham-injured ($n = 29$) were sacrificed at 15min ($n = 7$), 4h ($n = 8$), 24h ($n = 10$), 2d ($n = 10$), 4d ($n = 19$), or 7d ($n = 15$) post-injury. All brain-injured mice exhibited cortical tissue tears at 15min, cell loss by 24h, and a pronounced cavity by 7d post-injury. The cavity associated with moderate injury extended through all six cortical cell layers. Moderate injury resulted in loss of Nissl stained neurons over a larger rostral-caudal and medial-lateral extent of cortex compared to mild injury. Tearing of the ipsilateral subcortical white matter was commonly observed after moderate injury. In the hippocampus, loss of pyramidal neurons in the CA3 and hilar neurons in the dentate gyrus was observed by 24h after moderate brain injury and by 4d after mild injury. Loss of pyramidal CA1 neurons and granule cells in the dentate gyrus was observed between 24h and 7d after moderate brain injury only. These findings illustrate that both behavioral deficits and histological alterations are dependent on injury severity.

Support by NINDS Grants NS08803 and NS41561.

P455.

A CONFOCAL MICROSCOPIC EXAMINATION OF THE EFFECTS OF STRAIN (STRETCH) ON CULTURED NEURONS AND ASTROCYTES

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We examined the time course of cytoskeletal and morphological damage to cultured neurons and astrocytes grown on a silastic membrane and subjected to rapid biaxial strain (stretch). Injury was assessed using immunocytochemistry for MAP-2, GFAP, and BrdU and propidium iodide (PrI) uptake. Neuronal plus glial cultures subjected to moderate (6.5 mm) or severe (7.5 mm) membrane displacement and stained for MAP-2 demonstrated tortuous and beaded neuronal processes in many neurons immediately following injury. With time, the soma and processes of some injured neurons were less intensely stained with MAP-2. At 24 and 48 hr, many neurons showed PrI uptake and labeled poorly for MAP-2. 3D reconstruction of these cultures demonstrated that neuronal clusters sit upon a hill of astrocytic processes and 24–48 hr after injury, PrI labeled nuclei are seen on top of the astrocytic processes surrounded by degraded MAP-2 labeled cytoskeletal elements. Pure astrocytes or astrocytes of mixed cultures showed similar patterns of GFAP expression and morphological changes with injury. As the cells were increasingly stretched (4.5–8.5 mm), the confluent astrocyte bed retracted and with time the astrocytic processes demonstrated stellate morphology, swelling and hypertrophy. Astrocytes demonstrating both PrI uptake and GFAP staining were apparent at 15 min to 6 hr post-injury, but few astrocytes had PrI uptake at 24–48 hr. In addition, BrdU labeling increased in injured astrocytes of pure and mixed cultures at 24 and 48 hr post-injury, potentially signaling enhanced glial proliferation induced by injury.

In conclusion, injury of neurons first shows damage to neuronal processes (beading and potential deafferentation) and, with time, degradation of the soma with a concurrent increase of PrI uptake. Stretch injured astrocytes show the hallmarks of reactive gliosis—swelling, hypertrophy, increased GFAP immunofluorescence and glial proliferation.

Supported by NS 27214.

P456.

PROTEIN EXTRAVASATION, REACTIVE ASTROGLIOSIS, AND NEURONAL DAMAGE FOLLOWING MILD OR MODERATE TRAUMATIC BRAIN INJURY IN MICE.

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Spatiotemporal patterns and cellular mediators of posttraumatic neuronal or vascular damage may be related to injury severity. The patterns of vascular, glial and neuronal damage were evaluated in anesthetized C57BL/6 mice that were subjected to sham injury ($n = 4$), mild controlled cortical impact (CCI) injury (0.5mm depth at 5m/s; $n = 6$) or moderate CCI injury (1.0mm depth at 5m/s; $n = 7$). At 4hrs, 48hrs and 7days post-injury, 40μm coronal sections were examined immunohistochemically for IgG extravasation, glial fibillary acidic protein (GFAP), and microtubule-associated protein 2 (MAP2). After mild or moderate injury, the ipsilateral parietal cortex and hippocampus exhibited intense IgG labeling at 4 and 48 hrs. Hippocampal IgG labeling at 7 days and acute thalamic IgG extravasation was observed only after moderate injury. Both mild and moderate injury resulted in hypertrophy of hippocampal astrocytes at 4 hrs. At 2 days, a marked increase in GFAP immunoreactivity was detected in the ipsilateral cortex and hippocampus at both severities; however, astrogliosis in the ipsilateral striatum and thalamus was pronounced only after moderate injury. By 7 days in both mild and moderate injury, the cortex, hippocampus, striatum and thalamus all exhibited increased GFAP immunolabeling in the ipsilateral hemisphere. Both mild and moderate injury produced dendritic disruption and MAP2 loss in the cortex, and marked decreases in immunolabeling of the CA2, CA3, and dentate hilar regions of the ipsilateral hippocampus at all time points. Profound loss of MAP2 labeling was consistently observed in the hippocampal CA1 region after moderate, but not mild, injury. These data suggest that both mild and moderate CCI brain injury produce cellular and vascular damage in the cortex. Damage was more prolonged and involved additional brain regions with increasing injury severity. (Supported by NIH NS08803 and NS41561)

P457.

TRAUMATIC AXONAL INJURY DIFFERENTIALLY IMPAIRS FAST- VS. SLOW-CONDUCTING CORPUS CALLOSUM FIBERS.

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Moderate closed head injury reliably elicits diffuse axonal injury. Recent findings have demonstrated the complex nature of this pathology, revealing a multiphasic sequence of perturbations to axolemmal permeability, and to the structure and function of the axonal cytoskeleton and mitochondria. Evidence suggests subpopulations of axons may respond differently to injury, e.g., axonal swelling and neurofilament disruption do not always co-localize. Fiber size and degree of myelination are structural properties that may determine, in part, the specific injury response(s) of an axon. This study measured compound action potentials (CAPs) evoked through the corpus callosum fibers in brain slices from adult rats at 1 and 3 days following central fluid percussion injury (FPI), modifying a method of Baker et al. (2000). The biphasic CAP waveform is comprised of an early waveform component (generated largely by 'fast' myelinated fibers) and a later component (largely 'slow' unmyelinated fibers). Recording at 23 degrees C increased the response latencies enabling reliable quantifications of the fast wave component, which was partially embedded in the stimulus artifact when recording at 36 degrees C. Injury effects at 3 days postinjury were not significantly different from 1 day effects. FPI reduced the maximal amplitude of the fast wave by an average of 33%, compared to sham rats, but the slow wave amplitude was reduced by 73%. The mean duration of the fast wave was increased by 34%, whereas durations for the slow wave were not altered by injury. These results suggest these two subpopulations of fibers were differentially recruited into the injury process, possibly due to distinct structural properties. Supported by NS20193.

P458.

REGIONAL SPECIFIC ALTERATIONS IN NERVE GROWTH FACTOR (NGF) & NEUROTROPHIN-4/5 (NT-4/5) AFTER TRAUMATIC BRAIN INJURY IN RATS.

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Neurotrophins are required for the development, maintenance and regeneration of the central nervous system (CNS). These properties have stimulated an interest in these molecules as potential therapeutic agents for the treatment of brain injuries and neurodegenerative diseases. We evaluated the regional and temporal alterations in protein levels of NGF and NT-4/5, after moderate lateral fluid percussion traumatic brain injury (TBI) in anesthetized rats using an ELISA procedure, 3h, 24h, 72h and 1 week after surgery in the ipsilateral cortex and hippocampus of naive, sham and injured animals (n = 5–6 per time point and per group). NGF levels were significantly increased in the ipsilateral cortex compared to sham animals (comparison with a two-way ANOVA, injury effect $P = 0.0051$). No significant changes were observed in NGF levels in the hippocampus. NT-4/5 levels were significantly increased at 24h postinjury in the ipsilateral cortex compared to sham animals (mean ± S.E.M.:1.8 ± 0.3 versus 0.9 ± 0.2 pg/mg of protein, $P < 0.05$) and 72h (2.3 ± 0.5 versus 0.7 ± 0.0 pg/mg of protein, $P < 0.001$) and had returned to baseline by 7 days post-injury. NT-4/5 was also significantly increased in the ipsilateral hippocampus of injured animals compared to sham animals (comparison with a two-way ANOVA, injury effect $P = 0.0125$).

These studies suggest that region-specific alterations occur in NGF and NT-4/5 in the acute period following TBI. As NGF has been shown to be neuroprotective in this model when administered during the first two weeks following TBI, we hypothesize that alterations in NGF levels following TBI may play an endogenous neuroprotective role. Whether this is true for NT-4/5 remains to be determined.

These studies were supported , in part, by NIH NS 40978, NS 08803, GM 34690 and a Veterans Administration-DOD Consortium Merit Review grant.

P459.

NON-INVASIVE ASSESSMENT OF ICP FROM CEREBRAL BLOOD FLOW VELOCITY AND ARTERIAL BLOOD PRESSURE USING A FUZZY PATTERN CLASSIFICATION METHOD

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Objects: The authors previously introduced a method for a non-invasive assessment of I intracranial pressure (ICP, nICP). The underlying mathematical model established a linear relationship between certain hemodynamic parameters (TCD characteristics) and the quotient between mean ICP and arterial blood pressure (ICP/ABP). Some results suggested that this relationship might not be globally valid but might be influenced by additional parameters like, e.g., the patient's type of disease, the arterial CO2 pressure and the state of cerebral autoregulation (CA). In the current approach the former globally expressed relationship between TCD characteristics and the ICP/ABP ratio was specifically modified to certain subgroups of patients in order to adapt the model to individual cases. Methods: In 113 traumatic brain injured patients (3–76 years of age, mean age: 31 ± 16 years) signal data of cerebral blood flow velocity (FV), ABP and ICP was studied. TCD characteristics, calculated at several time points from FV and ABP recordings, together with time corresponding rations ICP / ABP ratios were sampled. CA was assessed by correlation of cerebral perfusion pressure and FV. A method called Fuzzy Pattern Classification was used to identify substructures (classes) in the samples of TCD characteristics. On each of these classes a specific relationship between TCD characteristics and ICP/ABP ratios was established. This construction facilitated the calculation of nICP as follows: Using FV and ABP the TCD characteristics were computed and related to the matching class(es). The estimator of ICP/ABP was calculated and multiplied by ABP resulting in nICP. Results: Median error between ICP and nICP was 6.9 mm Hg in patients with impaired CA (N = 66) and 4.5 mm Hg in patients with preserved CA (N = 56). Plateau waves, B waves and long-term trends of ICP could be visibly assessed. Conclusions: The class structure of facilitates nICP assessment in heterogeneous patient groups. Moreover, its modular structure enables a stepwise extension of the target patient group, without affecting the current validity. The results encourage further investigations of Fuzzy Pattern classification method in view of nICP assessment.

P460.

EARLY ONSET OF OXIDATIVE STRESS IN HUMAN TRAUMATIC BRAIN INJURY MAY BE RESPONSIBLE FOR FAILURES OF FREE-RADICAL SCAVENGER PHARMACOLOGICAL THERAPIES

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On the basis of the contradiction between data on experimental head trauma showing oxidative stress-mediated cerebral tissue damage and failure of clinical trials using free radical scavenger drugs, we monitored the time-course changes of malondialdehyde (MDA), ascorbate and dephosphorylated ATP catabolites in cerebrospinal fluid (CSF) of traumatic brain-injured (TBI) comatose patients suffering from severe TBI (Glasgow Coma Scale on admission of 6 ± 1). First CSF sample was collected within 2.95 hours from trauma (SD = 1.98) and during the next 48 hours once every 6 hours. All samples were analyzed by an ion-pairing HPLC method for the simultaneous determination of MDA, ascorbic acid, oxypurines and nucleosides. In comparison with values recorded in 10 herniated lumbar disk, non-cerebral control patients, CSF of TBI patients had high values (0.226 micromol/l; SD = 0.196) of MDA (undetectable in samples of control patients) and decreased ascorbate levels (96.25 micromol/l; SD = 31.74), already at the time of first withdrawal. MDA was almost constant in the next two withdrawals and tended to decrease thereafter, albeit after 48 hours from hospital admission (0.072 micromol/l; SD = 0.026) were still recorded. Ascorbate was normalized 42 hours after patient hospital admission. Evident changes in CSF values of ATP degradation products suggested neuronal energy metabolism derangement following TBI. These data demonstrate the early onset of oxidative stress in TBI patients, propose a valid explanation for the failure of clinical trials based on oxygen radical scavenger drug administration and suggest a possible rationale for testing the efficacy of lipid peroxidation "chain breakers" in future clinical trials.

P461.

RAPID UPREGULATION OF PHOSPHORYLATED-ERK SUGGESTS A ROLE FOR THE MITOGEN ACTIVATED PROTEIN KINASE PATHWAY IN TRAUMATIC BRAIN INJURY

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The mitogen activated protein kinases (MAPKs) cascades are well known membrane-to-nucleus signaling modules that have recently been implicated as mediators of cellular injury after cerebral ischemia and trauma. In this study, we investigated the involvement of the MAP kinase Erk, and the activated, phosphorylated form of Erk (p-Erk) in our controlled cortical contusion model of traumatic brain injury (TBI) in rats. Quantification of Erk and p-Erk in the contused cerebral cortex was made by western blot 10 min and 24 h after severe trauma. There was a strong increase in p-Erk 10 min after the injury. At 24 h after trauma, there was a marked accumulation of aggregated p-Erk in the lesioned tissue. The cellular identities of p-Erk expressing cells were studied by immunofluorescence double staining 24 h after trauma in formalin fixed frozen sections. At this time, numerous GFAP positive cells were double labeled with p-Erk, suggesting expressing astrocytes. Fewer cells were p-Erk expressing NeuN positive neurons. To investigate the potential connection between MEK inhibition and reactive oxygen species in this injury pathway we included animals treated with the MEK inhibitor U0126 and the free radical scavenger S-PBN, both with neuroprotective properties in TBI. Overall, the results implicate a significant role of the mitogen activated protein kinase Erk in the secondary injury cascade after traumatic brain injury.

P462.

EFFECT OF DURATION OF HYPOTHERMIA FOLLOWING CONTROLLED CORTICAL IMPACT IN IMMATURE RATS

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Rationale: Moderate hypothermia (HYPO) following experimental traumatic brain injury (TBI) has been shown to improve behavioral outcome in both adult and immature rats. Studies specifically addressing the effect of timing and duration of hypothermia on its efficacy in immature rats are lacking. The goal of this project was to begin to investigate the optimal timing and duration of therapeutic HYPO following TBI in immature rats.

Methods: Sprague-Dawley (postnatal day [PND] 7) rats were randomized to moderate HYPO 32-33OC applied for 1 h (target temperature achieved at time of injury) or for 4 h (delay to initiation of cooling for 15 min following CCI) vs. normothermia (NORM) 37°C (n = 10/treatment arm) and then injured using controlled cortical impact (CCI) (left, frontoparietal, 3mm tip, 4 m/s, 1.75 mm deflection). To test functional outcome following injury and treatment, the Morris water maze (MWM) paradigm was used on post injury days (PID) 11–17.

Results: Following CCI, treatment with HYPO for 1 h or 4 h significantly improved MWM performance as compared to NORM ($p < 0.05$). HYPO for 4 h additionally tended to improve MWM performance as compared to HYPO applied for 1 h though this difference was not statistically significant.

Conclusions: Moderate HYPO applied for 1 or 4 h after CCI in immature rats improved MWM performance as compared to NORM. In addition, HYPO applied for 4 h was still effective in improving functional outcome despite a 15 min delay in initiation following injury. The optimal timing and duration of HYPO after TBI in the immature rat needs to be further defined.

(Supported by NIH [NS01809, NS40049] and the Copeland Fund/Pittsburgh Foundation [PDA])

P463.

DELAYED TREATMENT WITH ANIRACETAM IMPROVES COGNITIVE RECOVERY AFTER TRAUMATIC BRAIN INJURY IN RATS

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In contrast to the acute, excitotoxic processes that dominate immediately after traumatic brain injury (TBI), research into the chronic post-traumatic alterations of neuronal processes supports a suppressed or hypofunctional neuronal state. If a reduced level of neuronal activity is a long-term consequence of TBI that contributes to persistent impairment of function, then the chronic, post-injury enhancement of neuronal activity should improve recovery after TBI. The purpose of the present experiment was to test the effectiveness of aniracetam in reducing the cognitive deficits produced by TBI. Aniracetam acts through the allosteric potentiation of AMPA-specific glutamate receptors. The consequences of this drug are a reduction of glutamate receptor desensitization and potentiation of metabotropic glutamate activity. Beginning 24 hr after midline fluid-percussion injury, either 25 (n = 9) or 50 mg/kg (n = 9) (p.o.) of aniracetam was administered daily for 15 days. On days 11–15 after TBI (n = 9) or sham injury (n = 10), rats were tested in the Morris water maze (MWM), and the latency to reach the goal platform was recorded. Results indicated that, compared to injured-untreated rats, both the 25 and 50 mg/kg doses of aniracetam significantly improved MWM performance ($p < 0.05$). In fact, the MWM performance of injured, aniracetam-treated rats did not differ significantly from sham-injured rats. These results demonstrate the efficacy of using a positive modulator of AMPA receptor function as a delayed treatment for the cognitive impairment produced by TBI. These data also support the hypothesis that a depression in neuronal activity contributes to the chronic deficits produced by TBI. Supported by the Commonwealth Neurotrauma Initiative Fund.

P464.

CASPASE INHIBITION AFTER TRAUMATIC BRAIN INJURY ALTERS AMYLOID PRECURSOR PROTEIN AND AMYLOID-BETA PRODUCTION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Traumatic brain injury (TBI) is a risk factor for Alzheimer's disease (AD). Brains from head-injured patients have frequently shown AD-specific pathological changes, including overproduction of amyloid precursor protein (APP) and amyloid-beta (A-beta). Although the mechanism for this acute upregulation of amyloid in TBI is unknown, several in vitro studies have suggested that caspases, which are known to be activated after TBI, may be involved in APP processing. We examined the effects of caspase inhibition on hippocampal production and processing of APP at 24 and 48 hours after TBI in "humanized A-beta" mice. These gene-targeted animals contain the APP Swedish mutations and have had their A-beta sequence changed from rodent to human, allowing detection of human A-beta production in mouse brain. We found that hippocampal APP expression was altered at 24 and 48 hours after weight-drop TBI. Production of A-beta increased after TBI, although no A-beta deposits were detected. Immediate post-injury administration of a single i.p. dose of 100 nM BAF, a pan caspase inhibitor, reduced A-beta production in injured brain compared to sham and vehicle-treated mice. These data imply that APP processing acutely after TBI results in production of amyloidogenic fragments, and that a mechanism for this altered processing may be caspase-dependent. Thus, the neuroprotective role of caspase inhibition may be through decreased production of neurotoxic A-beta as well as through inhibition of apoptosis. Supported by AG05133 and NS30318.

P465.

ATTENUATION OF OXIDATIVE STRESS AFTER ACUTE BROMOCRIPTINE TREATMENT IN TRAUMATICALLY BRAIN INJURED RATS

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Oxidative stress has been reported to be an important contributor to the secondary sequelae of traumatic brain injury(TBI). Therefore, pharmacotherapies that display antioxidant properties, such as the dopamine receptor (D2) agonist bromocriptine (BRO) may benefit outcome. We have recently reported that both acute and chronic BRO attenuates posttraumatic functional deficits (Massucci et al., 2001; Kline et al., 2002). In this study we examined the effects of acute BRO treatment on TBI-induced oxidative stress. Thirty-six isoflurane-anesthetized rats received BRO (5 mg/kg, i.p., Injury/BRO = 12, Sham/BRO = 6) or vehicle (Injury/VEH = 12, Sham/VEH = 6) 15 min prior to controlled cortical impact (2.7 mm impact at 4 m/s) or sham injury. At 1-hr post-surgery, rats were sacrificed and changes in lipid peroxidation, a major indicator of oxidative stress, was measured in the frontal cortex, striatum, and substantia nigra using thiobarbituric acid reactive substances (TBARS) assay. The data are expressed as nmol malondialdehyde per mg/tissue ± SEM. TBARS was increased in all regions examined in the Injury/VEH group vs. shams. In contrast, no differences were observed between the Injury/BRO and sham groups. A trend toward decreased TBARS was observed in the frontal cortex of the Injury/BRO vs. Injury/VEH groups (5.44 ± 0.44vs. 6.96 ± 0.77, $p = 0.06$). Significant decreases in TBARS were observed in both the striatum (4.22 ± 0.52 vs. 5.60 ± 0.44) and substantia nigra (4.18 ± 0.35 vs. 7.76 ± 2.05) of the Injury/BRO vs. Injury/VEH groups, respectively. These findings suggest that TBI-induced oxidative stress in the striatum and substantia nigra is attenuated by acute BRO treatment, which may explain the functional benefit previously reported by our group. (Supported by NIH NS33150 and NS40125).

P466.

INCREASED EXPRESSION OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) IN RAT BRAIN AFTER TRAUMATIC BRAIN INJURY

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Glial cell line-derived neurotrophic factor (GDNF), a member of the TGF-beta superfamily, plays important roles not only for the differentiation of neurons during normal development but also for the survival and recovery of many populations of mature neurons. GDNF is a potent and relatively specific neurotrophic factor for dopaminergic neurons. It has been reported that GDNF has protective effects on various injuries for central and peripheral nervous systems in vitro and in vivo. However, the effect of traumatic brain injury (TBI) on the expression of GDNF is currently unknown. To determine if there is alteration in GDNF after TBI, we examined the effect of controlled cortical impact (CCI) injury on GDNF protein levels at 1 day and 7 days following injury by utilizing a commercially available antibody specific to GDNF. Rats were anesthetized and surgically prepared for CCI injury (4 m/sec, 2.7 mm) and sham surgery. Injured and sham animals (N = 4 per group) were sacrificed at 1 day and 7 days, respectively, and perfused with 4% paraformaldehyde. Coronal sections (35 mm thick) were cut through the hippocampus. An increased expression of GDNF protein was observed by immunohistochemistry in the hippocampus and the cortex in injured rats compared to sham controls. The increased expression of GDNF is more evidently observed in the ipsilateral hippocampus and the area around the contusion in the cortex. In the cortex, GDNF immunreactivity appeared greatest in cells with glial morphology. However, in the hippocampus, GDNF immunoreactivity was greatest in neuronal-like cells. These changes were observed at both 1 and 7 days postinjury. We speculate that the up-regulation of the GDNF protein may reflect its neurotrophic and neuroprotective effect on dopaminergic system response to the TBI insult.

(Supported by grants: NIH-NS33150, NIH-NS30318, NIH-NS312296).

P467.

EXPLORATORY STUDY OF ACUPUNCTURE TREATMENT ON TRAUMATIC BRAIN INJURY (TBI) IN RATS

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Traumatic brain injury (TBI) is the leading cause of death and injury-related disability among young adults, making it one of the most tragic and prevalent of all neurological disorders. Acupuncture is a component of the health care system of China that can be traced back for at least 2,500 years. The effectiveness of acupuncture has been scientifically investigated in both human clinical trials and animal studies for the past 30 years. Since acupuncture is known to possess many effects, such as analgesia, promotion of homeostasis, and changes in the microcirculatory network as well as improvements in brain circulation, we believe that it is a logical to hypothesize that acupuncture treatment can reduce the pathological changes and will accelerate recovery of function following TBI in rat. To test this hypothesis, anesthetized male Sprague-Dawley rats were subjected to a controlled cortical impact (CCI) injury of moderate severity (4 m/sec, 2.7 mm deformation) and randomized into acupuncture treatment (N = 8) and control group (N = 8). Motor functions (beam balance and beam working) were evaluated on post-operative days 1–5. Rats were sacrificed at 28 days after TBI. Electroacupuncture (EA) treatment (2 Hz, 20 minutes of each, 2 hours apart) on bilateral Zusanli (St 36) starting at 1 hour after TBI for six days significantly reduces the motor deficit after TBI compared to control group ($P < 0.05$). EA treatment for 21 days significantly reduced the contusion (lesion) volume and the hemispheric tissue loss after TBI compared to the control group ($P < 0.05$). The data demonstrate the beneficial effect of post-injury acupuncture treatment on motor function and histopathological deficits caused by TBI in rats. The potential for acupuncture is just beginning to be understood. Thus, further studies of acupuncture to find the optimal therapeutic parameters and its mechanisms are warranted. (Supported by grant NIH-NS40125).

P468.

CHRONIC IMPAIRMENT OF EXTRACELLULAR K+ HOMEOSTASIS FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT.

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We have previously shown that, acutely following fluid percussion injury (FPI), rat hippocampal astrocytes are reactive, have decreased membrane potassium conductance that results in impaired extracellular K+ homeostasis which, in turn, contributes to abnormal neuronal excitability (1). However, it is not known how such acute impairment progresses overtime following injury. We have now assessed the efficiency of extracellular K+-homeostasis in rat hippocampal slices at subacute and chronic time points following moderate midline FPI. Moderate (3.5atm) midline FPI was induced. Hippocampal slices were obtained two days, two weeks or one month following FPI or sham operation. K+-selective microelectrodes were employed to measure K+ accumulation and evoked field potentials in CA3 stratum pyramidale during antidromic Schaffer collateral stimulation at 0.05Hz. We found that, during stimulation, baseline [K+]o was elevated at two days, two weeks and one month after injury. [K+]o was higher by 0.4 ± 0.04mM two days post-FPI (mean ± SD, n = 9; $p < 0.01$), and by 0.3 ± 0.04 mM two weeks post-FPI (n = 9; $p < 0.01$), and by 0.25 ± 0.02 mM one month post-FPI (n = 13; $p < 0.01$), in respect to K+ levels measured in similar manner in slices obtained from age-matched sham operated rats two days, two weeks or one month after surgery (n = 12, 6 and 8, respectively). We conclude that impaired extracellular K+ homeostasis persists at chronic time points following TBI, therefore contributing to chronic tissue hyperexcitability and to the likelihood of transition from interictal to ictal activity (2).

Supported by NIH, NS 40823 (RD).

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P469.

THE mGluR1 ANTAGONIST AIDA REDUCES POST-TRAUMATIC EMPTYING OF CALCIUM STORES IN NEURONS AND ASTROCYTES

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Using a cell culture model of strain (stretch) injury, we examined the effects of the mGluR1 antagonist (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA) on intracellular Ca2+ stores in astrocytes and neurons using fura-2. We have previously shown that AIDA blocks post-traumatic increases in astrocyte IP3, which can stimulate Ca2+ release from Ca2+ stores (Floyd et al., J. Neurotrauma 16: 961, 1999). We have also reported that in both astrocytes (Rzigalinski et al., J. Neurochem 70: 2377, 1998) and neurons (Weber et al., Cell Calcium 26: 289, 1999) elevation of [Ca2+]i by thapsigargin, which inhibits the sarcoplasmic-endoplasmic reticulum Ca2+-ATPase and allows release of Ca2+ stores, is abolished 15 min post-injury. This implies injury-induced depletion of Ca2+ stores. In the current study both pre- and immediate post-injury treatment with AIDA reduced depletion of Ca2+ stores 15 min post-injury in astrocytes and neurons, suggesting store depletion via activation of mGluR1.

As previously reported, in injured neurons the initially abolished [Ca2+]i increase by thapsigargin returns with time and is potentiated at 3 hr post-injury (Weber et al., J. Biol. Chem. 276: 1800, 2001). Using Ca2+-free medium has shown that the size of the neuronal Ca2+ stores is normal at 3 hr and that the enhanced response is due to extracellular Ca2+. The enhancement is also partially blocked by a store-operated channel inhibitor, SKF96365. In the current studies we found the enhanced neuronal response to thapsigargin at 3 hr was partially reduced by pre- or post-injury treatment with AIDA. In summary, our current findings implicate mGluR1 receptors in the initial post-traumatic depletion of astrocyte and neuronal Ca2+ stores and the delayed, potentiated capacitative Ca2+ influx in injured neurons. These findings provide insight as to the subcellular mechanisms by which AIDA produces its beneficial effect in in vivo and in vitro experimental trauma.

P470.

DIFFERENTIAL EFFECTS OF ACUTE AND CHRONIC EXERCISE ON PLASTICITY-RELATED GENES IN THE RAT HIPPOCAMPUS REVEALED BY MICROARRAY

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Exercise has a healing potential in the injured brain, but the lack of knowledge on the molecular mechanisms involved has hampered the implementation of exercise as therapeutical tool. A standing question for planning therapeutical applications is whether exercise provided for a short period of time can have the same benefit as long-term exercise. Hippocampal RNA from rats exposed to a running wheel for 3, 7, and 28 days were examined using a microarray with 1,176 cDNAs (Clontech). The expression of selected genes was quantified by Taqman RT-PCR or RNase protection assay. The largest upregulation was in genes involved with synaptic trafficking (synapsin I, synaptotagmin, and syntaxin), signal transduction pathways (Ca2+/calmodulin-dependent protein kinase II, CaM-KII; mitogen-activated/extracellular signal-regulated protein kinase, MAP-K/ERK I and II; protein kinase C, PKC-d), or transcription regulators (cyclic AMP response element binding protein, CREB). Genes associated with the glutamatergic system were upregulated (N-methyl-D-aspartate receptor: NMDAR-2A, NMDAR-2B, and excitatory amino acid carrier 1: EAAC1), while genes related to the GABA system were downregulated (GABAA receptor, glutamate decarboxylase GAD65). The temporal profile of gene expression seems to delineate a mechanism by which specific molecular pathways are activated along exercise performance. For example, brain-derived neurotrophic factor (BDNF) was the only trophic factor whose gene was consistently upregulated at all timepoints. These results, together with the fact that most of the genes upregulated have a recognized interaction with BDNF, suggest a central role for BDNF on the effects of exercise on brain plasticity. (Supported by NIH awards NS38978, NS39522, Alzheimer's Association, and UCLA Brain Inj. Res. Ctr.).

P471.

THE USAGE EFFICIACY OF PROLONGED VENTRICULAR DRAINAGE AND APRICOT JUICE ON MANAGEMENT OF CNS INJURY

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The efficacy of using of prolonged ventricular drainage and apricot juice three groups (with 21 patients on each) were almost identical, in mince of clinical- has been studied on 63 patients (divided in to three groups), with hard CNS injury. All neurological characteristic and age-gender attitude.

The patients of first group took usual traditional treatment, where to the patients of second group in addition to the traditional treatment, was mounted the prolonged ventricular drainage through laid fraise hole on Dandy drainage, which promote dosage effusion of liquor from stomach systems of brain in to the special container. The patients of third group, besides the traditional treatment and prolonged ventricular drainage, also took the natural apricot juice, the 80–100 ml/5–6 times a day, (using the stomach probe until the patient regains its consciousness), containing calium and diuretic efficacy.

The evaluation the effect of realized treatment was occurred by calculatinghe dynamics of clinical manifestations, sanation of liquor, the period of being at hospital department, mortality. Issue of traumas evaluated on Glasgow scale.

The positive neurological dynamics seen in first group for 11–12 days, in second and in third groups accordingly 9–10 and 8–9 days. The sanation of liquor composed accordingly—for 17–18, 12–13 and 10–11 days. Lasted period of being in hospital composed accordingly: 24.8, 22.9, and 21.3 days. The received trauma results improvement, on Glasgow scale were seen in 7 patients of first group, and 8 and 9 in second and third groups accordingly, moderate invalidization accordingly composed—7, 8, 8; hard invalidization accordingly—3, 2, 1; vegetative condition—1 patient on each; and mortality composed—4, 2, 2 accordingly.

Therefore, using the prolonged ventricular drainage and apricot juice in complex treatment of CNS injury promotes improving the results of treatment, decreasing the invalidization and mortality of patients.

P472.

A HIGH-FAT SUCROSE DIET (HFS) EXACERBATED TRAUMATIC BRAIN INJURY (TBI) -INDUCED IMPAIRMENTS IN COGNITION AND NEURONAL PLASTICITY

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Traumatic brain injury (TBI) results in long-lasting functional impairments in cognitive function, but the molecular mechanisms remain unknown. Although TBI patients have elevated susceptibility to subsequent insults, the effects of nutritional factors on neural healing following TBI have not been experimentally scrutinized. We have recently reported that a HFS diet decreases brain-derived neurotrophic factor (BDNF) and its downstream effectors in the hippocampus, resulting in impairments in neuroplasticity and cognition. Based on the roles of BDNF on neuroprotection and excitability, we have examined the neuroplasticity after TBI and the possibility that a HFS diet may reduce the capacity of the brain to react to injury by affecting BDNF-related neuroplasticity. Male Sprague-Dawley rats were maintained on HFS diet or a low-fat, complex-carbohydrate (LFCC) for 4 weeks before a fluid percussion injury (FPI) or sham surgery were performed. All rats were killed after one week. The mRNA levels of BDNF, synapsin I, and cyclic AMP-response element-binding protein (CREB) were determined in the hippocampus by Real-time quantitative RT-PCR. The cognitive function was assessed after surgery using a water maze. Results showed that (1) FPI decreased BDNF mRNA level in HFS-fed rats, but not in LFCC-fed rats; (2) FPI decreased synapsin I and CREB mRNA levels in both LFCC and HFS-fed rats with a stronger effect in HFS-fed rats; (3) FPI impaired cognitive function in both LFCC- and HFS-fed rats with a worse outcome in HFS-fed rats. Results showing that TBI induced impairments in cognition and neuroplasticity are exacerbated by HFS suggest that HFS decrease the capacity of the brain to compensate for traumatic injury. (Supported by NS38978, NS39522, Alzheimer's Association, and UCLA Brain Injury Research Center).

P473.

IS MINOCYCLINE REGULATING GLUTAMATE TOXICITY AFTER TRAUMATIC BRAIN INJURY?

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Objective: Former experiments proved a positive effect of minocycline after ischemia that led to reduced infarct sizes. We were interested if this holds also true after tbi and what possible mechanisms underlying a potential beneficial effect of minocycline after trauma.

Model: Severe tbi was induced in male Wistar rats using the controlled cortical impact device introduced by Dixon et al. with a velocity of 7 m/sec an da depth of 2 mm.12 h and 24 h after tbi minocycline was applied in a dosage of 90 mg/kg/bw followed by 45 mg/kg/bw twice daily for 1–4 days. Animals were then sacrificed and the brains processed for DNA-array analysis. Control animals received saline instead. Animals without surgery served as absolute controls.

Results: The DNA arrays demonstrated that 72 h and 96 h after tbi animals treated with minocycline had different expressions of the brachend chain amiotransferase, which plays an important role in glutamate metabolism.

Discussion: Former experiments using minocycline after ischemia demonstrated beneficial effects on infarct volume. Our group and other also showed a significantly lower number of apoptotic neurons after tbi when using minocycline. It is known that minocycline has a direct effect on interleukine-B1-converting enzyme, that plays a role in apoptosis. Our results showed that minocyline may act beneficial after tbi by influencing the glutamate pathways.

P474.

ALZHEIMER'S DISEASE PATHOLOGY IN SURVIVORS OF SEVERE BRAIN INJURY

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Extracellular amyloid-beta (A-beta) plaques and neurofibrillary tangles (NFT) are the major pathological hallmarks of Alzheimer's disease (AD). In a subset of individuals who died from traumatic brain injury (TBI), postmortem brain examination shows Ab deposits similar to AD plaques. The present study determined the extent and dynamics of AD-related changes in cerebral cortex from 18 people (age range 18 to 64 years) who survived severe (Glasgow Coma Score, GCS<9) TBI. Most of these subjects underwent surgical treatment within 16 hours post-injury; two subjects had longer TBI-to-surgery intervals (58 and 74 hours). Samples of surgically removed temporal cortex were processed for immunohistochemistry using antibodies against A-beta (total, 1–40, 1–42), and markers of neuronal degeneration (tau, ubiquitin, synuclein alpha, and synuclein alpha beta gamma). Cortical A-beta deposits were found in one third of examined subjects (age range 35 to 62 years), as early as 2 hours after injury. Plaque-positive and plaque-negative cases had similar age, GCS, and TBI-to-surgery intervals; notably, the two subjects with longer post-injury intervals were plaque-free. A-beta 42 plaques were more abundant than A-beta 40. In addition to A-beta deposits, initial neurodegenerative changes were frequently evident after severe TBI. Most of the cases had ubiquitin- and synuclein-positive neurons. Although a number of subjects showed diffuse tau-positive neuronal staining, NFT-like changes were found only in two cases who were of advanced age and without A-beta deposits. Our results demonstrate that after TBI, A-beta plaques and early neurodegenerative changes can develop rapidly in-vivo, while development of NFT might require more chronic process. Supported by AG5133 and NS30318.

P475.

ASSESSING COGNITIVE AND PSYCHOLOGICAL PATTERNS IN POST-TRAUMATIC HEADACHE FOLLOWING SEVERE BRAIN INJURY.

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Post-traumatic headache (PTH) is often reported following minor and moderate head injury. Sex, repeated head injury and skull fracture are the most relevant predictive features in epidemiological studies. PTH occurs more frequently after minor head injury than after severe brain-injury and in patients with impaired cognitive functions in some studies, or in less cognitively impaired patients in others. Therefore, data on this issue are not conclusive. We evaluated the incidence of PTH after very severe traumatic brain-injury and assessed cognitive and psychological features in the same population.

We examined the headache occurrence in 500 patients suffering from very severe traumatic brain injury (Glasgow Coma Scale (GCS) < 8 and coma duration of at least 2 weeks), consecutively admitted to Santa Lucia Hospital (year 1990 to 2001). Clinical features of head trauma and presence of neuropsychological disorders were investigated. Some patients suffering from PTH of migraine type were studied by means of transcranial doppler (TCD), and compared with patients suffering from idiopathic migraine.

The incidence of headache was reported in about 10% of patients, skull fracture or craniotomy, post-traumatic epilepsy and a good recovery of the cognitive functions being the most frequent features associated with the presence of headache. Tension-type headache was the commonest in these patients, whereas migraine occurred in the minority of the patients. All patients were affected by anxiety or depression.

The low frequency of headache following severe traumatic brain-injury may be secondary to the diffuse impairment of cerebral structures with a pivotal role in the affective component of pain. In fact, only patients with good cognitive recovery may develop PTH. The presence of anxiety and depression is possibly related to the awareness of disabilities due to trauma. Therefore, PTH may be considered as an adaptative disorder in which both altered biological and psychological features play a pathophysiological role.

P476.

COMBINED MUSCARINIC AND NMDA RECEPTOR ANTAGONISM REDUCES HYPERGLYCEMIC EXACERBATION OF POST-TRAUMATIC CEREBRAL ISCHEMIC HYPERSENSITIVITY

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Using a model of mild concussive TBI with secondary ischemia we have demonstrated posttraumatic brain ischemic hypersensitivity. Under our experimental conditions, antecedent mild TBI changes the pathology of a 6 minute forebrain ischemic insult into the pathology of a 10 minute forebrain ischemic insult. Posttraumatic cerebral ischemia often occurs during a time of high serum catecholamines and hyperglycemia which exacerbates the response of the brain to primary or secondary cerebral ischemia. Under fasted conditions, mild TBI and secondary ischemia (T+I) produces selective CA1 hippocampal neuronal death and cognitive deficits, but under hyperglycemic conditions (serum glucose >=400 mg%), results in secondary seizures and widespread neuronal loss. Pretreatment with combined scopolamine and MK-801 reduces posttraumatic ischemic sensitivity in this model under fasted conditions and the purpose of the present study was to determine if comparable receptor blockade was also effective with hyperglycemic T+I.

Using a Wistar rat model of mild fluid percussion TBI with 6 min of imposed forebrain ischemia, we examined if the increased brain damage occurring with posttraumatic but preischemic serum hyperglycemia of >=400 mg% could be reduced with combined 1 mg/kg sccopolamine and 0.1mg/kg MK-801 antagonism given before the insults. Two groups of fasted rats (N = 4/group) were given 6 min forebrain ischemia preceded by mild TBI 1 hr before. Thirty minutes before ischemia, rats received either i.p. glucose solution or saline. Hyperglycemic rats with T+I developed status epilepticus within 24 hr after combined injury with extensive intra- and extra-hippocampal damage. However, scopolamine and MK-801 pretreated hyperglycemic rats with T+I had a reduction in postischemic seizures and histopathological damage. These data suggest that TBI increases the sensitivity of the brain to hyperglycemic ischemia in part by excitatory neurotransmitter cascades. Supported by NS35365.

P477.

BRAIN TISSUE PO2, INTRACRANIAL PRESSURE, ADENOSINE AND PURINE DEGRADATION PRODUCTS AFTER SEVERE HEAD INJURY IN ADULTS: A PRELIMINARY ANALYSIS

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The usage of brain tissue oxygenation (PbtO2) as an invasive monitoring tool in severe traumatic brain injury (TBI) has increased. Although ischemic threshold values have been determined for survival1, correlations to physiological parameters have been limited2,3, and the relationship between PbtO2 and purine degradation products (PDP) remain unclear. Continuous PbtO2 monitoring was performed in adults (n = 6) with severe TBI (GCS<9), utilizing Licox probes placed within the non-lesioned white matter of the frontal lobe. Ventricular cerebrospinal fluid (CSF) samples (n = 97) were obtained every 4h for the first 24h and then every 6h for 4d. CSF adenosine, inosine, hypoxanthine, xanthine, lactate, and lactate/pyruvate ratio (L/P) were quantified by HPLC, and correlated to physiological parameters [mean arterial pressure (MAP), end-tidal CO2 (ETCO2), intracranial pressure (ICP), cerebral perfusion pressure (CPP), and rectal temperature (TEMP)] at the time of the CSF sampling. Mean PbtO2 was 32.7 ± 16.6 mmHg for all patients, significantly correlating with ICP ($r = -.514$, $p = 0.001$), ETCO2 ($r = .331$, $p = 0.02$), and TEMP ($r = .266$, $p = 0.015$). PbtO2 also correlated to PDP, xanthine ($r = -.36$, $p = 0.001$), and indicators of anaerobic metabolism, lactate ($r = -.344$, $p = 0.001$), and L/P ($r = -.247$, $p = 0.022$). Two patients exhibiting profound reductions in PbtO2 (<10 mmHg), had dramatic concurrent increases in adenosine and hypoxanthine. Our data support the hypothesis that critical reductions in PbtO2 are accompanied by energy failure, as reflected by concomitant increases in adenosine and PDP. 1Crit Care Med 26(9):1576, 1998; 2Acta Neurochir 71:153, 1998; 3Neurosurg Rev 23(2):94, 2000. Support: NS 38087 and NS 30318.

P478.

MULTICENTER STUDY OF CONTINUOUS VS INTERMITTENT CEREBROSPINAL FLUID DRAINAGE AFTER SEVERE TRAUMATIC BRAIN INJURY IN CHILDREN: EFFECT ON BIOCHEMICAL MARKERS

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Cerebrospinal fluid (CSF) drainage is routinely used in the treatment of severe traumatic brain injury (TBI)—either continuously or intermittently in response to increases in intracranial pres-sure (ICP). We have previously reported that levels of a variety of markers of injury or repair are increased in CSF after severe TBI (1). We hypothesized that these markers would be reduced in CSF drained continuously vs intermittently. We compared CSF levels of markers of neuronal injury (neuron specific enolase, [NSE]), glial injury (S-100B), inflammation (interleukin-6 [IL-6]), and regeneration (vascular endothelial growth factor [VEGF]) (ELISA) in 19 severely in-jured children whose CSF was drained continuously (n = 13) vs intermittently (n = 6) as part of standard care in two institutions. Mean level of each marker was nearly two-fold lower in CSF drained continuously vs intermittently, though only statistically significant ($p < 0.05$) for NSE. When controlled for all confounding variables, however, the difference between drainage methods was significant for all four markers. S-100B and VEGF were inversely associated with time post-trauma. Child abuse as a mechanism of injury was directly associated with level of NSE, and inversely associated with level of IL-6. We conclude that the method of CSF drainage greatly affects levels of CSF markers after TBI. The marked reduction in CSF levels of NSE in patients receiving continuous CSF drainage suggests the possibility that this method of drainage may more effectively reduce neuronal death. Alternatively, the difference may reflect increased clearance of CSF biochemical substances with continuous drainage. Correlation of CSF markers with physiologic (ICP) and clinical outcome is required.

(1) Kochanek et al, Ped Crit Care Med, 2000.

SUPPORT: HD40686, NS30318, NS38087, U. of Pgh CIRCL/CDC, Eric Bundy Memorial Fund, Medic First Aid Foundation.

P479.

DOWN REGULATION OF AQUAPORIN-4 IN AREA ADJACENT TO BRAIN INJURY IN A TRAUMATIC RAT BRAIN MODEL.

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Aquaporin-4 (AQP4) plays a significant role in the regulation of brain water homeostasis. Several studies have shown that this molecule is up-regulated following non-traumatic brain injury. This study investigated the regulation of AQP4 following a focal cortical contusion injury in the rat. Twenty five adult male Wistar rats received a unilateral parietal cortical injury (craniectomy followed by contusion with a 1 mm diameter sphere using a 800 g-cm force over a 3 mm vertical depression). Five received a craniectomy with no trauma (sham injury). Procedures were performed under sodium pentobarbital anaesthesia with controlled body temperature. Animals were sacrificed at 4 and 24 hours. Brains were examined for water content by comparing the wet and dry weight of each hemisphere. AQP4 mRNA was measured by RT-PCR. AQP4 mRNA expression on the lesioned side compared to the control hemisphere was calculated for each animal at the injury site (parietal cortex), adjacent to the injury (occipital cortex) and distant from the injury (frontal pole cortex). Brain edema was significantly increased at the injury site. AQP4 mRNA was significantly increased at the injury site, significantly decreased adjacent to the injury site in the occipital cortex, and not significantly different at a site well distant from the injury in the frontal pole. The magnitude of AQP4 mRNA up-regulation at the injured parietal cortex correlated ($R2 = 0.74$) with the degree of down-regulation in the adjacent occipital cortex. This study demonstrates (i) an up-regulation of AQP4 at the site of traumatic brain injury, and (ii) a down-regulation of this molecule adjacent to the site of injury. This down-regulation may be a protective mechanism reducing the development of brain edema following trauma.

P480.

GENDER INFLUENCES ON CEREBROSPINAL FLUID PATHOPHYSIOLOGY AFTER TRAUMATIC BRAIN INJURY

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Female sex hormones have been shown to affect some aspects of secondary traumatic brain injury (TBI) pathophysiology, including excitotoxicity, cerebral edema, and blood flow. As such, we investigated gender differences in TBI related excitotoxicity and ischemia by evaluating cerebral spinal fluid (CSF) levels of glutamate and lactate in a clinical population with severe TBI. We evaluated 123 patients (30 female, 93 male) with severe TBI (GCS<8) admitted between 1995–2001. A portion of this population was treated as a part of a randomized controlled clinical trial evaluating moderate hypothermia treatment (32–33°C for 48 hours). The remaining population, meeting clinical criteria, received 24 hours of hypothermia as standard treatment. Maximum CSF glutamate concentration and lactate/pyruvate ratio were determined at twelve-hour intervals for both 24 and 48 hours after injury. Repeated measures multivariate analysis, (adjusting for age, sex, time, injury severity and type, hypothermia status, and gender interactions) were used to determine the relationship of gender to CSF glutamate concentration and lactate/pyruvate ratio. Results showed a significant gender effect on overall CSF glutamate production ($p = 0.0023$) and a significant interaction between glutamate, gender, and time ($p = 0.0035$) within the first 24 hours after injury. Females had lower levels of CSF glutamate compared to males, especially in the first twelve hours after injury ([4.26] vs. [7.49] micromolar) and a faster return of CSF glutamate to normal levels by 48 hours after injury. Additionally, there were significant gender differences in CSF lactate/pyruvate ratios ($p = 0.0006$) and a significant relationship between lactate/pyruvate, gender, and time ($p = 0.0045$) throughout the first 48 hours after injury. Females had lower lactate/pyruvate ratios than males, particularly within the first 12 hours after injury (24 vs 37). Hypothermia reduced glutamate levels in both males and females. These results signify the importance of studying how gender impacts TBI pathophysiology and efficacy with therapeutic interventions. R03HD41399-01, CCR310285-08.

P481.

NEURONAL SURVIVAL AFTER CENTRAL NERVOUS SYSTEM INJURY REQUIRES AUTOIMMUNE T CELLS: TOLERANCE TO MYELIN ANTIGENS DIMINISHES NEUROPROTECTION

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After trauma to the central nervous system (CNS), neurons that escaped the primary insult nevertheless undergo degeneration, spreading the area of damage beyond the epicenter. Immune activity (especially autoimmune activity) has traditionally been considered as detrimental for neuronal survival, but studies from our laboratory contradict this view. We show here that strains of rats and mice which are inherently resistant to secondary degeneration after CNS injury are endowed with an endogenous mechanism of neuroprotection, and that a key component of this mechanism is a population of autoimmune T cells. We further show that tolerance to myelin self-antigens, long thought to be the preferred state for autoreactive cells, is to the individual's disadvantage in coping with injury-induced stress. Neonatal immunization of rats with whole spinal cord homogenate diminished the ability of the adult animal to respond to myelin immunization, and resulted in significantly worse recovery from severe optic nerve crush (assessed in terms of neuronal survival) or from spinal cord contusion (assessed by locomotor ability). We further show that CNS insult breaks down tolerance to CNS antigens and activates autoimmune T cells. The loss of neurons was greater in strains in which activation of autoimmunity is constitutionally delayed. Autoimmune neuroprotection could be boosted by immunizing rats with the self-antigen or by depleting their endogenous suppressor T cells (e.g. CD4+CD25+ regulatory T cells). These findings demonstrate that the autoimmune response to CNS injury, if well-controlled, is beneficial for neuronal survival after trauma. Boosting of this response by depletion of regulatory T cells or by safe immunization with altered self-peptides is likely to have clinical implications.

P482.

MECHANISM OF PRO-REGENERATIVE VACCINE UNLIKELY TO INVOLVE ANTIBODIES AGAINST GROWTH-INHIBITORY PROTEINS

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We have previously shown that a spinal cord homogenate (SCH) vaccine stimulates axon regeneration of adult rat retinal ganglion cells (RGCs) following optic nerve microcrush. This vaccine does not promote survival of injured RGCs. To examine if antibodies against growth inhibitors are important for RGC regeneration after vaccination, sera of vaccinated animals were tested by Western blot and ELISA against known growth inhibitory proteins. We were unable to detect serum antibodies to myelin-associated glycoprotein (MAG), NogoA, Nogo-66 receptor, or chondroitin sulfate proteoglycans (CSPG). However, antibodies to myelin basic protein, an abundant myelin protein, were detected. We also examined the ability of sera to override RGC growth inhibition on myelin or CSPG culture substrates. Pre-incubation of substrate with sera from SCH-vaccinated animals promoted growth on myelin but not on CSPG. Our results suggest that the growth promoting effect of the SCH vaccine is not mediated by antibody blocking of growth-inhibitory proteins, but by antibodies binding to major myelin proteins. Supported by the CIHR and the FRSQ.

P483.

THE RISK OF BLADDER DENERVATION DURING ANTIREFLUX SURGERY: A RELIABLE NEUROPHYSIOLOGICAL MODEL

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Introduction and Objectives: Previous anatomical studies (Leissner et al. J. Urol. 2001) demonstrating the close relationship between uretero-vesical junction and urinary bladder innervation prompted us to develop a supporting animal model to demonstrate the risk of partial or complete neuronal damage caused by urinary bladder innervation during antireflux surgery.

Material and Methods: Laminectomy was performed on 5 male Göttinger minipigs and a modified Brindley electrode was implanted at S2 for sacral anterior root stimulation (SARS). The lower urinary tract was exposed by abdominal midline incision. The nerve bundle of the pelvic plexus was identified about 1 cm dorsal-medial to the uretero-vesical junction.

The bladder was filled with 150 cc NaCl solution and intravesical pressure monitored with an intraurethral catheter. A blue longitudinal line down the middle of the bladder dome enabled exact visualisation of bladder contraction. Bilateral and unilateral SARS was performed, the nerve fibers of the pelvic plexus were either blocked by Xylocaine or cut under vision.

Results: Bilateral SARS evoked a bilateral detrusor muscle contraction with an intravesical pressure rise up to 39 cmH2O. Unilateral stimulation evoked an exclusively unilateral bladder contraction with maximum intravesical pressures of 13 to 14 cmH2O. Xylocaine injection at the uretero-vesical junction completely blocked the response to SARS. Neurotomy of the nerve bundles in close proximity to the ureter produced the same results. Unilateral neurotomy evoked unilateral detrusor decentralization at the site of nerve damage, whereas bilateral neurotomy evoked detrusor acontractility. Consecutive cutting of the nerve fibers, starting 8 cm from the uretero-vesical junction and proceeding up to 1 cm away from it, led to a drop in detrusor pressure with SARS from 31 cmH2O to 12 and 0 cmH2O, depending on the closeness to the uretero-vesical junction.

Conclusions: Similar to human cadavar studies, the urinary bladder is innervated strictly unilaterally, the nerve supply emanating from the pelvic plexus runs in close proximity (dorsal-medial) to the uretero-vesical junction. Dissection away from the ureter during antireflux surgery and/or purse-string sutures due to bleeding or anchor stitches (Vest) bear high risk of injury to the pelvic plexus which may result in uni- or bilateral detrusor decentralization.

P484.

MECHANICALLY ELONGATED PNS AXONS SUSTAIN HIGH GROWTH RATES: IMPLICATIONS FOR NERVE REPAIR

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Repair of nerve damage has traditionally relied on donor nerves and is limited by availability and associated donor site morbidity. As an alternative strategy, we propose that mechanically elongated axons grown in culture could be used to bridge even extensive nerve damage. Here, we evaluated the ability of rat dorsal root ganglion cells (DRGs) to rapidly grow under continuous mechanical tension. DRGs were plated on adjoining substrates and during a static growth period, axons cross the interface of the two substrates. Using a microstepper motor system, we then progressively separated the two substrates further apart from each other resulting in two populations of DRGs connected together via elongated fascicular axon tracts. Axon growth appears highly strain dependent early in the elongation phase due to the initially small axon lengths. As axons elongate, the rate is slowly increased based on a constant strain value until a maximal elongation rate is realized and growth can be maintained. We previously reported that CNS axons were limited to stretch induced growth of 1 mm/day. Here, we found that DRGs could be grown 6mm/day reaching at least 3 cm in length. Immunocytochemical analysis revealed that elongated axons consistently expressed phosphorylated neurofilament, polymerized beta-tubulin and tau proteins. Despite this enormous growth rate, the expression of these cytoskeletal proteins in the area of elongation actually exceeded that found in adjacent non-stretched axons. These findings may represent a fundamental shift in our understanding of axonal cytoskeleton assembly during growth. We are currently using electron microscopic examination and proteomics to reveal more detail about cytoskeletal assembly during stretch growth. Furthermore, we are elongating DRG axons on substrates suitable for transplantation to repair peripheral nerve injuries. Supported by NIH grants, AG 21527 & NS 38104.

P485.

IMPLANTABLE NEUROCYBERNETIC INTERFACE WITH MECHANICALLY ELONGATED AXONS

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Though once relegated to science fiction, neurocybernetic interfaces have now become a tangible strategy for restoring loss of function following trauma. These interfaces must generally be non-invasive, yet integrate with the host's neural network. Here, we used a new technique to mechanically elongate numerous axons interconnecting two populations of neurons to create such a device. Rat dorsal root ganglion cells (DRGs) were plated on a multi-electrode array (MEA) and an adjoining towing substrate. The two populations of neurons were allowed to integrate, including the growth of axons across the interface of the two substrates. Using a microstepper motor system, we then progressively separated the two substrates further apart from each other resulting in two populations of cell bodies connected together via elongated fascicular axon tracts. Previously we found that sustained mechanical tension induced CNS axons to grow 1 cm in length over a period of 10 days. Here we elongated DRGs to lengths over 3 cm at a rate of 6 mm per day. Immunocytochemistry of these tracts revealed a normal appearing cytoskeleton, including expression of phosphorylated neurofilament, tau and tubulin proteins. The design of this interface allows for implant of neurons at one end into sensitive nervous tissue areas while MEA can be conveniently located for an electronic interface outside the body. We are currently evaluating electrophysiological interactions between the MEA and the elongated cultures. Supported by NIH grants, AG 21527 and NS 38104.

P486.

RESULTS OF PERIPHERAL NERVE RECONSTRUCTION BY AUTOGRAFT

Viktor Matejčík, M.D. (Department of Neurosurgical Clinic of the Medical Faculty of Comenius University, Academician L. Dérer Faculty Hospital, Bratislava, SK).

The purpose of this retrospective clinical study is presents the results achieved in microtechnique surgeries performed during a 15-year-long period (1985–1999). By performing surgeries on 60 patients, 63 nerves were treated.

In 42 patients with injuries of peripheral nerves of upper extremities, 45 nerves were reconstructed by autografts. 14 patients were subjected to reconstructive surgeries on peripheral nerves of lower extremities. In 4 patients we reconstructed the facial nerve by means of autograft. The analysis of surgical effects has been made in dependence on indicators as follows: period elapsed from injury to surgery, age of patient, nature of injury, length of autograft, location of injury, kind of nerve inflicted.

When assessing the results of reconstructive surgeries of peripheral nerves of lower and upper extremities we observed a big difference on the behalf of upper extremities. High efficiency can be seen in tibial nerve surgeries of lower extremities. In general we achieved good results in facial nerve reconstructions.

The crucial factor that has an impact on the result of surgery is that of the time which elapsed from injury to reconstructive surgery. The factor is especially marked in younger patients.

P487.

OUR EXPERIENCES WITH SURGICAL TREATMENT OF INJURIES OF NERVUS ISCHIADICUS

Viktor Matejčík (Department of Neurosurgical Clinic of the Medical Faculty of Comenius University, Academician L.DÈrer Faculty Hospital, Bratislava, SK).

This report presents the results of 44 surgical interventions performed in 44 patients during the period of 15 years, from 1985 to 1999. The report presents the basic lines of surgical treatment performed on a total number of 50 peripheral nerves of lower extremities—nervus ischiadicus and its rami.

In the whole group of 44 patients, external neurolysis was performed in 23 individuals on 26 nerves. Remaining 21 patients were treated by reconstruction surgery that included 24 injured nerves. In this subgroup, suture of peripheral nerve was performed in 8 treatments on 9 nerves and neural graft was performed in 13 treatments of 15 nerves in cases of complete and persisting neurological deficit and in the absence of action potentials as revealed by EMG. Complete or severe motoric defect and the absence of spontaneous recovery during the period of several months were the indications for the treatment. The analysis of the efficiency of surgical treatment was performed with respect to following parameters: period between the injury and operation, patient age, character of the injury, type of injured nerve, and type of surgical intervention.

The best results were obtained for external neurolysis which was applied in traumatic lesions of the least severity. The effective degree of recovery M3 was observed in 21 patients (91.3%). With respect to reconstruction surgery, more favourable results were obtained for treatments involving suture (in 6 patients, 75%) than for nerve grafts used for the treatment of the most severe injuries associated with a loss on nerve tissue. In the latter cases, improvement was observed after a delay and the extent of recovery did not always meet the expectations. The effective degree of recovery was observed in 4 patients (30.8%). Good and excellent results were typical for n. tibialis and they were not dpendent of the type of surgical intervention, character and location of the injury, period from the injury or patient age.

Our results demonstrate that late and inappropriate treatment of injured peripheral nerves have severe and disturbing consequences for the patient. If a complete treatment of the injured nerve is not possible by the first contact physician, it should be performed in the shortest possible time by the specialist trained for microneurosurgical techniques of the treatment of peripheral nerves.

P488.

EXERCISE INCREASES THE REGENERATIVE POTENTIAL OF SENSORY NEURONS VIA NEUROTROPHINS

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Physical activity promotes functional recovery after spinal cord injury, but molecular mechanisms involved are still puzzling. We investigate mechanisms by which voluntary exercise helps the regenerative capacity of neurons in adult rats. Animals were exposed to exercise for 3 and 7 days and changes in mRNA levels in the DRG cells were analyzed in vitro by quantitative real-time PCR. DRG neurons from exercised rats showed increased axonal length, associated to increased levels of BDNF and NT-3 mRNAs, and a 2 fold increase in synapsin I mRNA (downstream effector of BDNF and NT-3 actions). In addition, GAP-43 mRNA levels showed a consistent increase in exercised rats. All changes were dependent on neurotrophins as injection of K252a in vivo blocked plastic changes. Lastly, to evaluate the effects of exercise on axonal regeneration, rats were exercise conditioned for 7 days and then sciatic nerve crush injury was performed. Two days later, the sciatic nerve was transected 0.5 cm distal to the crush site and regenerating axons were labeled by fluorogold (a retrograde axonal marker). The exercise-conditioned animals showed approximately 2 fold more fluorogold-labeled DRG cell bodies than sedentary animals, indicating an increased capacity for axonal regeneration in vivo. These findings indicate that physiological forms of activity, such as exercise, cause changes in expression of neurotrophins and this enhances the ability of these neurons to compensate for insults. (Supported by NS38978, NS39522, UCLA Brain Injury Research Center, and Roman Reed Awards).

P489.

SURGICAL TREATMENT OF INJURIES OF N. FIBULARIS

Viktor Matejčík, M.D. (Department of Neurosurgical Clinic of the Medical Faculty of Comenius University, Academician L.Dérer Faculty Hospital, Bratislava, SK).

In this retrospective study, we present the results of 40 surgeries of 40 patients that within the period of 15 years, i.e. from 1985 to 1999 were provided the treatment of 40 lesions of n. fibularis, historically treated as problematic in terms of successful healing. The work provides the fundamental lines of their surgery treatment.

From the total number of 40, external neurolysis was performed to 20 patients. The remaining 20 patients were provided with reconstruction surgeries of the injured nerves, while 8 surgeries was done by suture of peripheral nerve and 12 surgeries were performed by nerve graft, in cases of complete and persisting neurological deficit and absence of action potential at EMG. The mechanism of lesion included the damages of nerve from elongation, with or without fracture, "sharp" or "blunt" lesions, lesions of shooting, compressions and iatrogenic injuries. If the spontaneous adjustment did not occur within the period of 2–6 months after the lesion, the patients underwent surgery and with 27 of 40 an effective adjustment was achieved preventing the sagging of the foot trace and with 25 of 40 protective sensitivity appeared. We performed the analysis of the efficiency of the surgical intervention depending on the following parameters: period of surgery from the lesion, patient's age, nature of lesion, degree of lesion, type of surgery intervention.

After neurolysis with 18 of 20 patients (90 %) we achieved effective degree of adjustment in spite of heavy pre-surgical motor deficit. With 8 patients an "end to end" suture was performed and with 6 (75 %) the degree of adjustment was 3 or higher. 12 patients requested reconstruction surgeries with the help of nerve grafts, the length of grafts varied from 4 to 20 cm. The grafts were shorter than 5 cm with 2 patients, 1 with cut lesion and 1 patient with iatrogenic lesion. With both patients the function was adjusted to the degree M4. With 1 of 4 patients (20 %) with the graft of 6 to 12 cm and with none of 6 with the grafts from 13 to 20 cm the adjustment of the degree 3 or higher was not achieved. In this cases, however, we noticed partial adjustment of trophic and tonus, however at the absence of motor adjustment.

Similarly as with other nerve injuries, the perfect pre-surgery examination and timely surgery are needed for achieving optimum results. The excellent results of proximal injuries of n. fibularis in comparison with more distant in the area of knee are worth noting.

P490.

SURGICAL TREATMENT OF LESIONS OF NERVUS FACIALIS

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The study presents the results of reconstruction surgery of lesions on n. facialis performed in our clinic in the time period 1998–2000. Four patients were treated by anastomosis of n. facialis with n. hypoglossus (HFA), 1 patient by anastomosis of n. facialis with n. accessorius (AFA) while nervus facialis in 3 patients was reconstituted by neural graft. In the last group, neural graft in one patient originated from nervus auricularis magnus and in two patients from nervus suralis. All operations were performed under the microscope; HFA and AFA anastomoses were sewed without tension at perineurium. The techique of suturation of facial nerves did not differ from the suturation of peripheral nerves in extremities. During neural graft surgery, the grafts were loosely deposited between two nerve endings in such a way that the graft overlapped the nerve endings by 1–3 mm (depending on transplant length). Fascicles or groups of fascicles were connected by value 8.0 sewing material.

The results were objectivized by a VI grade Brudny modification of House-Brackman classification1 introduced originally for scaling of the outcome of hypogosso-facial anastomoses. In this study, this classification has been used for the objectivization of all reconstruction microsurgical interventions of n. facialis.

The results of neural graft treatments were superior to the results of cross anastomoses HFA or AFA. Grade II was achieved by all patients treated by neural graft. There were no symptoms of glossal hemiatrophy or atrophy of m. sternocleidomastoideus and m. trapesius in this group that were observed in patients treated by cross anastomosis with n. hypoglossus or n. accessorius. Minute synkineses in the region of labial angle and chin occurred in the excited emotional state or during a long-lasting extensive speech. Improved mimics was apparent here compared to the group treated by HFA and AFA. Reconstruction surgery by HFA and AFA resulted in all cases in grade III of the scale. Synkineses in the region of lower eyelid were manifested in patients treated by HFA and they were even more pronounced in patient with AFA anastomosis. Major diskineses were not observed in any of reported treatments.

Compared to AFA anastomosis, HFA anastomoses resulted in improved mimics and synkineses present here were finer. We prefer HFA anastomosis also because the discomfort caused by atrophy of m. trapesius and m. sternocleidomastoideus was apparently more perceived by patient treated by AFA than the negative effects of hemiatrophy reported by patients treated by HFA.

P491.

SPINAL CORD INJURY EXPRESSION PROFILING: FEATURES OF NEURONAL DAMAGE ARE ASSOCIATED WITH CELL CYCLE PROGRESSION AND DEPRESSION OF NEURITOGENESIS

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Spinal cord injury is a major cause of disability. and it is known that much of the functional deficit results from delayed cellular consequences of injury repair mechanisms. To define the temporal series of gene expression changes following a spinal cord injury. rats were subjected to a controlled impact injury at T8-T9 by weight drop (10gm. 17.5 mm). Rats were sacrificed at four time points (30 min. 4h. 24h and 7 days). with 4 to 6 individual rat spinal cords expression profiled at each time point (total 26 profiles) using the U34A Affymetrix genechip containing 8700 probe sets. Genes showing 40% or more "present" calls in 26 profiles by Affymetrix analyses were retained for further analysis (data scrubbing). and p values (>0.05) and fold changes (2 fold threshold) correlated. with temporal and functional clustering. Specific RNAs were verified by QMF-RT-PCR using infrared primers. protein level quantified by western blot and localized by immunocytochemistry. We found induction of DNA damage-inducible genes and genes favoring cell cycle progression at 4 and 24 hours after injury; at these times there was also depression of genes associated with neuritogenesis. Changes in mRNA expression were associated with changes in respective proteins as shown by western blots and immunocytochemistry. Cell cycle and DNA damage related proteins were frequently localized in neurons showing signs of DNA damage and apoptotic features. We conclude that gene associated with DNA damage and progression of cell cycle were significantly upregulated in response to a low-moderate level of spinal cord injury and may contribute to subsequent apoptosis. Such changes were temporally associated with suppression of genes implicated in neuritogenesis.

P492.

UPREGULATION OF EPHRIN LIGANDS AFTER SPINAL CORD INJURY

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Spinal cord injury (SCI) generates a cascade of events that lead to inhibition of axonal regeneration. The molecular and biochemical changes represent the presence of repulsive factors that may restrict or block neurite outgrowth after SCI. One class of factors with inhibitory activity for axonal outgrowth are the Eph receptors and their ligands, the ephrins. These molecules are involved in cell migration, axonal pathfinding, target recognition and synapse formation, by repulsive interactions after receptor-ligand binding. However, the pattern of expression and role of ephrins in adult injured spinal cord is unknown. Adult Sprague Dawley rats received a contusion to the thoracic vertebra T10 with the NYU Impactor device. Stantdardized semi-quantitative RT-PCR analysis of some ephrins genes were performed from injured and control spinal cord tissues. Our results indicate that the expression levels increased 7 days after SCI, that returned to basal level by day 14 in the case of ephrinB2 but remains elevated until day 28 for ephrinA1. Immunohistochemistry studies confirmed the upregulation data obtained at the mRNA level after SCI. The immunoreactivity was localized in the ventral region of the white matter. Ongoing studies will determine the phenotype of cells expressing the ligands and role of these molecules after spinal cord injury. The previous data suggest that ephrins may contribute in creating the non-permissive environment for axonal regeneration after spinal cord injury. This work was supported by NIH/NINDS (NS 39405), RCMI (G12RR03051), PR-EPSCOR (EPS-9874782), KSCHIR Trust (8-29), Norton Healthcare, and MBRS SCORE (2 SO6 6M8224).

P493.

METHYLPREDNISOLONE INHIBITS INTERLEUKIN-1 AND INTERLEUKIN-6 PRODUCTION IN THE SPINAL CORD FOLLOWING COMPRESSION INJURY IN THE RAT

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Introduction: Cytokine proteins are present in normal spinal cord tissue and their production is increased after tissue injury. We sought to evaluate the effect of methylprednisolone (MP) on the production of IL-1 and IL-6 protein in spinal cord tissue, following T7 compression spinal cord injury (SCI).

Methods: Sprague-Dawley rats were treated randomly with saline IM or methylprednisolone (30 mg/kg) IM. No lesions were produced in animals in the control groups (saline control, MP control). SCI was induced by extradural placement of a 55 g aneurysm clip at T7 for one minute. SCI animals were treated with saline or MP immediately after clip removal. Spinal cord sections at T7 were processed by enzyme-linked immunosorbant assays (ELISA) to measure IL-1 and IL-6 protein, expressed as mean ± sd.

Results: In the Saline and MP Control animals, the IL-1 levels were 37.72 ± 7.25 pg/mcg and 43.01 ± 14.36 pg/mcg, respectively. An increase in IL-1 was seen in the SCI + Saline animals (67.47 ± 19.99 pg/mcg, $p < 0.02$). Compared to SCI + Saline animals, those in the SCI + MP group had a decrease in IL-1 (32.65 ± 6.64, pg/mcg, $p < 0.01$). In the Saline Control and MP Control animals, the IL-6 levels were 20.01 ± 5.91 pg/mcg and 19.25 ± 4.10 pg/mcg, respectively. An increase in IL-6 was seen in the SCI + Saline animals (29.65 ± 5.46 pg/mcg, $p < 0.01$). Compared to SCI + Saline animals, animals in the SCI + MP group had a decrease in IL-6 (17.73 ± 5.93 pg/mcg, $p < 0.01$).

Conclusions: Spinal cord compression produced an increase in IL-1 and IL-6, which was inhibited by MP, confirming the anti-inflammatory role of MP. Further studies are warranted to discover if inhibiting cytokine production affords therapeutic benefit or renders spinal cord tissue more compatible to host neural cell transplantation.

P494.

VASCULAR INDUCTION OF HEME OXYGENASE-1 AS A NOVEL APPROACH FOR STABILIZING BARRIER FUNCTION AFTER SPINAL CORD INJURY

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Heme oxygenase (HO) catalyzes the breakdown of heme to carbon monoxide, iron and bilirubin. There is recent evidence that HO-1, the inducible HO, can alter vascular function as evidenced by its ability to attenuate inflammation, vasoconstriction and vascular proliferation. In this study we have developed a method for the selective induction of HO-1 in blood vessel and have used this approach to begin to identify the role of HO-1 in abnormal vascular induction of HO-1 stabilized the barrier after spinal cord injury. We found that HO-1 was specifically induced in spinal cord vasculature by systemic administration of stabilized hemin, as evidenced by both western immunoblots and immunocytochemistry. We next examined the extent to which induction of HO-1 prior to spinal cord injury influenced barrier permeability. 24hours after systemic administration of either vehicle or stabilized hemin, adult, male mice were subjected to a moderate level of contusion injury. Luciferase, a marker of barrier permeability, was given intravenously 30 min prior to euthanized at 24 hours post injury. Luciferase was quantified in tissue prepared from the lesioned epicenter. There was a significant attenuation of barrier permeability to luciferse in the stabilized hemin as compared to the vehicle treated groups. There findings offer a novel role for HO-1 in limiting early vascular dysfunction after spinal cord injury.

Supported by NS39278 and NS 39847.

P495.

RECONSTRUCTION OF ANTERIOR COLUMN DEFICIT FOLLOWING CERVICAL SPINE INJURIE USING THE HARMS TITANIUM MASH CAGE

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Objectives: the optimal treatment of cervical fractures is still controversial. Anteriror plating sistem combined with an artificial vertebral body implant is the surgical method to provide biomechanical stability in injured patients following partial or total corporectomy.

Methods: clinical data, X-ray, CT and MRI scan records were reviewed retrospectively in 14 injured that were treated by vertebrectomy combined with the titanium mesh cilinders and plate implants. These 11 males and 3 females of the mean age of 45 years (range 16–72) sustained cervical spine fractures in automobile accidents, by falling from one level to another and jumping in shallow sea.

The spine lesions were classified according to Magerl—5 patients had type A, 9 type B lesion. A preoperative and 12 months postoperative Frankel score and functional status were assesed.. Preoperatively 6 patients had grade A, 2 grade B, 3 grade C, 1 grade D and 2 patients were neurologically intact.

Results: there was no neurological deterioriation. Bone fragments were removed completely in all cases. Good spine realignment was achieved in 13 cases and in 1 patient spine deformation occured—a kyphosis with an angle of 9 degrees.

Neurological status improved for one grade in one patient, two or more in 2 patients and remained unchanged in 11 patients.

Conclusions: anteromedial approach can provide significant eliminitaion of bone fragments, disc et haematoma. Good spine alignment and solid fusions are achieved by instrumentation—plating and Harms cages.

P496.

EFFECT OF P75NTR DELETION ON WHITE MATTER PROTECTION AFTER EXPERIMENTAL SPINAL CORD INJURY.

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Background: Despite advances in medical and surgical care, spinal cord injury (SCI) remains a devastating event. Novel therapies are needed. Our laboratory has recently shown that members of the tumor necrosis factor receptor family death receptors (specifically, Fas and p75NTR) are temporally and spatially associated with post-SCI oligodendroglial apoptosis. While p75NTR is associated with post-SCI oligodendroglial apoptosis, little is presently known about the specific role of p75NTR in secondary injury. As such, we have started an investigation of the effect of p75NTR deletion on axonal integrity and white matter preservation after SCI.

Methods: To further delineate the role of p75NTR in post-SCI apoptosis we subjected mice null for p75NTR to extradural clip compression SCI at T6. One centimeter of spinal cord (centered at the injury epicenter) was extracted at 0 d (uninjured), 1d, 3d, 7d, and 14d after injury (n = 3/time point), homogenized with protease inhibitors and used for western immunoblots. Immunoblots were probed with antisera to NF200. an axonal cytoskeletal protein whose degradation inversely correlates with axonal integrity and functional outcome after SCI.

Results: Preliminary results do not show a significant difference in the pattern of NF200 degradation between p75NTR null animals and strain-matched wild-type controls.

Conclusions: Our early results suggest that downregulation of p75NTR activity may not inhibit axonal degradation after SCI. This result needs to be further clarified by immunohistochemical analysis of axonal degeneration after SCI in p75NTR null animals. Further detailed investigation of the role of p75NTR in delayed cell death after SCI is warranted.

P497.

ESTROUS CYCLE MEDIATED EFFECTS ON SPINAL CORD INJURY

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A recent study suggested that female rats have smaller spinal cord lesion volumes than males after a standardized weight-drop contusion injury. We hypothesize that these differences may be due to the neuroprotective effects of estrus that other scientists have reported. To test this hypothesis, we compared spinal cord lesion volumes in female rats during proestrus, estrus, and diestrus; comparisons were also made with male rats. Estrogen levels should be highest during proestrus, intermediate during estrus, and lowest in diestrus and in male rats. If estrogen accounts for the difference between male and female rats, we expect to see the smallest lesion volumes in female rats during proestrus and these lesion volumes should be significantly less than females in diestrus and male rats. We studied a total of 26 female (8 proestrus, 9 estrus, 9 diestrus) and 8 male Long-Evans hooded rats. The rats were all injured with a 10 gram weight dropped 25 mm onto the T13 spinal cord and euthanized at 6 hours after injury. The rats were 77 ± 3 days of age. Lesion volumes were calculated from the potassium concentrations at and around the injury site. In addition, we collected messenger RNA from the tissues for later analyses with large-scale microarray to identify genes that may be responsible for any neuroprotective effects. The results indicate that female rats undergoing proestrus have significantly smaller spinal cord lesion volumes, compared with rats undergoing diestrus and male rats. We are assessing gene expression of the rats at each of estrous stages to identify potential gene expression changes that may explain the neuroprotective effects of proestrus.

Supported by SCI Project, Rutgers University Summer Undergraduate Research Fellowship, and Douglass College SUPER Project.

P498.

GLUTAMINE ADMINISTRATION HELPS TO MAINTAIN BASAL GLUTATHIONE CONCENTRATIONS IN RAT SPINAL CORDS FOLLOWING ACUTE INJURY.

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Glutathione (GSH) has been previously demonstrated to decrease oxidative stress resulting from spinal cord injury. Compounds designed to increase GSH production such as L-2-oxothiazolidine-4-carboxylate (OTC) have been shown to be neuroprotective following spinal cord injury. Glutamine supplementation of parentral diets in rats has also demonstrated increased plasma GSH levels. This study was designed to examine the effect of administration of glutamine on GSH concentrations in spinal cord tissue. Wistar rats underwent surgeries in which a laminectomy was performed and a 50g aneurysm clamp used to pinch the spinal cord at the level of T6. In sham animals only a laminectomy was performed. Thirty minutes after surgical intervention 5mmol/kg glutamine or saline was administered into the peritoneal cavity, subsequently every 12 hours. Animals were sacrificed 24 hours after surgical intervention and C3, T3, T5, T6, T7, T9 and L4 spinal cord segments were collected. GSH was measured by HPLC. In the T5, T6 and T7 segments of spinal injured animals administered glutamine, GSH concentrations were similar to sham values and were significantly higher than those of the saline treated animals. GSH concentrations were not increased in glutamine treated sham animals over saline treated sham animals. This was expected, as glutamine is known to be rate limiting only when GSH concentrations are decreased and in the presence of cysteine. These results suggest that glutamine is an effective agent for maintaining basal GSH concentrations and therefore decreasing oxidative stress after spinal cord trauma. This research is funded by the Christopher Reeves Foundation and S. T. Rigley holds a College of Medicine Scholarship.

P499.

NOVEL ROLE OF PROSTACYCLIN IN COMPRESSION-INDUCED SPINAL CORD INJURY IN RATS.

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Although spinal cord injury (SCI) is a serious condition which produces lifelong disabilities, only limited therapeutic measures are currently available for its treatment. We previously reported that prostacyclin (PGI2) reduced the motor disturbances subjected to SCI in rats, probably by inhibiting the leukocyte accumulations at injury site and PGI2 could be a new therapeutic agent for patients with SCI (Taoka et al., J. Neurosurg, 1997). However, the precise mechanisms of PGI2 against SCI are not well known. The present study was conducted to clarify these mechanisms by using the compression-induced SCI model in rats. 6-keto-PGF1alpha level, a stable PGI2 metabolite, in injured spinal cord tissue significantly increased, peaking at 2 hr after the induction of SCI. Subcutaneous administration of indomethacin (IM), a non-selective cyclooxygenase inhibitor (5 mg/kg), completely inhibited this increase but significantly exacerbated the motor disturbances following SCI, and enhanced leukocyte accumulation as determined by myeloperoxidase (MPO) activity, TNF-alpha production which is a potent activator of leukocytes, and mRNA TNF-alpha expression at injury site in rats subjected to spinal cord trauma. Compression-induced motor disturbances were significantly reduced in animals given iloprost, a stable analog of PGI2 and in those with nitrogen mustard-induced leukocytopenia. Iloprost and leukocytopenia also significantly inhibited the IM-induced exacerbations of SCI. NS-398, a selective inhibitor of cyclooxygenase-2, did not attenuate the motor disturbances, and the increases in MPO activity, TNF-alpha production, and mRNA TNF-alpha expression induced by trauma. These observations indicate that the increase in trauma-induced PGI2 in spinal cord tissue, mainly mediated by cyclooxygenase-1, appears important in preventing the motor disturbances following SCI by inhibiting leukocytes activation.

P500.

LONGTERM RESULTS AFTER 36 MONTHS FOR CHRONIC BILATERAL NEUROMODULATION

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INTRODUCTION AND OBJECTIVES: Sacral root neuromodulation can be a beneficial treatment option in patients suffering from therapy-resistent detrusor instability or detrusor hypocontractility.

The implantable neuromodulation system as described by Tanagho and Schmidt enables unilateral sacral nerve stimulation. The electrode is inserted unilaterally into the sacral canal via the sacral foramen (S3). Reports have been made on sacral neuromodulation failures of up to 50% in patients undergoing this procedure.

We preferred bilateral electrode implantation and tailored laminectomy in order to achieve better effectivity of the chronic sacral neuromodulation.

MATERIAL AND METHODS: After assessment of the beneficial effect by means of PNE test, 32 patients (18 with detrusor instability, 14 with hypocontractile detrusor) underwent tailored laminectomy for bilateral electrode placement. Minimally invasive laminectomy was performed. The electrodes were bilaterally positioned. Laminectomy allows optimum electrode placement and fixation.

RESULTS: In the patients with detrusor instability the incontinence episodes were reduced from 8.6 to 0.9 per day and the bladder capacity improved from 270 to 375 ml. In patients with hypocontractile detrusor, the initial residual urine level of 340 ml (170 to 489) dropped to 54 ml (40 to 66). Maximum detrusor pressure during micturition rose from initially 12 cmH2O (7 to 15) to 36 cmH2O (29 to 48). The average followup period was 36 months. There was no sign of deterioration in the effect of modulation in any of the patients.

CONCLUSIONS: Chronic sacral bilateral neuromodulation results in optimal longtermresults in either hyper- or hypocontractile detrusors.

P501.

THE INTRAVENOUS ADMINISTRATION OF AUTOLOGOUS BONE MARROW CELLS INTO THE RAT DEMYELINATED MODEL.

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The regenerative potential of the bone marrow cells were studied in the demyelinating model rat. Although both the focal injection and the intravenous administration of bone marrow cells isolated from bone marrow repaired the demyelinated spinal cord in the adult rats, the ideal protocol in terms of the administration method and the cell number remains unknown. This study was to examine how we should transplant the bone marrow cells and how many cells are required to establish the sufficient numbers of remyelinated axons in the demyelinated spinal cords. A focal demyelinated lesion was created in the dorsal columns of the rat spinal cord using X-irradiation and ethidium bromide injection (EB-X). A suspension of bone marrow cells (1 × 102–1 × 108) collected from the same rat was directly transplanted into the middle of the EB-X-induced lesion or was injected into a femoral vein 3 days after the EB injection. Lesions were histologically examined 3 weeks after transplantation. Light microscopic examination revealed the demyelinated axons were extensively repaired by autologous bone marrow cells. The numbers of the repaired axons following bone marrow transplantation were in proportion to those of the transplanted cells. In addition, the effectiveness of the focal injection is 100 times more than the intravenous administration. These results demonstrate that the intravenous administration of the autologous bone marrow cells may be a better strategy for the injured CNS.

P502.

TRANSPLANTATION OF SCHWANN CELLS (WITH OR WITHOUT OLFACTORY ENSHEATHING GLIA) AFTER SPINAL CORD INJURY (SCI): CAN PRETREATMENT WITH THE NEUROPROTECTIVE STRATEGY OF CO-ADMINISTERED METHYLPREDNISOLONE AND INTERLEUKIN-10 ENHANCE RECOVERY?

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Methylprednisolone (MP) and interleukin-10 (IL-10) have been demonstrated to be protective when given acutely after spinal cord injury (SCI) and recent work from our laboratory has shown that a combination of these agents offers additive neuroprotection. The current study examined if acute neuroprotection with MP and IL-10 could increase the efficacy of Schwann cell (SC) grafts or combination grafts of SC plus olfactory ensheathing glia (OEG) transplanted 1 wk after moderate contusive injury. Efficacy of each strategy was determined by tracing of axonal regeneration, immunohistochemical analysis and behavioral testing (BBB score) 8 wk post-transplantation. Combination SC/OEG grafts displayed reduced astrogliosis (GFAP) and chondroitin sulfate proteoglycan expression (CS56) in both the graft and host tissue compared to SC grafts. Furthermore, the presence of significant numbers of Reca1-positive cells and 5-HT fibers within and rostral to the SC/OEG grafts, but not the SC grafts, indicated that these grafts were better vascularized and supported the re-growth of brainstem neurons. Behaviorally, SC/OEG transplanted animals were significantly better than those transplanted with only SCs. The acute administration of MP and IL-10 after SCI, however, failed to enhance behavioral recovery in either transplantation paradigm and no benefit was observed in any of the histological parameters examined. The cell transplantation procedure could induce a second inflammatory response associated with the injection surgery or immune intolerance of the transplantated cells that damages any tissue that is spared from the acute neuroprotective strategy. Future combination strategies may require a less invasive transplantation procedure or a re-administration of the protective compounds during transplantation. (Supported the NINDS09923, POINS38665 and The Miami Project)

P503.

DIFFERENTIAL RESPONSES OF PAIN AND SENSORY ABNORMALITIES TO I.V. BARBITURATES AND LIDOCAINE IN SPINAL CORD INJURED PATIENTS

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The incidence of pain reported after spinal cord injury (SCI) varies between 7.5–94%. In some studies chronic pain is more disabling than paralysis, bowel or bladder dysfunction. SCI pains may arise from multiple and often co-existent pathophysiologic mechanisms of peripheral and/or central origin. SCI pain is difficult to treat and has been reported to respond fairly poorly to opioids, but much better to IV barbiturates. Cutaneous hyperesthesia responsive to barbiturates has been shown to be of central origin in other neuropathic pain patients. Spontaneous pain and sensory abnormality alterations were investigated via IV normal saline-controlled infusions of sodium amobarbital (SA), a medium action barbiturate, and lidocaine (L), a local anesthetic-type of drug, in 6 SCI patients (5 with thoracic and one with cervical spine lesions, 4 males, 2 females, mean age 35 yrs, mean pain duration 4.9 yrs). Spontaneous pain was reduced by 74% on average after SA infusion and 45% after L infusion, while sensory abnormalities were modified in [4/6] patients with SA and [1/5] patient with L infusions. The sensory abnormalities modified under the drugs consisted primarily of hyperesthesia to touch and pinprick, while dense hypoesthesia (at and below the level of lesion) failed to change. The analgesic effect of IV SA is far superior to that obtained with IV L in SCI patients. The non-competitive NMDA receptor antagonistic action of SA may be responsible for the substantial alteration of cutaneous (centrally mediated) hyperesthesia.

P504.

FUNCTIONAL RECOVERY AFTER MODERATE CONTUSIVE INJURY IN THE MOUSE: ROLE OF DHEA IN IMPROVING BLADDER FUNCTION.

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We have previously demonstrated that dehydroepiandrosterone (DHEA) promotes differentiation of motor neurons and facilitates axonal growth in the developing CNS. Most recently, we have shown that DHEA promotes functional recovery after spinal cord injury, as evidenced by improved locomotor performance, gait pattern and reduction of foot faults on an inclined ladder. The present study extends these previous observations by focusing on the role of DHEA in bladder function after spinal cord injury. Whereas DHEA treated animals developed a functional bladder by 10 days post injury, bladders of vehicle treated animals remained dyssynergic for an extended period of time. DHEA-treated animals exhibited a significantly smaller bladder, coincident with a significant reduction in urine volume, as compared to vehicle-treated animals. Interruption of controlled voiding of the bladder in response to atraumatic neuropathy has been shown to induce distension of the bladder wall that is correlated to a change in the extracellular matrix (ECM) composition of the layer muscularis of the detrusor. We found that spinal cord injury also increases the ratio of collagen type III to collagen type I in the layer muscularis. Moreover, this ratio in the spinal cord injured, DHEA treated group was similar to that of the naÔve animal. We next examined the relationship between previous measures of functional recovery and controlled voiding using cluster analysis. We found that early recovery of controlled voiding is predictive of motor recovery. Together, these findings emphasize the unique and beneficial role that DHEA plays in restoration of function after spinal cord injury. This work was supported by the Charitable Columbia foundation, the Roman Reed Program and NIH grant NS41998.

P505.

TARGETING THE RHO SIGNALING PATHWAY TO PROMOTE REPAIR AFTER SPINAL CORD INJURY

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The activation state of Rho is an important determinant of axon growth and regeneration in neurons. We have investigated the use of antagonists to Rho or Rho associated kinase (ROK) to overcome growth inhibition and promote axon regeneration after spinal cord injury (SCI). In primary culture, inactivation of either Rho with C3 or ROK with Y-27632 promoted neurite growth on inhibitory myelin or chondroitin-sulfate proteoglycan substrates. To examine how the environment influences Rho activation states, we have isolated active Rho in tissue homogenates by pull down assay. Inhibitory substrates activated Rho when cells were plated in culture. In vivo, SCI activated Rho in homogenates of spinal cord. The increased Rho activation was blocked by treatment with the Rho antagonist C3-05. To examine the use of Rho or ROK antagonists after SCI, a lesion was made in mouse spinal cord at T7–T8, and the antagonists were applied in a fibrin gel. Three weeks to three months post-lesion, the corticospinal tract (CST) of injured mice was anterogradely labelled with WGA-HRP, and axon regeneration was detected in longitudinal cryostat sections of the spinal cord. Animals treated with either Rho or ROK antagonists showed long distance regeneration. Untreated animals showed retraction of CST axons from the lesion site. Functional recovery was scored by the BBB open field test. Treated animals showed a remarkable 24 hr recovery and continued to recover over next month with BBB scores significantly higher than untreated mice. Examination of the histology at 24 hours showed fewer Tunel-labeled cells after Rho inactivation. These results demonstrate that Rho is abnormally activated after spinal cord injury, and that inactivation of Rho is both protective and promotes axon regeneration. Supported by the Canadian Institutes of Health Research.

P506.

DIFFERENTIAL TEMPORAL EXPRESSION OF MATRIX METALLOPROTEINASES DURING WOUND HEALING IN A MURINE MODEL OF SPINAL CORD INJURY

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We have previously shown that Matrix Metalloproteinase-9 (MMP-9) promotes the infiltration of neutrophils after spinal cord injury and that attenuation of neutrophil infiltration by blockade of MMPs promotes locomotor recovery and preservation of white matter. MMP-9 and other MMPs are integral to angiogenesis and thus may also be critical during wound healing in the injured spinal cord. We therefore examined the extent to which MMP-9 and MMP-2 are altered in the acutely injured cord (1 day post injury), during revascularization (7–14 days post injury), and after re-establishment of the blood-spinal cord barrier (28 days post injury). Gelatinolytic activity, defined by in situ zymography, was restricted to meninges and blood vessels in shams and identified in glia and macrophages at all time points after spinal cord injury. In addition, unusually large diameter blood vessels expressed gelatinase activity at 7 to 28 days post-injury. The active form of MMP-9, identified by gelatin zymography, was most prominent at 1 day and returned to control values by 14 days post injury. In contrast, the active form of MMP-2 activity was not identified until 7 days post injury; activity declined thereafter, but remained elevated over sham controls for the duration of the study. These findings suggest that although MMP-9 and -2 exhibit overlapping expression during revascularization, the former is primarily associated with acute injury responses and the latter with wound healing. Supported by NS39278 and NS39847.

P507.

THE EFFECT OF THE L-TYPE CALCIUM CHANNEL AGONIST, BAYK8644, ON REGENERATION OF CULTURED RAT SYMPATHETIC NEURONS

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Purpose: To investigate the effect of the L-type Ca2+ channel agonist, BayK8644, on the regeneration of transected rat sympathetic neurites in an in vitro model of spinal cord injury.

Hypothesis: BayK8644 enhances regeneration of cultured rat sympathetic neurites. Methods: Sympathetic neurons were harvested from the superior cervical ganglia of neonate rats. Injury of 14 day-old neurites was with a motorized rubber impactor. Various concentrations of BayK8644 were administered 20 min. pre-neuritotomy (1, 2.5, 5, 15, and 30 micromolar).

The 30 micromolar solution was also administered 5 min. post-neuritotomy. Regeneration was assessed by measuring neurite density and length at 2 and 24 hr. post-neuritotomy using BIOQUANT imaging software.

Results: The 1 micromolar solution of BayK8644 enhanced neurite length at 2 and 24 hr. post-neuritotomy ($p = 0.026$ at 2 hr; $p < 0.001$ at 24 hr). Conversely, the 2.5 micromolar solution reduced neurite density and length at 24 hr. post-neuritotomy ($p = 0.009$ for density; $p < 0.001$ for length). The 5 micromolar solution reduced neurite density and length at 2 and 24 hr. post-neuritotomy ($p = 0.003$ for density at 2 hr; $p < 0.001$ for density at 24 hr. and length at 2 and 24 hr). The 15 micromolar solution reduced neurite density at 2 hr. post-neuritotomy ($p = 0.0116$) and length at 24 hr. post-neuritotomy ($p = 0.02$). Similarly, the 30 micromolar solution reduced neurite density and length at 2 and 24 hr. post-neuritotomy ($p < 0.001$). The 30 micromolar solution administered 5 min. post-neuritotomy reduced neurite density and length at 2 and 24 hr. post-neuritotomy ($p = 0.005$ for density at 2hr; $p < 0.001$ for density at 24hr. and length at 2 and 24 hr).

Conclusions: The differential effect of BayK8644 on neurite regeneration suggests that increasing Ca2+ influx through L-type Ca2+ channels enhances regeneration, but excessive influx inhibits regeneration. Further experiments are required to establish the effects of L-type agonists on neurite regeneration.

P508.

IDENTIFICATION OF PROLIFERATING EPENDYMAL CELLS IN THE RAT SPINAL CORD FOLLOWING TRAUMA

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In lower vertebrates such as amphibians and lizards. the ependyma of the spinal cord plays a significant role in neuronal regeneration. After spinal cord injury (SCI), the ependymal cells rapidly proliferate, migrate, and differentiate to regenerate the cord. In adult mammals, limited proliferative activity has been reported in the normal ependymal canal. However, after SCI, ependymal cells become activated with specific characteristics of precursor cells, as we have previously shown by an increased bromodeoxyuridine (BrdU) labeling index and immunoreactivity to nestin, a marker for neural precursor cells. The purpose of this study is to specifically label the ependymal cells and compare their proliferative capacity following spinal cord trauma of varying severity. We have examined one minute clip compression injuries of mild (2.4g) and moderate (20g) severity at T8 level, and lateral stab wounds which do not disrupt the central canal. To label the ependymal layer along the neuraxis, a 0.2% (w/v) solution of DiI in dimethylsulfoxide was stereotactically injected into the lateral ventricle 24 hours prior to injury. We found that intraventricular injection of the liphophilic DiI specifically labeled the ependymal cells of the spinal canal, as shown by fluorescence and DiI photoconversion with diaminobenzidine. Double-labeling with nestin demonstrated DiI labeled, nestin positive ependymal cells after spinal cord injury. We are currently investigating the proliferative capacity of ependymal cells following varying degrees of spinal cord trauma. Double-labeling for BrdU and DiI will be used to identify proliferating and migrating ependymal cells, and cell-type specific markers will be used to identify resulting progeny.

P509.

AXONAL PRESERVATION WITHIN DESCENDING VASOMOTOR PATHWAYS AND CARDIOVASCULAR CONTROL AFTER SPINAL CORD INJURY

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The association of cardiovascular abnormalities in individuals with traumatic spinal cord injury (SCI) and the severity of demyelination and axonal preservation was investigated. We focused on two areas of descending vasomotor pathways (DVP) in animals and humans which have been described in dorsolateral aspect of the lateral horn (Area I). and dorsal portion of the lateral funiculus (Area II). Data were analyzed using ANOVA and Student t-Test. Five individuals (2M. 3F; age 31 to 67 y; mean of 51.4 y) with well-documented cardiovascular abnormalities and neurological outcomes of SCI were analyzed. All subjects had cervical SCI (ASIA A 3. B 1. C 1). The mean survival in the post-injury period was 11.6 mos. (3.5 to 36 mos.). Severe hypotension immediately after SCI was observed in 3 individuals. Two of these subjects also developed autonomic dysreflexia. The histopathological findings from these individuals were compared to findings from 2 individuals with no cardiovascular abnormalities. There were no significant differences in the severity of demyelination (demyelinated areas: 24.7% vs. 9.3%; $P = 0.2$) between two groups. The number of axons within areas I & II of DVP in individuals with cardiovascular dysfunctions was significant lower than subjects without cardiovascular abnormalities ($P < 0.001$). Moreover. area I in these individuals also had fewer axons compared to area II ($P < 0.001$). These data suggest that severity of destruction of DVP could contribute to cardiovascular abnormalities after SCI. (Supported: Christopher Reeve Paralysis Foundation; Heart & Stroke Foundation of Ontario).

P510.

ECG FINDINGS IN ACUTE SPINAL CORD INJURY IN HUMANS

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Significant impairment of hemodynamic parameters occurs after acute spinal cord injury (SCI). However. the relationship between ECG changes the level and severity of injury is poorly understood. We conducted an analysis of ECG recordings of 17 consecutive patients (F 4. M 13; age 31–83 y.o.) in order to examine the incidence of abnormalities in heart rate and ECG in individuals with acute SCI. ECG data [atrial (APC) and ventricular (VPC) premature contractions; changes in T. ST. Q waves; ventricular tachicardia (VT). atrial fibrillation (AFIB). and supraventricular tachicardia (SVT)] were compared at day 1 and day 7 after SCI. Based on the level and severity of SCI subjects were divided into three groups: group1 ($n = 6$)—high cervical injuries. ASIA A-B; group2 ($n = 6$)—high cervical injuries. ASIA C-D; group3 ($n = 5$)—low lumbar injuries. ASIA A-D. Abnormalities in heart rate and ECG were predominantly observed in high cervical injuries (group 1): APC—16% (day 1); ST changes—50% (day 1) and 25% (day 7); T wave changes—16% (day 1) and 50% (day 7); AFIB—25% (day 7). This represents a significant clinical concern during acute phase of SCI. the correlation between the severity of injury of descending cardiovascular pathways and changes in heart rate and ECG are under investigation. (Supported by Christopher Reeve Paralysis Foundation; Heart & Stroke Foundation of Ontario).

P511.

CERVICAL SPINAL INJURY WITH ESOPHAGEAL RUPTURE: REPORT OF TWO CASES

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Two consecutive cases of cervical spinal injury complicated with esophageal rupture were encountered recently. Two cases were all dislocated burst fracture. one at C5-6. another C6-7. The C5-6 patient was operated in Thailand and transferred to us 11 days later. The C6-7 patient was operated by us. The operation was anterior discectomy. open reduction. corpectomy. autogenous bone graft and plate instrumentation. The presenting symptoms and signs were dysphagia. delayed subcutaneous emphysema and wound infection. In a period of 10 years retrospective study of surgical treatment of cervical spinal injury in our hospital. we found 46 cases of fracture dislocation. 35 burst fracture. 28 dislocation and 12 compression fracture. No esophageal rupture was found until recently. Both were proved by endoscope 15 and 17 days after surgery respectively. Esophagogram could not be performed due to dysphagia. Preoperative endoscopic examination sometimes cannot find the rupture in acute stage but should be done when patients have dysphagia or subcutaneous emphysema.

P512.

HP184 IS NEUROPROTECTIVE AND IMPROVES LOCOMOTOR FUNCTION AFTER MODERATE ACUTE SPINAL CRUSH INJURY IN RATS

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After central nervous system injury. neuronal ATP production is reduced. thereby compromising the maintenance of the sodium ($Na+$) gradient across the cell membrane. Thus. reduction of energy demand by down-modulation of voltage-gated $Na+$-channels has been tested as a rational strategy for neuroprotection. HP184 (Aventis) is a voltage-dependent blocker of potassium currents in PC12 cells and a use- and frequency-dependent blocker of sodium channels. To test whether HP 184 is neuroprotective in acute spinal cord injury. rat spinal cords were compressed to a moderate level (Gruner et al.. Brain Res.. 729:90–101. 1996). Within 15 minutes of crush (day 1). rats in HP 184 designated groups received ip injections or po gavage of 20. 10. 5 or vehicle. This administration was repeated on days 2 and 3. Methylprednisolone (MPSS). on the other hand. was administered at 60 mg/kg ip at 15 minutes. and at 30 mg/kg ip at 2 hours. 4 hours. and 6 hours on day 1 after crush. This MPSS dosing schedule has been described as optimal in the literature. and mirrors the dosing performed in humans. HP184 significantly improved locomotor function in open field walking task. hind limb placement and foot orientation testing as compared to vehicle control. as did the MPSS treatment. Behaviour was evaluated on days 1. 2. 3. 7. 10. 14. 21 and 28. Most of the functional improvement occurred within the first 24 hours. consistent with a neuroprotective effect of HP184.

P513.

INFLUENCE OF CRANIOPLASTY ON CEREBRAL BLOOD FLOW AND CARDIAC FUNCTION

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Objective: Cranioplasty is usually performed for aesthetic. protective and patient comfort reasons. However. there are many theories suggesting that an underlying physiological alteration may occur that may require the correction of the bone defect. The objective of this study is to prove that cranioplasty improve the cerebral blood flow by decrease the cardiac after-load. Methods: Twenty-two patients who had taken bone removal to prevent uncontrollable intracranial hypertension were included in this study. Arterial flow velocities were checked in MCA and ICA by TCD. and cardiac function was checked by echocardiogram. And the last ten patients. cerebral blood flow was measured perfusion CT. Results: The blood flow velocity of the lesion side was decreased from 48.8 ± 16.0 to 36.4 ± 14.2 cm/sec at the MCA and from 32.9 ± 8.9 to 28.1 ± 7.5 cm/sec at the ICA ($P < 0.05$). And the opposite side it was decreased from 56.7 ± 14.5 to 40.6 ± 14.2 cm/sec at the MCA and from 32.2 ± 7.6 to 26.7 ± 6.4 cm/sec ($P < 0.05$) at the ICA. On the cardiac function evaluation by measuring the stroke volume. was increased from 66.1 ± 18.6 to 73.4 ± 18.8ml/bt. ($P < 0.05$). Cerebral blood flow evaluated by perfusion CT shows increased values. from 30.2 ± 9.3 to 45.4 ± 12.1 ml/100g/min. on lesion side and from 29.9 ± 6.8 to 44.7 ± 12.7 ml/100g/min. on opposite side ($P < 0.05$). But there was no correlation between the changes of blood flow velocity and the stroke volume changes ($P > 0.05$). Conclusion: We can conclude that disappeared atmospheric pressure after cranioplasty. lessened the peripheral resistance and this can increased the cerebral blood flow and stroke volume without alteration of the systemic blood pressure. It appeared that skull bone defect be repaired as soon as possible. because cranioplasty has not only aesthetic or protective but also systemic therapeutic effect. (This study was supported by a grant of the Korea Health 21 R&D Project. Ministry of Health & Welfare. Republic of Korea.HMP-00-CN-01-0018).

P514.

VOLUMETRIC PROTON MR SPECTROSCOPY OF MILD TRAUMATIC BRAIN INJURY

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Introduction: Proton MRS can assess biochemical changes of traumatic brain injury (TBI). Several single volume spectroscopy studies have evaluated metabolite levels at a few specific locations within the brain [1–2]. In this study. we used a volumetric proton MR spectroscopic imaging (MRSI) method to observe distributions of N-acetyl aspartate (NAA). creatine (Cr) and choline (Cho). providing neurochemical information from the entire brain. Methods Twelve subjects (6 patients with Glasgow Coma Score (GCS) _14 and 6 controls) were studied at 1.5T. between 2 and 24 days after injury. A 70 ms TE 3D spin-echo excitation sequence was used [3]. with 1.15 cm3 nominal voxel. Ratios of NAA. Cr. and Cho areas from 25 different regions in 5 MRSI slices were obtained using an automated spectral analysis method [4]. Student's one tail t-test was used. Results and Discussion Our volumetric method allowed at least five contiguous 15 mm-MRSI slices to be obtained within the brain. Some regions were excluded because of strong B0 inhomogeneities. In one patient. NAA loss was seen in the immediate neighborhood of regions of MRI-observed injury. No evidence of increased lactate was seen. Metabolite ratios calculated from 25 locations in five slices showed no statistically significant difference between patients and controls. References 1. Ross. B.D. et al. J. Magn. Reson. Imag.. 8. 829. 1998. 2. Brooks. W.M.. et al J. Neurotrauma. 17. 629. 2000. 3. Ebel. A. et al. Magn. Reson. Med.. 46. 1072. 2001. 4. Soher. B.J. et al.. Magn Reson. Med.. 40. 822. 1998. Acknowledgments This study was supported by PHS grants AG12119 and NS38029.

P515.

BRAIN TISSUE OXYGEN REACTIVITY IN SWINE UNDER DIFFERENT CO2 AND BLOOD PRESSURE LEVELS

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Introduction: Brain tissue oxygen tension (PbrO2) measurements are being used increasingly in the treatment of traumatic brain injury (TBI). PbrO2 is known to be responsive to changes in mean arterial pressure (MAP). arterial CO2 (PaCO2) and inspired oxygen concentration (FiO2). Because low PbrO2 values are associated with poor outcome. increasing the FiO2 to improve PbrO2 has been proposed as a therapeutic intervention. However. the physiologic and metabolic consequences of this are not fully understood. We examined the effects of increasing FiO2 in uninjured swine brain at various levels of PaCO2 and MAP. Methods: PbrO2 . MAP. cerebral blood flow (CBF). and blood gases were monitored in swine (n = 6). FiO2 was adjusted to obtain PaO2's of 100. 300. and 500 mm Hg at each PaCO2 level of 25. 40. and 60 mm Hg at a constant MAP (85–90 mm Hg). The FiO2/PaO2 "ramp" was repeated for MAP's of 40. 60 and 150 mm Hg and a constant PaCO2 (38–42 mm Hg). Results: With MAP and PaCO2 at normal levels. increasing the PaO2 from 100 to 500 mm Hg increased PbrO2 from 19 ± 7 mm Hg to 44 ± 18 mm Hg. At a PaO2 of 500 mm Hg. PbrO2 decreased during hypocapnia (PaCO2 25) and hypotension (MAP 40) to 33 ± 15 mm Hg and 11 ± 9 mm Hg respectively. Elevations in PbrO2 occurred during hypercapnia (PaCO2 60. PbrO2 82 ± 27 mm Hg) and hypertension (MAP 150. PbrO2 83 ± 21 mm Hg). PbrO2 also increased during mild hypotension (MAP 60. PbrO2 73 ± 16 mm Hg). Conclusion: As anticipated. the reaction of PbrO2 to increased PaO2 levels is altered at different blood pressure and arterial CO2 levels. The unexpected finding of increased reactivity during mild hypotension indicates a complex relationship between oxygen carrying capacity and flow. It also underscores the importance of further elucidating this relationship prior to implementing increased FiO2 as a therapeutic intervention for patients with TBI.

P516.

FUNCTIONAL MAGNETIC RESONANCE IMAGING AFTER ACUTE MILD TRAUMATIC BRAIN INJURY

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In the context of a large-scale study of physical and neurocognitive recovery from concussion in high school football. functional magnetic resonance imaging (fMRI) was used to study 15 players with Grade 2 (n = 10) or 3 (n = 5) (American Academy of Neurology guidelines) concussion within 12–18 hours of injury. Teammates matched to the injured players on a preseason neurocognitive measure also underwent fMRI studies. Whole brain. echoplanar imaging was used to investigate changes in blood oxygenation level dependent (BOLD) signal changes during two fMRI protocols: (1) A verbal working memory task (Sternberg paradigm) was used to investigate the effect of memory load on brain activation. Use of similar tasks in functional imaging studies have shown a pattern of increased activation in mild traumatic brain injury. presumably due to recruitment of increased attentional resources for task performance in the injured compared to the control participants. (2) Coherence in spontaneous low frequency BOLD signals between a region of interest in the right and left hemisphere during rest was studied as a measure of interhemispheric connectivity. The region of interest in each hemisphere was defined functionally during a separate fMRI protocol using finger movement as the activation protocol. Imaging findings showed a similar pattern of functional activity in the injured and control participants in both the working memory and resting scan protocols. These findings were consistent with neuropsychological testing completed at the same time interval. which also failed to show differences between injured and noninjured players. Despite the suggestion of quick cognitive recovery. injured players continued to report postconcussive symptoms for approximately one week after injury. Overall these findings suggest that individuals with Grade 2 and mild Grade 3 concussions recover neurocognitive capabilities and related neurophysiological function (as measured with fMRI) relatively quickly following injury. and that this recovery precedes recovery of postconcussive symptomatic complaints.

P517.

PRIMARY AND DELAYED ISCHEMIA IN SEVERE TRAUMATIC BRAIN INJURY

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The frightening growth of patients with severe forms of Traumatic Brain Injury (TBI) demands more investigations for understanding of the pathophysiology of that dangerous disease. For the in-time detection and continuous monitoring of Cerebral Blood Flow Velocity (CBFV) infringements in 78 patients with severe forms of TBI we used the method of Transcranial Ultra-sound Dopplerography (TCD). For further analysis we put the records of CBFV quantitative characteristics in M1 MCA and Basilar Artery. The TCD recordings of CBFV were performed starting first hours of TBI onset and were continued during next 14th–17th days to reveal any disturbances of flow velocity if they were present. Our investigations reveal succeeding each other similar processes of cerebral hypoperfusion (expressed decrease of CBFV). hyperemia (sufficient increase of CBFV). and cerebral vasospasm development in all patients with severe forms of TBI. The hypoperfusion stage lasts during first hours and day after TBI. and leads to very dangerous low cerebral circulation (mean speed in M1 MCA was decreased about two times. equal to 30–40 cm/sec). We find the straight correlation of the CBFV drop level and risks of brain ischemia development. On days 2nd–3rd there was develop hyperemia. The increase of CBFV in two times means the expressed narrowing of the diameter of the artery. and indicate vasospasm. and can leads to secondary brain tissue ischemia on days 6th–9th due to insufficient blood supply. The TCD signs of vasospasm were detected on days 4th–5th meanwhile the clinical signs usually develop during subsequent second-third days. So. first days and beginning of the second week of TBI onset are the most dangerous days of cerebral ischemia development and sufficient exacerbation of the disease. The mechanisms of ischemia are different but clinical signs are similar and demand steadfast observation and applicable treatment.

P518.

DIFFERENTIAL ALTERATIONS OF VASCULAR REACTIVITY FOLLOWING TRAUMATIC BRAIN INJURY

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Objective: Alterations of cerebral microcirculation may contribute to the development of secondary tissue damage after traumatic brain injury (TBI). The pursuit of the study was to characterize alterations of cerebrovascular reactivity following experimental TBI. Methods: In male SD rats anesthetized with chloralhydrate (360 mg /100 g i.p.) global TBI was induced by weight drop (lesion parameters: weight. 350g; height. 28.5cm). After 24h or 48h rats were killed and ring segments prepared from the basilar artery (BA) and the middle cerebral artery (MCA) for measurement of isometric force. Vasoactive stimuli included i) contraction by incubation in 124 mM K+-Krebs solution or endothelin (ET)-1. ii) endothelium-dependent nitric oxide (NO)-mediated relaxation by acetylcholine (ACh. BA). bradykinin (Bk. MCA) and the selective ETB-receptor agonist. sarafotoxin-6c (S6c) and iii) endothelium-independent relaxation by sodium nitroprusside (SNP) and 8-bromoguanosine cyclic-monophosphate (cGMP). Results: Contraction due to 124 mM K+-Krebs (reference contraction) was markedly decreased after trauma (MCA/BA: control. 2.1 ± 0.8 / 5.1 ± 2.0 mN; 48h after TBI. 1.3 ± 0.4* / 3.2 ± 1.9* mN; *$p < 0.001$) as were contractions upon serotonin (5-HT. BA) and the thromboxane mimetic U46619 (MCA). Relaxation upon ACh and Bk was significantly enhanced whereas endothelium-independent relaxation induced by SNP and cGMP were not significantly altered. However. ET-1 induced contraction was significantly enhanced after TBI (MCA/BA; % reference: control. 104 ± 40 / 95 ± 17; TBI. 166 ± 34* / 142 ± 52*; *$p < 0.05$) while S6c induced relaxation was shifted to higher concentrations (MCA/BA; pD2: control. 9.6 ± 0.5 / 10.0 ± 0.7; TBI. 7.9 ± 0.4* / 8.2 ± 0.7*; *$p < 0.001$). Conclusion: TBI results in differential alterations of cerebrovascular reactivity. Contraction to a variety of stimuli was decreased. while NO-mediated relaxation appeared preserved or even enhanced. In contrast ET-1 induced contraction was increased. probably due to a decrease of ETB-receptor mediated relaxation.

P519.

CASPASE INHIBITION ATTENUATES MITOCHONDRIAL RELEASE OF CYTOCHROME C AND APOPTOSIS-INDUCING FACTOR AFTER TRAUMATIC BRAIN INJURY IN RATS.

Paula D. Nathaniel, Xiaopeng Zhang, Patrick M. Kochanek, C. Edward Dixon, Robert S. B. Clark. (Depts. of Critical Care Med. Pediatrics, and Neurological Surgery, Safar Center for Resuscitation Research, U. of Pittsburgh, PA USA).*

The pathobiology of traumatic brain injury (TBI) includes activation of multiple caspases followed by cell death with a spectrum of apoptotic phenotypes. Recently. activated caspase-2 was shown to have the capacity to induce release of cytochrome c (cyt c) and apoptosis-inducing factor (AIF) from mitochondria. establishing itself as a direct effector of the mitochondrial apoptotic pathway and adding an additional level of regulation into the caspase cascade. We examined the biochemical. histopathologic. and functional outcome effects of the pancaspase inhibitor boc-aspartyl(OMe)-fluoro-methylketone (BAF) after controlled cortical impact (CCI) with secondary insult in rats. First. rats were randomized to receive 0.1. 0.5. or 1 _mol BAF or vehicle (DMSO) via single ipsilateral hippocampal injection 1 min after CCI (n = 4-6/group). At 24 h. caspase-3 activity. TUNEL+ neurons. and hippocampal neuronal loss were reduced in a dose dependent manner. To define the effect of BAF on intracellular trafficking of apoptogenic factors. Western blots on subcellular protein fractions were performed using antibodies against cyt c. AIF. and caspase-2. 1 _mol BAF reduced cytosolic cyt c. nuclear AIF. and proteolysis of caspase-2 vs. vehicle. An outcome study testing the effect of hippocampal injection of 1 _mol BAF with or without nerve growth factor (NGF. 12.5 ng/h x 14 d i.c.v. via osmotic pump) did not reveal differences in motor function (d 1–5). Morris water maze performance (d 14–20). hippocampal neuron survival. nor contusion volume (n = 9–11/injured group; n = 5/sham group). These data suggest that pancaspase treatment with BAF reduces acute cell death by inhibiting mitochondrial release of cyt c and AIF. possibly via a mechanism involving caspase-2. This effect appears temporary: however and supports further study using chronic administration of caspase inhibitors. Support: NS 38620 and 30318.

P520.

TEMPORAL SEQUENCE OF POLY (ADP-RIBOSE) POLYMERASE EXPRESSION IN TRAUMATIC BRAIN INJURY IN HUMANS

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Poly (ADP-ribose) polymerase (PARP) catalyses covalent post translational modification of nuclear proteins during an eukaryotic cellular response to DNA breakage. In acute brain injury however. there is over-activation of PARP which depletes its substrate nicotinamide adenine dinucleotide (NAD) and then adenosine triphosphate (ATP) storage. leading to cellular energy depletion and cell death. Additionally. PARP is a substrate for caspases in apoptosis. We sought to determine the temporal characteristics of PARP expression in human peri-contusional tissue. Of 19 patients who had surgery for traumatic contusions (Marshal's Class 5 on CT). 12 were suitable for analysis. Ethics approval was obtained from our local institutional ethics committee. These patients were managed according to a standard severe TBI protocol. Demographics. opening ICP. ICP at 12 and 24 hours and PARP expression characteristics were studied PARP was expressed in 8 of 12 specimens (67%). Differences were noted with regards to the predominant site of PARP expression. In patients who were operated early (less than 4 hours after event). PARP expression was predominantly cytoplasmic versus patients operated late (>4 hours) which showed more nuclear expression ($p < 0.05$). There were no difference in demographics and ICP values in these 2 groups. It appears that there is a temporal difference in site of PARP expression. Early expression of PARP appears to be more cytoplasmic versus late expression which tends to be predominantly nuclear. PARP cleavage products have been shown in in vitro cellular assays to translocate from the nucleus to the cytoplasm during apoptosis. This may suggest that PARP may mediate cell death via apoptosis rather than necrosis in the initial period following brain injury.

P521.

INCREASED PHOSPHORYLATION AND NUCLEAR TO CYTOSOLIC TRANSLOCATION OF FORKHEAD TRANSCRIPTION FACTOR IN RAT CORTEX AND HIPPOCAMPUS AFTER TRAUMATIC BRAIN INJURY.

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The Protein kinase B (PKB) signaling pathway may play a critical role in the regulation of cell survival after traumatic brain injury (TBI) and cerebral ischemia. Forkhead transcription factor (FKHR) is one substrate of activated PKB. and is a positive regulator of Fas ligand gene expression. We previously showed increased phosphorylation of FKHR concordant with decreased Fas ligand in human brain after TBI. To investigate intracellular trafficking of FKHR in vivo. adult rats (n = 25) were subjected to controlled cortical impact (CCI) with imposed secondary hypoxemic insult. At 2. 6. 24 and 72 hours after injury. animals were sacrificed and dorsal cortices and hippocampi were dissected. NaÔve rats were used as controls. Proteins from whole cell lysates. nuclear and cytosolic enriched fractions were prepared and Western blots using antibodies against PKB. phosphorylated PKB (pPKB; Ser473). FKHR and phosphorylated FKHR (pFKHR) were performed. Total PKB levels from ipsilateral cortex and hippocampus were similar in controls and all timepoints after CCI. In contrast. pPKB was increased after injury in both ipsilateral cortex and hippocampus vs. control. A significant amount of PKB was observed within the nuclear fractions from both cortex and hippocampus. Relative protein levels of FKHR were increased in the cytosolic fraction of both cortex and hippocampus as early as 2 h after injury. There was a decrease in FKHR in cortical nuclear fractions but an increase in FKHR in hippocampal nuclear fractions after injury. Total pFKHR was increased in both cortex and hippocampus after injury vs. control. These preliminary results suggest that after TBI. the transcription factor FKHR may translocate from the cell nucleus to cytosol. were it has the potential to be phosphorylated by PKB. sequestering it in the cytosol and thereby reducing transcription of its target genes that include Fas ligand. Support: NS 38620 and 30318.

P522.

AGE-DEPENDENT SUSCEPTIBILITY TO OXIDATIVE STRESS AFTER TRAUMATIC BRAIN INJURY IN THE DEVELOPING BRAIN

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Children less than 4 years have worse outcome after traumatic brain injury (TBI) compared to older children and adults. This increased susceptibility may in part be due to differences in the response to oxidative stress. We hypothesized that the immature brain does not have an adequate compensatory response to injury from oxidative stress. We examined glutathione peroxidase (GPx) activity in cortical and subcortical regions in postnatal day 21 (P21) and adult mouse brain following a controlled cortical impact. Brain dimensions were measured to adjust the parameters of the impact accordingly. No significant differences were found between P21 and adult brain in the dimensions studied. except that the cortical mantle of the P21 brain was thicker than adult ($p = 0.01$). Injury was assessed by measuring brain edema through changes in water content. and the response to oxidative challenge was identified by changes in GPx activity. The P21 brain exhibited more prominent edema compared to adult ($p < 0.0001$). GPx activity in the adult brain was increased at 3 hours (ANOVA. $p < 0.05$ over the injured cortex) and remained high until 24 hours post-injury whereas there was no compensatory change in GPx activity in P21 brain. although absolute levels had reached adult levels developmentally. These findings support our hypothesis and illuminate the important role of oxidative stress after TBI in the developing brain. (This research supported by the UC Neurotrauma Program and NS 41256.)

P523.

PEDIATRIC CCI ALTERS THE PHOSPHORYLATION STATUS OF TWO KEY PROTEIN KINASES, p70S6K AND p90RSK

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Previous data from our laboratory showed phosphorylation changes of protein kinase B (PKB) and two key protein synthesis initiation factors. eukaryotic initiation factor 4E (eIF4E) and eukaryotic initiation factor 2a(eIF2a) after pediatric TBI suggesting an acute upregulation followed by a longer lasting downregulation of PKB dependent pathway protein synthesis regulating activity. Two other serine/threonine kinases. p70S6K and p90RSK kinase. are also important in developmental protein synthesis control. p70S6K is a mitogen activated protein kinase that is activated by target of rapamycin kinase (mTOR). atypical protein kinase C zeta (PKCz). phosphoinositide dependent kinase 1 (PDK1) as well as the mitogen activated protein kinases. ERK $^1/_2$. p90RSK is a ribosomal protein kinase activated by MAPKs such as ERK $^1/_2$. When activated p70S6K and p90RSK in turn phosphorylates ribosomal S6 kinase. important in both the translation of 5' terminal oligopyrimidine tract (5'TOP) mRNA and cap-dependent (eIF4E pathway) mRNA resulting in selective expression of growth associated proteins such as ribosomal proteins and elongation factors. We evaluated the level and distribution of brain phospho-p70S6K and phospho-p90RSK activity in injured or sham PND 17 rats at 6. 24 or 72 hr after moderate (4 m/ sec. 2.0 mm deflection) controlled cortical impact (CCI)) using immunohistochemistry (n = 5/group). Increases in the phosphorylation of p70S6K and p90RSK activity in CA1 pyramidal cell bodies only were found at 6 hr with decreased activity at 24 and 72 hr in all pyramidal neuron sectors following CCI. Decreased phosphorylation (activation) of p70S6K and p90RSK should decrease both cap-dependent and 5'TOP translation at 1–3 days after CCI providing additional evidence that nutritive and trophic factor enhancement of protein synthesis should be useful after pediatric CCI for both normal developmental growth as well as injury repair. (Supported by NIH NS40049 and NS01809).

P524.

DIFFERENTIAL EXPRESSION OF GENES RELATED TO CELLULAR SIGNALING, SYNAPTIC FUNCTIONING AND ION CHANNELS POST-INJURY: A COMPARISON OF MODERATE AND SEVERE INJURY EFFECT ON GENE EXPRESSION IN HIPPOCAMPUS

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Cognitive deficits following traumatic brain injury (TBI) are often related to the function of hippocampus. The systematic study of molecular events within this structure following traumatic brain injury (TBI) is essential for better understanding of neuropathology after TBI. Lateral fluid percussion injury was administrated to adult rats and gene expression profiles in hippocampus of moderately (n = 12) and severely (n = 12) injured brains were examined using the microarray technique. Of ~8.700 genes and expressed sequence tags (ESTs) examined. 760 genes and ESTs showed dynamic changes (>2 fold) at either 30 min. 4 h or 24 h following severe injury. while 342 genes and ESTs were altered after moderate injury. One fundamental difference in gene expression profiles found between moderate and severe injury was that majority of genes with altered expression were up-regulated following severe injury while they were down-regulated following moderate injury. Genes that have altered expression participate in diverse cellular activities. Differential time courses were observed in expression of most genes between moderate and severe groups. Among the differential expressing genes are those related to cellular signaling. synaptic functioning and ion channels. NS30308. NS37365. NS27544.

P525.

GENE EXPRESSION PROFILING FOLLOWING TRAUMATIC BRAIN INJURY IN WILD TYPE AND ALZHEIMER TRANSGENIC MICE

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The nature and extent of recovery after traumatic brain injury (TBI) is heterogeneous and not fully explained by known demographic and injury prognostic features. suggesting that additional factors such as genetic influences modulate the secondary injury/recovery pathways. We and others have shown that the e4 allele of the apolipoprotein E gene influences recovery following TBI. and variation in other genes influential in neurogenesis. neurodegenerative or neural repair mechanisms will likely modify recovery. Changes in gene expression that occur as a response to TBI implicate those genes and their encoded proteins in the cell injury and/or recovery process and identify targets for possible therapeutic intervention. Experimental modeling of TBI in wild type and transgenic mouse models of human disease and neurodegeneration. provide an environment in which some of the confounding variables (e.g. injury severity. patient demographics) can be controlled. and the influence of specific genes can be investigated. Using microarray technology we are generating age-. time- and genotype-dependent gene expression profiles in wild type mice and mouse models of Alzheimer's disease. following TBI or sham-injury. and correlating these with measures of blood flow and behavior. In addition to genes which have already been reported to respond to TBI (e.g. those encoding heat shock proteins. neurotrophic factors. metalloproteinases) we have identified responses from genes encoding neurospecific peptides and proteins which implicate previously unreported cell pathways as important in the neurodegenerative and neuroregenerative sequelae following brain injury. Supported by: The Roskamp Foundation and the Center for Traumatic Brain Injury Studies.

P526.

GENE EXPRESSION PROFILES FOLLOWING MODERATE AND SEVERE BRAIN INJURY IN RATS: IMPLICATION OF ENERGY SHORTAGE AND CELLULAR VULNERABILITY IMMEDIATELY AFTER INJURY.

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Immediately following traumatic brain injury (TBI). there is an activation of ionic pumps resulting in an increase in glucose metabolism. which appears responsible for the reduction in ATP content within injured tissue. In addition. levels and durations of ATP reduction were associated to injury severity. In this study. adult rats (n = 8) sustaining a moderate or severe lateral fluid percussion injury were used to assess how these acute physiological responses are related to injury-induced genetic alterations. Using oligonucleotide GeneChip array (Affymetrix). we screened for the gene expression profiles of injured rats within the ipsilateral cortex and hippocampus and compared them to that of sham-injured controls. Thirty minutes after injury. a large number of genes was induced in the severely injured group for both cortex and hippocampus. The number of induced genes was significantly reduced 30 min following moderate injury. compared to that of severely injured animals. Five probe sets each encoding either alpha or beta subunits of Na+. K+-ATPase were induced up to 7.5 fold in hippocampus following severe injury. along with genes encoding many other voltage-gated ion channel proteins. Only one probe set for the Na+. K+-ATPase was induced in cortex following severe injury. A few genes that fall into this category showed an attenuated expression in moderately injured hippocampus. These findings suggest that the activation of ionic pumps after severe injury is regulated at the level of mRNA expression and provide a potential guide to reduce the level of energy crisis by gene therapy. NS30308. NS37365. NS27544.

P527.

MICROARRAY GENE EXPRESSION ANALYSIS OF POSTNATAL DAY 26 RAT CORTEX AFTER LATERAL FLUID PERCUSSION INJURY

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Our laboratory has previously described the distinct physiological. metabolic. behavioral and plasticity responses of postnatal day (PND) 26–30 rats to traumatic brain injury. The current study was conducted to screen for changes in gene expression following TBI in "juveniles." PND26 Sprague-Dawley rats were given either sham surgery (n = 8) or moderate fluid percussion injury (n = 8). At 4 and 24 hours after injury. animals were sacrificed. S1 cortex was dissected and frozen. Total RNA was isolated. labeled and hybridized to Rat Genome Arrays (Affymetrix). Injury severities between 4 and 24 hr groups did not differ significantly as reflected by apnea (96 ± 43.9s and 113 ± 41.5s. respectively) or time to toe pinch response (229 ± 95.3s and 194 ± 52s. respectively). Overall changes in gene expression at 4 hours reveal >=2-fold change in expression in 52 genes with 15.4% showing downregulation and 84.6% upregulation. By 24 hours post injury >=2-fold change in gene expression was detected in 34 genes (38.2% decrease. 61.8% increase). Genes related to metabolism (lactate dehydrogenase B. HMGCoA reductase) showed upregulation at 4 hours. but returned to sham levels by 24hrs. There was an early induction of several transcription and stress-related genes (NFkappaB. hsp70. grp78). but glia-related gene (GFAP. vimentin. s100) were only induced at 24 hours. This approach will help identify those genes that are involved in the pathophysiological response of the juvenile brain to TBI. NS30308. NS37365. NS27544 and the Lind Lawrence Foundation.

P528.

CHARACTERISATION OF A HIGHLY ADAPTABLE, NEW MODEL OF DIFFUSE TRAUMATIC BRAIN INJURY IN RODENTS

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Diffuse injury is a major feature of clinical traumatic brain injury. Despite this. few experimental models of diffuse traumatic brain injury exist. Those that do are limited in terms of their suitability for use in smaller animals. be they simply using immature animals for developmental studies or using the model in a smaller animal species (1,2). We have therefore developed a highly controlled model of diffuse traumatic brain injury that offers tremendous utility in its application to rodents. An adaptation of the Marmarou impact -acceleration model (1). the injury device delivers a hydraulically controlled. high-velocity impact to a steel disc cemented onto the rodent skull. The distance the impactor travels after contacting the steel disc is under user control thus varying the injury severity. Force of impact is recorded on an oscilloscope. The head is decelerated after impact using a molded gel-filled base upon which the animals head is supported during injury. Using an 18 mm injury. we demonstrate that the model results in moderate to severe motor and cognitive deficits. Diffusion weighted magnetic resonance imaging shows edema development within the first few hours after trauma with a maximum at 24 h. Phosphorus magnetic resonance spectroscopy confirmed that there was no energy depletion or pH changes after trauma. although significant declines in brain free magnesium concentration were observed as has been described in other models of diffuse traumatic brain injury. The diffuse appearance of amyloid precursor protein. considered to be a marker of axonal injury. was apparent in immunohistochemistry studies. These results confirm that this new model produces biochemical and neurological changes consistent with the diffuse axonal injury produced in other models. but with the added utility of being adaptable to various rodent sizes. (1) Marmarou. A.. Foda. M.A.A.. Van den Brink. W.. Campbell. J.. Kita. H. and Demetriadou. K. (1994) J. Neurosurg.. 80: 291–300. (2) Smith. D.H.. Chen. X.H.. Xu. B.N.. Mcintosh. T.K.. Gennarelli. T.A. and Meaney. D.F. (1997) J. Neuropath. Exp. Neurol.. 56: 822–834.

P529.

EXTRA- AND INTRACRANIAL PRESSURE PULSES DURING FLUID PERCUSSION INJURY

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Fluid percussion injury (FPI) is one of the most commonly used animal models of traumatic brain injury (TBI) and is well characterized in many aspects. However. the pressure pulse delivered to the brain is often estimated from recordings of the extracranial pressure wave. Here. we sought to compare the extra- and intracranial pressure waves measured simultaneously during FPI. To measure the pressure delivered from the FPI device into the skull cavity we used thin. flexible optic pressure probes (Samba Sensors. Göteborg. Sweden) with a diameter of 0.4 mm inserted into either the ipsilateral or the contralateral lateral ventricle. The pressure in the FPI-device was measured in the nozzle. 65 mm from the exposed dura mater. Simultaneous measurement of the pressure pulses extracranially and intracranially was done using two Samba Sensor 3000 optic pressure monitors connected to a computer with recording software with a frequency of 500 Hz. The FPI-device was set to deliver a pulse with 2.8–3.0 atm pressure. resulting in a moderate to severe injury with an apnea duration of 15–20 s. The extracranial pressure pulse showed a steep incline with a mean peak value of 2.92 ± 0.15 atm. followed by a gradual return towards the baseline over the ensuing 1200 ms. The intracranial pulse showed a similar curve pattern with a mean peak value of 2.77 ± 0.30 atm. We found no significant difference between the pressure pulse in either ventricle compared to the extracranial signal. This suggests that the transient pressure pulse during FPI is similar intracranially and extracranially.

P530.

RELATIONSHIP OF APPROPRIATE HISTOLOGICAL PROCESSING TECHNIQUES AND ACCURATE EVALUATION OF EXPERIMENTAL BRAIN INJURY

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Accurate assessment and quantitation of large brain lesions has proven difficult for a variety of reasons. including the inability to accurately evaluate spatial and volumetric parameters. We propose a novel technique for reliable. consistent and reproducible paraffin-embedded histological sectioning and volumetric lesion analysis. Our technique has allowed for accurate qualitative and quantitative lesion assessment following clinical and experimental brain alterations such as trauma or therapeutic intervention. Nine Yorkshire domestic piglets. between the ages of 5 day old and 4 month old. underwent a scaled cortical impact injury to the fronto-parietal cortex. The subjects were then perfusion fixed. and the brains removed and processed for routine histology. Following a specific trimming technique. we were able to accurately compute lesion volume analyses through the use of parallel coronal 5mm sections and the use of AIS Software (Analytical Imaging Station). Lesion quantitations were computed by expressing the area of the lesion as a ratio of the injured area divided by the uninjured area of the contralateral hemisphere. This method was then used to determine the average lesion size across 5 comparable serial sections throughout the injured zone. both within and between subjects of different ages at specific time points. Using this protocol. edema. hemorrhage. asymmetry and various Magnetic Resonance Image signal abnormalities can be defined and co-registered with other parameters. such as histology. Our methodology permits accurate quantitative assessment and co-registration of histologic. radiologic and immunohistochemical images. It is now clear that the accurate co-registration of these parameters is one of the more important and efficient methods for understanding central nervous system (CNS) disease. injury and therapeutic mechanisms.

P531.

CLINICORADIOLOGICAL CLASSIFICATION OF TRAUMATIC INTRACEREBRAL HEMATOMAS (THI).

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Based on the clinical and CT data of 94 consecutive patients with traumatic intracerebral hematomas the classification is offered. The classification takes into account the following criteria: 1. Location of TIH: cortical-subcortical—settle down in white substance of hemispheres and grasp cortex of a brain with (or without) diffusions in subdural space (19 %); subcortical—locate in the white substance of hemispheres of the brain (74 %); central—location medially of a putamen (7 %); the cerebellum (3 %). 2. Size of TIH: small—the maximal diameter in most demonstrative scan on CT is equal or more than 1.5 cm and less than 3 cm (volume of the sphere is about 2–15 cm3) (18 %); average—the diameter is equal or more than 4.5 cm(15 –45 cm3) (57 %); large—the diameter 4.5 cm or more (25 %). 3. Peculiarities of the formation TIH: in the focus of contusions (88 %); without any sings of the contusions (12 %); 4. Time of formation TIH: primary—are formed directly after a trauma (93 %); deferred—24 hours and later after a trauma (7 %). 5. Combination TIH: single (58 %); multiple (19 %); with subdural or epidural hematomas (23 %); with the focus of contusions of a brain on a distance (34 %). 6. Type of clinical current THI: without a light interval (13 %); without light interval (40 %); with the complete light interval (16%); with gradual restoration of the consciousness after its primary loss (31%).

P532.

CLINICAL CRITERIA OF SORTING, PROGNOSIS OF OUTCOMES AND TREATMENT OF THE PATIENTS WITH A CRANIOCEREBRAL TRAUMA AT STAGES OF MEDICAL EVACUATION

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This work is based on the results of the unified complex study of 561 patients with a serious cranio-cerebral trauma (CCT). verificated by outcomes from neirosurgery clinics of 4 countries (Uzbekistan. Russia. Lithuania. Estonia) with use of the Moscow scale of a coma and mark estimation of a condition of the patients (Shakhnovich A. R.. 1986. Mamadaliev A.M. 1987. 1988) 175 patients were subjected to surgical treatment. By outcomes patients are distributed to four groups: lethal outcome -129. outcome with rasping neurologic infringements—71. with the moderate neurologic transgressions—103 and with good restoration of function down to the compensated condition—108 patients. The data about 150 patients served for realization "of examination" on the computer. On the unified base of the clinical data on CCT is found out. that the scale of a coma. which used. is a simple and adequate method of estimation of a condition of consciousness. The terminal coma determined on presence of a bilaterial fixed mydriasis and a muscular atony. per all days after a trauma is absolutely difficult to predict. Among the patients acted in hospitals in a condition of a deep coma 2/3 of patients die. and in a condition of a moderate coma—only 1/3 die; and at acting in a condition of a moderate coma the different outcomes down to restoration of function of organism with indemnification of a condition are possible. At a deafenation and sopor the probability of favourable outcome sharply raises also this tendency is brightly shown at the fifth day after a trauma. The developed system of dynamic forecasting of outcomes among the patients with CCT for various parts of practical public health services represents a package of 15 prognostic tables realized in the computer. The system provides forecasting of lethal and favorable outcomes at the first day after a trauma with reliability on the average 83 %. in 5–7 days 95 %. differential forecasting of different categories of recoveries with reliability 80–87 % in the first day and 84 % in 5–7 day. At possible mass defeats is recommended to use the system of sorting. offered by us. rendering of a medical care and evacuation of the injureds depending on character and seriousness of CCT. We developed new ways of treatment (invention patents are obtained) Craniocerebral trauma using endolumbar injection of ozone and nootropes. autoplasty of defects of a skull using fan-shaped titanium device.

P533.

PRIMING EFFECT IN REPETITIVE CONCUSSION: PROLONGED PERSISTENCE OF SENSITIZATION TO REINJURY

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A rat model of repeated concussion was developed to determine to what extent and for how long a first concussion sensitizes the brain for subsequent concussive injury. Methods: Thirty rats were subjected to impact-acceleration head injury calibrated to a righting time of 8–12 minutes. After the initial injury, separate groups were given a second, milder injury either 3, 5, or 7 weeks later, using 10% less weight. Sham-injured and single-injury animals were used as controls. Spatial learning and memory were measured using the Morris Water Maze test (6 trials/day for 3 days) starting 17 days after the final injury. Results: Sham and single injury groups had final goal-latency times averaging 15 and 26 seconds, respectively. Animals subjected to a prior concussion all showed markedly more severe cognitive deficits from a second injury. The goal latency times of the double-injured animals were increased to 94 and 98 seconds with a 3 or 5 week inter-injury interval. With a 7 week inter-injury interval, goal latency was partially improved to 53 seconds. The learning curves demonstrated severe impairment in the double injury animals on all trials. Conclusion: The data of this study suggests that a first injury profoundly sensitizes an animal to severe cognitive impairment after a second injury, far worse than after a single injury alone. This sensitization effect partially dissipates with time, but still persists far longer than the observed cognitive effects of the first injury. The neurological basis for this sensitization, and whether it persists indefinately or eventually clears needs to be further investigated. Awareness of this priming effect should influence guidelines for management of concussive head injury in athletes. Supported by: NINDS.

P534.

ROLE OF MICRODIALYSIS IN CLINICAL TRIALS FOR SEVERE TBI

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Introduction: Over 200 Phase III drug trials have failed to show any clinical benefit in severe TBI. We have recently tested the role of microdialysis as a pharmacokinetic tool to improve clinical trials design. Material and Methods: 20 severe head injured patients (GCS<9)received topiramate. a drug that inhibits glutamate release. In order to study central nervous system (CNS) drug delivery and to relate the extracellular fluid (ECF) concentration to its "neuroprotective" effect. topiramate and glutamate were simultaneously recovered from cerebral ECF. using a microdialysis probe. Results: Patients receiving 11.4mg/Kg of topiramate showed significantly higher levels of unbound drug in the brain compared to patients receiving 5.7mg/Kg ($p < 0.05$). however. this did not double the steady state concentration (Css). suggesting an active transport mechanism across the BBB that may be partially exhausted. Doubling the dose of topiramate also resulted in a tenfold decrease in Emax for dialysate glutamate ($p < 0.05$) and in a mean r-value for top/glut correlation of -0.5 ($p < 0.0001$). Conclusions: Microdialysis gives valuable information on temporal pattern of drug penetration across the BBB in the "in vivo" injured brain. A "neuroprotective effect" may be inferred from the dose-dependent glutamate lowering effect of the drug. This type of pharmacokinetic-pharmacodynamic analysis may be a powerful tool in clinical trial design in the future.

P535.

PRELIMINARY EXPERIENCE WITH DECOMPRESSIVE VENTRICULOSTOMY BY CONTIUOUS VENTRICULAR CEREBROSPINAL FLUID DRAINAGE IN POSTTRAUMATIC DIFFUSE BRAIN SWELLING.

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In head injured patients. high intracranial pressure (ICP) due to brain swelling is the main factor that can lead to death itself and. together with Glasgow Coma Score. the main prognostic factor. Several ways of intracranial pressure management have been used in the treatment of these patients. from clinical measures in the ICU to large decompressive surgeries. which can evolve to uncal herniation at the opposite side or even the herniation of the sick brain parenchyma through out the craniotomy with venous obstruction and hemispheric venous infarct. The truth cause of brain swelling is yet poorly understood. Although the widespread theory of brain swelling as a increase in cerebral blood volume due to microvasculature dilatation. having edema just a minor play in the genesis of High Intracranial pressure (HIP). Marmarou et al (2000) have proved through total brain water analysis that brain swelling genesis is mainly due brain edema and not due to an increase in brain vascular volume. Intracranial pressure monitoring with a ventricular catheter has showed to be very useful in head injured patients. not only because of being a trustful way to intracranial pressure measurement but also to allow a quick way to intracranial pressure relief by cerebrospinal fluid (CSF) drainage. Continuous CSF drainage with a ventricular catheter leads to a pressure gradient which causes a change in CSF flux direction with the edematous parenchyma water being drained into the ventricle and so ICP relief. as it causes a continuous drainage of pro-inflammatory substances present in post-trauma CSF. In this work we report the initial experience in the use of Decompressive Ventriculostomy as treatment of HIP in 40 patients with severe head trauma. admitted at the neurosurgery emergency room of Sao Paulo's Medicine college clinical Hospital. discussing the pathophysiology of brain swelling and proposing a new and useful management in these patients.

P536.

CNS PROTECTION BY ANTI-OXIDANTS: PROMISING APPROACHES FOR HEAD TRAUMA IN A RAT MODEL

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There are few treatments that prevent secondary damage following head trauma. Our previous work has shown that both administration of a procysteine compound (OTC) and the iron-chelating flavonoid quercetin prevented much of the secondary damage following spinal cord trauma. The objectives of this study were to determine whether these approaches are also protective following head trauma. Forty-two adult male Sprague Dawley rats were submitted to moderate fluid percussion injury in the anterior midline. Animals were divided into 3 groups: Group 1: 12 mmol OTC 30 minutes after injury. 4mmol OTC in 12hr intervals; Group 2: 0.025mmol quercetin/kg. starting 1 hour after injury and then every 12hr; Group 3: saline vehicle ($n = 13$ per group). Compound Action Potentials (CAP) were recorded 24hr ($n = 5$) and 72hr ($n = 5$) after injury on vibrotome sections of corpus. Three brains per group were used for histological. immunocytochemical and biochemical analysis after sacrifice at 24hr. CAP were significantly higher in both treated groups. as compared to saline controls. at both 24hr and 72hr after injury; this indicates that corpus callosal function was better retained in the drug-treated groups. S-100beta in brain tissue at the injury site was significantly lower in saline controls than in non-injured animals. but significantly higher in animals of both treated groups. Administration of either OTC or quercetin significantly prevents decrease of glutathione levels in brain tissue at the injury site. We conclude both therapeutic approaches hold promise for treating head trauma patients. Supported by HSURC & Neurotrauma Initiative Saskatchewan and the Ontario Neurotrauma Foundation.

P537.

DELETERIOUS EFFECT OF SECONDARY INSULTS ADDED ON TRAUMATIZED ORGANOTYPIC CULTURE IS MORE PROMINENT IN MILD TO MODERATE THAN IN SEVERE DEGREES OF INJURY

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The present study aimed to elucidate the effects of secondary injury mechanisms on the different severity of primary insults in traumatic brain injury (TBI). Cultures of organotypic rat hippocampal slice (OTC) were subjected to TBI by using a centrifugal system. To test the possibility that exposure of cultures to TBI decreased cell survival from following secondary insults. we deprived of serum from media or added of hydrogen peroxide after TBI. Cellular injury of OTC was evaluated by measuring the fluorescence of propidium iodide. A graded diffuse injury was observed all over the slice. depending on the level of force. from 30 × g to 3.000 × g. Hippocampal pyramidal cells of the CA1 region and granule cells of the upper limb of dentate gyrus demonstrated a selective vulnerability to secondary injury. The extent of secondary cell injury of sham-injured or in low centrifugal forces (100 × g) was significantly greater than in high forces (3.000 × g). Pretreatment with N(omega)-nitro-L-arginine methyl ester (L-NAME) (an inhibitor of nitric oxide synthase) or free radical scavengers reduced the extent of secondary cellular damage in low level of TBI. but (+)-MK-801 hydrogen maleate (MK-801) (an N-methyl-D-aspartate antagonist) was without effect on the injury. regardless of the severity. In conclusion. the extent of cell death after secondary insults is strongly correlated with the amount of survival cells in culture system from primary TBI and no increased vulnerability of traumatized cells is found.

P538.

SPECIFIC INHIBITION OF APOPTOSIS AFTER DIFFUSE BRAIN INJURY BY MODERATE POSTINJURY HYPOTHERMIA

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Object: This study explores variant processes of apoptosis after different diffuse brain injury and the inhibition of apoptosis by moderate postinjury hypothermia. Methods: Diffuse brain injury was induced by trauma device reported by Marmarou. Using a terminal deoxynucleotidyl transferase-mediated deoxyuridine 5′triphosphate-biotin nick end labeling technique (TUNEL). the neuronal cells with DNA fragmentation in cortex and hippocampus regions of the brain of rats subjected to brain injury were detected. Using agarose gel electrophoresis. the internucleosomal fragments of DNA in apoptotic cells were examined. Using electron microscopy apoptotic morphological specialty were observed. Results: 1)TUNEL: Apoptotic cells were increased according to injury degree. Their numbers peaked at 48 hours and then declined afterwards. In the mild injury apoptosis was located in hippocampus CA2 and CA3. in the severe injury apoptosis increased evidently. located in all of hippocampus. frontal and parietal cortex region. The hypothermia-treated rats had some apoptotic cells; however. even at 24. 48 and 72 hours there were significantly fewer of these cells than not treated. 2)Electron microscopy:At 24 and 48 hours after injury. the cells were characterized by a round and shrunken morphology. the nuclei were round and condensed. At 48 hours the apoptotic cells is more than at 24 hours. The hypothermia-treated rats had no apoptotic cells. 3)Gel: electrophoresisTypical internucleosomal DNA fragmentation at interrals of 185 to 200 bp was revealed in the injured cortex and hippocampus after severe injury. At 24 and 48 hours this fragments displayed a characteristic DNA "ladder" by gel electrophoresis. There were no DNA "ladder" at the other time point. mild injury and hypothermia-treated groups. Conclusions: The data suggest that apoptosis occurs after diffuse brain injury and apoptotic cells were increased according to injury degree. Moderate hypothermia showed specific effect an inhibition of aoptotic cell death after diffuse brain injury in rats.

P539.

NEUROPROTECTIVE EFFECTS OF AMINOGUANIDINE IN A RAT MODEL OF LATERAL FLUID-PERCUSSIVE BRAIN INJURY

Dr Jia Lu. (Defence Medical Research Institute, Singapore, SG).

The present study examined the effects of a selective inducible nitric oxide synthase (iNOS) inhibitor on neuronal cell survival and post-traumatic recovery in rats following a lateral fluid percussion injury. in which brief displacement and deformation of brain had resulted from the rapid epidural injection of saline into the closed cranial cavity. Daily treatment of aminoguanidine (AG) at the dosage of 100 mg/kg or normal saline was given intraperitoneally into rats starting 2 hours before or 30 minutes after the head injury. Animals were sacrificed at 24. 48 and 72 hours post-injury. Treatment with iNOS inhibitor AG significantly reduced lesion volumes in rats after fluid percussion. as evaluated by high-resolution magnetic resonance imaging (MRI). Immunohistochemical analysis showed a marked induction of iNOS expression in macrophages at the injury site in cerebral cortex and cerebral ventricles ipsilateral to the injury in rats. In parallel with the appearance of iNOS positive macrophages. apoptotic neurons were observed in the ipsilateral cerebral cortex by in situ terminal transferase d-UTP nick-end labelling (TUNEL). In rats receiving prophylactic or post-injury treatment of AG. the number of degenerating neurons was markedly reduced in the cerebrum compared to those receiving saline injection. The location and extent of these pathologic changes correlated with MRI findings. The neurobehavioral studies showed both total and ambulatory locomotor responses were reduced in rats subjected to the traumatic brain injury (TBI). Administration of AG significantly improved the locomotor performance. Present results showed that inhibition of iNOS synthesis by AG improved the histopathological outcomes. It is suggested that nitric oxide (NO) may be involved in neuronal apoptosis following TBI.

P540.

DIFFERENTIAL RESPONSES BY AXONAL MICROTUBULES AND NEUROFILAMENTS FOLLOWING RE-WARMING AFTER TRAUMATIC AXONAL INJURY

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Amelioration of axonal pathology after TAI is greatest with post-traumatic hypothermia followed by slow re-warming. But this has been shown only by labelling for β-APP and 1 hr of cooling using light microscopy. The present study tests the hypothesis that a longer period of cooling, followed by slow re-warming, provides for amelioration within the ultrastructure of the axonal cytoskeleton.

Stretch-injury (load 180–210 g over 19–21msec) was induced in the right optic nerve of adult guinea pigs (weight 750 ± 35g). Animals were cooled to 32–32.5°C as rapidly as possible and maintained for 4 hrs. Rapidly re-warmed animals were returned to a core temperature of 38.3°C within 45min, slow re-warmed animals over 120 min. All animals were maintained at 38.3–38.5 °C until culling 4 hrs later. Stereological analysis of transverse thin sections of axons was undertaken.

Results:

At nodes of Ranvier:

- Slow re-warming provides protection against compaction of neurofilaments
- Both fast and slow re-warming protect against loss of microtubules
- At Internodes 0.5–1.0 μm diameter
- Slow re-warming protects against compaction of neurofilaments
- Slow re-warming protects against loss of microtubules while fast re-warming does not
- At Internodes 1.01–1.5 μm diameter
- Slow re-warming protects against compaction of neurofilaments
- But neither slow or fast re-warming provides protection against loss of microtubules
- At Internodes 1.51–2.0 μm diameter
- Slow re-warming protects against compaction of neurofilaments
- Slow re-warming protects against loss of microtubules while fast re-warming does not

Conclusions:

This study provides quantitative evidence that 4 hrs post-traumatic mild hypothermia followed by slow re-warming provides protection against pathology for neurofilaments but only partial protection for microtubules.

P541.

MODULATION OF HYPOTHERMIC DELAY, DURATION AND REWARMING RATES POSITIVELY INFLUENCE THE GENESIS OF TRAUMATIC AXONAL INJURY (TAI)

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Recently. we have shown that posttraumatic hypothermia significantly reduces TAI in rats. suggesting that hypothermia is neuroprotective. In the clinical setting. however. reports of hypothermia's neuroprotection have been mixed. with a recent large clinical trial reporting no benefit. In these clinical studies. confounds existed in terms of passive versus active rewarming. differing times of initiation and duration. and patient inclusion criteria. In this communication. we have revisited issues related to hypothermic initiation. duration and rate of rewarming in a rodent model of (TAI). Male Sprague-Dawley rats were subjected to impact-acceleration injury. In Experiment 1. the temporalis muscle and rectal temperature were maintained at 37 C in the normothermic group. while the hypothermic group employed a 1h period of hypothermia (32 C). induced 2h postinjury. In Experiment 2. the period of hypothermia induced 2 h postinjury was prolonged to 2 h. In Experiment 3. post hypothermic rewarming to normothermic levels was accomplished either over a 20-minute period (rapid rewarming group) or a 90-minute period (slow rewarming group). Twenty-four hours postinjury the animals' brains were processed for visualization of amyloid precursor protein (APP). a marker of TAI. The number of APP-positive axonal profiles per mm2 was calculated. In Experiment 1. hypothermia provided no protection. However. in Experiment 2. prolonged hypothermia (2h) significantly reduced the number of the APP profiles in comparison to the normothermic group. Further. in Experiment 3. the APP-positive axonal density in the slow rewarming group was significantly reduced in comparison to the rapid rewarming group. Collectively. the results of this and previous studies show that early initiation. prolonged duration and slow rewarming enhance the benefits of hypothermic intervention. at least within an animal model of TAI. Supported by NIH NS20193.

P542.

ENHANCED GASTRIC TOLERANCE TO INDOMETHACIN FOLLOWING NEUROTRAUMA

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Recent studies suggest that indomethacin may be considered as one of the frontline agents for correction of intracranial pressure and cerebral perfusion pressure following head injuries. However. head injury as well as indomethacin have been shown to exert deleterious effect on gastric microcirculation which may lead to gastric and duodenal ulcers. The present investigation was undertaken to study the gastrointestinal tolerance in rats following concomitant exposure to indomethacin and concussive head injury (CHI). Three groups of Albino rats weighing 220 ± 10 g were fasted overnight before the head injury using a controlled cortical impact device. The rats in group 1 and 2 received 45mg/kg of indomethacin orally whereas animals in group 3 received water only. The CHI was produced in group 2 (30 minutes after indomethacin) and group 3. Six hours after the CHI. the rats were sacrificed under light ether anaesthesia. the stomachs were removed and opened along greater curvature and the gastric lesions were quantified. The stomachs were analysed for thiobarbituric acid reactive substances (as a marker of oxidative stress) and myeloperoxidase (indicator of neutrophil infiltration). There were no stomach ulcers in the rats exposed to CHI alone; whereas. all the animals treated with indomethacin (with no CHI) had gastric lesions mainly in the glandular stomach with a mean ulcer score of 18.3 ± 2.3. Only one out of six rats exposed to CHI plus indomethacin had minor ulcerative change (score 0.66 ± 0.66). Our results clearly suggest that the gastric toxicity of indomethacin is significantly reduced in the rats with CHI.

P543.

THE ROLE OF ION TRANSPORTERS IN POSTTRAUMATIC CYTOTOXIC BRAIN EDEMA.

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The pathological increase in intracellular osmolarity is known to be involved in the formation of cytotoxic edema following traumatic brain injury (TBI). We wanted to determine which transporter(s) could be targeted to decrease cytotoxic edema. We used severe (4atm) midline FPI in the rat. Acute brain slices were obtained 2 days post-FPI or sham operation. and randomly assigned to a control chamber and a test chamber. where they were incubated for 2 hours in the specific drug(s). Water content was computed as 100 * (Wet Weight–Dry Weight) / Wet Weight. Furosemide. (2.5mM). a non selective blocker of Na+/K+/2Cl- cotransporter. caused water content elevation by 0.46 ± 0.14% in control ($n = 5$; $p < 0.01$). and by 0.32 ± 0.26% in post-FPI slices ($n = 8$; $p = 0.01$). Its selective blocker. bumetanide (50uM). did not affect water content in control (0.07 ± 0.18%; $n = 8$; $p = 0.32$) and in post-FPI slices (−0.07 ± 0.08%; $n = 5$; $p = 0.13$). DIDS (200uM). a non selective blocker of Na+/2HCO3- cotransporter. did not affect water content in control (−0.11 ± 0.15%; $n = 7$; $p = 0.09$). and slightly decreased it in post-FPI slices (−0.23 ± 0.21%; $n = 6$; $p = 0.04$). SITS (500uM). a non selective blocker of Cl-/HCO3- exchanger and Na+/2HCO3- cotransporter. did decrease the water content in control (−0.13 ± 0.05%; $n = 4$; $p = 0.01$) and in post-FPI slices (−0.17 ± 0.03%; $n = 4$; $p < 0.01$). Amiloride (100uM). a selective blocker of Na+/H+-exchanger. caused water content elevation by 1.16 ± 0.24% in control ($n = 6$; $p < 0.01$) and by 1.25 ± 0.33% in post-FPI slices ($n = 5$; $p = 0.01$). These results suggest that 1) no major ion transporter can be singularly targeted to completely and significantly control posttraumatic cytotoxic edema; 2) furosemide causes edema in normal brain and worsens edema following FPI. although it has previously been found to reduce cytotoxic edema in brain slices following an acceleration/hypoxia model of TBI. The previously observed beneficial effect of furosemide may depend on the traumatic animal model used. Supported by NIH. NS40823 to RD.

P544.

MODULATION OF NEURONAL AND GLIAL GROUP I MGLURS PREVENTS STRETCH-INDUCED ENHANCEMENT OF NMDA RECEPTOR CURRENT

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Neuronal stretching in culture has been used to model diffuse axonal injury caused by acute head trauma and activation of N-methyl-D-aspartate receptors (NMDAR) has been implicated in the pathophysiology of such injury. Here we report the effects of modulating injury severity and the group I metabotropic glutamate receptor subtypes 1 (mGluR1) and 5 (mGluR5) on N-methyl-D-aspartate receptor (NMDAR) activity. Following mild stretch. cortical neurons plated upon a confluent layer of astrocytes (NG). exhibited both increased maximal current (INMDA) and reduction in the voltage-dependent Mg2+ block. In contrast. neurons grown without an astrocyte monolayer (PN) only exhibited increased INMDA. In NG. prior activation of either mGluR1 or mGluR5 decreased the stretch-induced enhancement of INMDA. Similarly. prior inhibition of mGluR5 limited the stretch-induced INMDA changes. whereas inhibition of mGluR1 had no effect. In contrast. in PN inhibition of mGluR1 and mGluR5 prior to injury limited the stretch-induced enhancement of INMDA. whereas prior activation of mGluR1 or mGluR5 had no effect. In both culture conditions. activation of either mGluR1 or mGluR5 did not diminish the stretch-induced reduction in the Mg2+ block. In contrast. inhibition of mGluR1 exacerbated the stretch-reduced Mg2+ block in NG. but prevented the stretch-reduced Mg2+ block in PN. Inhibition of mGluR5 limited the stretch-reduced Mg2+ block in both culture conditions. Severe stretch had no effect on INMDA or the Mg2+ block in either culture condition. despite a correlation between injury severity and the release of lactose dehydrogenase measured post injury. None of the mGluR compounds used had any direct effects upon the NMDA receptor. Combined. these data suggest that during mild stretch-induced injury. significant neuronal/glial interactions underlie group I mGluR mediated enhancement of NMDAR activity. We conclude that both neuronal and glial group I mGluRs regulate NMDAR activity during mild stretch-injury by modulating both the Mg2+ block and INMDA.

P545.

INJURY-INDUCED CHANGES IN NMDA RECEPTOR SUBUNIT COMPOSITION CONTRIBUTE TO PROLONGED CALCIUM-45 ACCUMULATION IN INTACT CORTEX

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The NMDA receptor's (NMDAR) physiological properties are imparted by its NR2 subunit composition. Receptors with a lower NR2A:NR2B ratio are more sensitive to glutamate. conduct larger currents. and are open for a longer time. A potential mechanism of the prolonged calcium accumulation following lateral fluid percussion injury (LFP) is an injury-induced alteration of the NMDAR subunit composition. To characterize the NMDAR subunit composition following LFP. 5 sham-injured and 6 mild-moderate LFP-injured rats were studied at each of 4 time points (1. 2. 4. and 14 days post-injury). Quantitative western blotting performed on region of interest homogenates using antibodies to NR2A and NR2B showed that the greatest alteration in the NR2A:NR2B relative ratio occurred in the ipsilateral parietal ($p < 0.05$) and occipital cortices at 1 day (20.5–33.2% decrease) and 2 days (8.8–21.2% decrease). normalizing to sham levels by 4 days. To investigate if this injury-induced subunit composition alteration contributes to the post-traumatic accumulation of calcium. 30 rats were subjected to mild-moderate LFP and at 1 or 2 days were treated with either saline (1d n = 5. 2d n = 5). MK-801 (n = 5.5; 0.3 mg/kg i.p.; inhibits the NMDA-associated ion channel in a non subunit-specific manner). or ifenprodil (n = 5.5; 30 mg/kg i.p.; inhibits NR2B subunit-containing NMDARs) followed by injection of calcium-45(1μCi/g i.v.). After a five-hour uptake. brains were processed for autoradiography and optical densitometry. In regions where LFP does not alter NR2A:NR2B (ipsilateral frontal cortex). NR2B-specific ifenprodil blocked 25.4–41.9% of the NMDAR-associated calcium-45. However. in regions with a reduced NR2A:NR2B (ipsilateral parietal and occipital cortices). ifenprodil blocked 74.8–83.7% of NMDAR-associated calcium-45 flux. demonstrating that the ifenprodil-sensitive proportion of calcium influx is increased in regions with a decreased NR2A:NR2B. These results suggest that LFP induces a regional. acute change in NMDAR subunit composition that is associated with a change in receptor function. Supported by NS30308 & NS27544.

P546.

ASSESSMENT OF AGRIN EXPRESSION DURING TRAUMA-INDUCED SYNAPTIC PLASTICITY

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Agrin. a heparin sulfated proteoglycan located in the synaptic basal lamina. is known for its regulatory role in the formation of the neuromuscular junction. Interestingly. agrin mRNA is widely distributed throughout the adult rat brain. and has been associated with synapse formation (O'Connor et al., 1994; 1995). For example. agrin levels increase just prior to synaptogenesis in cultured hippocampal neurons (Ferreira, 1999) and are required for the development of normal synapses. By contrast. suppression of agrin results in abnormal synapse formation in vitro. Given that traumatic brain injury (TBI) profoundly affects synaptic activity and that CNS agrin levels may be regulated in an activity-dependent manner (Rupp et al., 1996). we have assessed agrin expression during postinjury intervals of synaptic recovery. The present study examined agrin mRNA expression induced by either unilateral entorhinal cortex (UEC) lesion or moderate central fluid percussion TBI. RNA was isolated from rat hippocampal tissue at 7 days after trauma and RT-PCR was performed using specific primer pairs designed to produce a 200 bP agrin fragment. Contralateral unlesioned hippocampi served as UEC controls and paired Sham-injured rats as controls for the TBI animals. Results showed that a 200 bP DNA fragment was produced in all cases. Initial analysis of this PCR fragment showed a 49% increase ipsilateral to UEC lesion when compared with the contralateral side. and a 26% increase after TBI relative to Sham-injured controls. These observations suggest that agrin may play a role in trauma-induced synaptogenesis. Supported by NS 12587; NSO 7288-13.

P547.

MITOCHONDRIAL GENE EXPRESSION FOLLOWING TRAUMATIC BRAIN INJURY: ANALYSIS OF THE ND4 SUBUNIT OF COMPLEX I.

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We have recently shown that traumatic brain injury (TBI) increases expression of the mitochondrial gene cytochrome c oxidase II, a protein integral to cytochrome c binding at complex IV of the electron transport (ET) chain (Harris et al., 2001). Given that the mitochondrial genome (mtDNA) appears to be sensitive to pathology elicited by TBI. and that elevations in ET chain proteins are associated with neuroplasticity (Yang et al., 2001). we have probed for changes in other mtDNA coded ET enzymes during postinjury intervals exhibiting synaptic recovery. The present study reports our current results from RT-PCR analysis of the ND4 subunit of ET complex I in rats subjected to moderate central fluid percussion injury. RNA was isolated from hippocampal tissue at 7 days after injury and RT-PCR was performed using specific primer pairs designed to produce a 200 bP ND4 fragment. Sham-injured animals were run in parallel as controls. RT-PCR was optimized by varying the concentration of both the RNA and cDNA templates, primer pairs and dNTPs, as well as cycle number. Results showed that a 200 bP DNA fragment was produced in both TBI and Sham samples. However, no significant difference in level of PCR product was observed after injury. This fragment. when excised and sequenced. was 100% homologous with the selected segment of ND4 mtDNA. These results suggest that transcription of ND4 is not altered during periods of recovery following TBI, and support the hypothesis that ET complex proteins may exhibit a differential vulnerability to the pathology induced by brain injury. Supported by NS12587.

P548.

AGE-RELATED CELL PROLIFERATION IN THE RAT CNS FOLLOWING TRAUMATIC BRAIN INJURY

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It has long been known that juvenile mammals recover much more completely than adults after traumatic brain injury (TBI). We have previously shown that TBI, in the form of a lateral fluid percussion injury (FPI). greatly enhanced the total number of proliferating cells in both the subventricular zone (SVZ) and the hippocampus in adult rats. We, therefore, hypothesized that differences in neurogenesis may exist between adult and juvenile rats that could account for the improved functional recovery of the CNS in juveniles after injury. To test this, both juvenile (P28) and adult rats were subjected to a moderate FPI or sham injury. Following this injury (2. 7. or 14 days), each animal received three i.p. injections of BrdU (50mg/kg) and was sacrificed 24 hours after the last injection. Coronal sections of brain were then stained for BrdU and the total number of BrdU-positive cells quantified. Preliminary results showed that there was a significant increase ($P < 0.01$) in the number of BrdU positive cells in the CNS of juvenile rats as compared to adult rats. This enhanced proliferation was localized to regions of the subventricular zone, corpus callosum, striatum and septal nuclei and was significant for up to 7 days post injury. These results, together with ongoing experiments aimed at determining the cell types of proliferating cells in the CNS in both juvenile and adult animals following FPI, may explain the fundamental differences in recovery as it relates to age. Supported by the Virginia Neurotrauma Trust.

P549.

SEVERE HEAD INJURY MANAGEMENT IN LATVIA

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Since 1998 when Neurosurgery Clinic of P.Stradins Hospital became a part of the international collaboration project with Brain Trauma Foundation (BTF). New York. USA there is a gradual improvement in the management of severe head injury (SHI) patients. BTF Guidelines have been widely used in the treatment of neurotrauma patients in Latvian hospitals. Patients with Glasgow Coma Scale (GCS) 3–8 admitted in our hospital within 12 hours after trauma have been managed observing BTF Guidelines and entered into internet database. In 1999–2000 123 patients were entered in Traumatic Brain Injury Survey database (Intracranial pressure (ICP) monitored in 83% patients). in 2001–2002 51 patients were entered in Traumatic Brain Injury-trac Quality Assurance Program (ICP monitored in 88% patients). GCS was used to compare early outcomes (10–14 days after trauma) in 2001–2002 and 1999–2000. GCS 13–15 31% (36% in 1999–2000). GCS 9–12 12% (11%). GCS 6–8 18% (13%). GCS 3–5 22% (11%). death 17% (29%). During four years of collaboration the death rate after SHI has decreased by 12%. The BTF Guidelines use has helped Latvia to develop the Head Trauma System thus improving the care for SHI in Latvian hospitals that admits neurotrauma patients. ICP monitoring is the key of postsurgical management. is routinely performed in patients with SHI.

P550.

TRAUMATIC SUBARACHNOID HEMORRHAGE, EVOLUTION AND PROGNOSIS.

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Introduction: In head injury patients (HI) the craneal tomography scan (CT) image can change along the time and thus. the initial prognosis could be modified. Objective: To know the evolution along the time of the CT image in HI patients and traumatic subarachnoid haemorrhage (tSH). To determine the prognosis value of tSH seen at initial CT and those of the worst evolutive tSH image. Material and Methods: We studied prospectively 370 head trauma patients admitted consecutively to Intensive Critical Unit in 7 universitary hospitals in Catalonia between February 1998 and January 1999. A CTwas performed at admission. 24 hours later and when it was considered accorded to the best clinical practice. The presence. and evolution along the time of tSH was assessed according to the Fisher scale. Initial and worst grade of tSH for each case were evaluated. A multivariate analysis adjusted for Glasgow Coma Scale. pupillary reactivity and age. was made in order to know the prognosis value of tSH. A chi-squared test and odds ratio (OR) with 95% confidential interval was made. in order to compare the hospital mortality calculated between the initial and the worst tSH degree. Results: 190 patients showed tSH Fisher I. 51 Fisher II. 45 Fisher III and 84 Fisher IV at admission. tSH worsened in 13 cases (3.5%). From tSH Fisher I. 4 cases changed to Fisher II and 3 to Fisher IV. From tSH Fisher II. 2 cases changed to Fisher IV. From tSH Fisher III. 4 cases changed to Fisher IV. All Fisher IV were the worst image. The hospital mortality among each group at admission was 15.5%. 19.6%. 37.7%. and 29.7% ($p < 0.005$). If tSH was present at admission. a highest mortality was observed (OR 2.21). The hospital mortality according to the worst degree of tSH was 13.7%. 20.7%. 39.1% and 31.9% for each group ($p < 0.005$. OR 2.26). There was not differences statistically significant between the hospital mortality according to the tSH admission classification and the worst evolutive degree (p = ns). Multivariate analysis showed that tSH was an independent prognosis variable. Conclusions: The tSH at admission rarely worsened. The presence of tSH is an independent prognosis variable. The prognosis value of tSH. can be calculated following the first CT scan.

P551.

NMDAR-PSD95 INTERACTION MEDIATES SECONDARY TRAUMATIC NEURONAL INJURY.

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Neuronal damage from mechanically-induced trauma is comprised of "primary" and "secondary" components.Primary" injury is the physical damage leading to cell death arising as the direct consequence of mechanical tissue deformation.Secondary" injury arises from subsequent events at the tissue. cell and molecular levels that are triggered by. but are distinct from. the primary injury. As primary injury. by its nature. is not treatable. research is focused on understanding and treating secondary events in order to improve outcomes for CNS injury patients. Because primary and secondary traumatic injuries are intimately related. the mechanisms of secondary injury are difficult to study in isolation. By inducing stretch to cultured cortical neurons grown on flexible membranes. we developed a model in which stretch has no deleterious effect on neuronal survival (no primary damage). but renders the cells more vulnerable to subsequent insults (secondary injury). Using this model. our observations indicate that secondary injury occurred by distinct signaling via the NMDA subtype of glutamate receptors. as insults with sub lethal concentrations of NMDA and L-glutamate. but not kainate or the Ca ionophore A23187 produced secondary neuronal damage in stretched cells. The vulnerability of neurons specifically to NMDA toxicity occurred without stretch causing increased presynaptic glutamate release. without increased synaptic activity. and without effects on NMDA receptor-mediated ionic currents. Thus. this increased vulnerability occurred due to mechanisms downstream from. but specifically associated with. NMDA receptors. We investigated the role of the cytoskeleton in mediating the increased vulnerability of stretched neurons to NMDA toxicity. Actin and microtubule depolymerization did not reduce the vulnerability of stretched neurons to NMDA. Disrupting the NR2b-PSD95 linkage results in an attenuation of the vulnerability of stretched neurons to NMDA. These data indicate that neurons subjected to sublethal traumatic injury become more vulnerable to a glutamatergic insult via a NR2b-linked signaling pathway.

P552.

SYSTEMIC HEMORRHAGE AND THE TYPE OF RESUSCITATION IMPACTS HIPPOCAMPAL FUNCTION FOLLOWING BRAIN TRAUMA

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Clinical databases of human traumatic brain injury point to an association between hypotension and poorer outcome. Traumatically brain injured patients frequently suffer hemorrhagic shock secondary to systemic injuries. However. there is controversy regarding the optimal fluid resuscitation and hemodynamic targets of multi-system trauma patients with hemorrhagic shock. Controlled under-resuscitation is now advocated by some in the prehospital phase. This study was designed to use the functional evaluation of a model of traumatic brain injury to demonstrate and quantify the impact of hemorrhage. with the view of more closely calibrating the nature and target of optimal hemodynamic resuscitation. Male sprague-Dawley rats weighing 200–250gm were anesthetized and subjected to moderate intensity (1.8–2.0 atm) lateral fluid percussion injury or sham procedure. Immediately following. the animals were phlebotomized 50% of their calculated blood volume. After 30 min two groups of animals were resuscitated to near pre-injury blood pressure with either the hemorrhaged volume of blood or three times this with normal saline. After 2 hours the animals were decapitated. and hippocampal slices were studied at 36.5°C. The evoked population spike (PS) was extracellularly recorded in the CA1 pyramidal cell layer after stimulation of the Schaffer collaterals and the amplitudes compared between groups. At the maximum stimulating current (1.5mA). the PS amplitudes of sham. injured only and injured plus hemorrhaged animals were significantly different from each other at (mV ± SD) 8.6 ± 2.26; 4.9 ± 1.46; and 3.30 ± 0.98. The PS amplitudes from animals that received blood and normal saline resuscitation were 5.5 ± 1.49 and 3.57 ± 1.59. Hemorrhage following traumatic brain injury exacerbates the immediate hippocampal functional deterioration. Early resuscitation with blood prevented this while hemodynamic resuscitation with normal saline did not. Both the presence of systemic hemorrhage and the type of resuscitation impacted hippocampal function following brain trauma.

P553.

EXPERIENCE OF DIAGNOSTICS AND TREATING OF A SEVERE TRAUMATIC BRAIN INJURY (STBI), COMBINED WITH OPENED DAMAGES OF A CHEST.

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Experience of diagnostics and treating of a severe traumatic brain injury (STBI). combined with opened damages of a chest. OBJECTIVE. Intention of this study was to analyse of results of surgical treatment of the patients with STBI in a combination to opened damages of chest and determination of rational surgical tactics. METHODS. 10 consecutive multiple traumatic patients with severe head injury were treated between 1996 and 2001. The age of the patients was from 20 till 60 years. all—males. At 4 patients the bullet wounds of a skull were combined with bullet wounds of a chest. at 5—STBI (3—closed. 2—opened) was combined with penetrating knife wound of a chest and abdomen. at 1—there was a combination opened STBI and thoracicoabdominal wound as a result of fall from height of 12 meters. A traumatic and hemorrhagic shock III-IV of a degree is marked at 6 patients. Diagnostics of multiple injured TBI was spent based on study of the mechanism of a trauma. clinical symptoms. radiographic and ultrasonic analysis. tapping of cavities. RESULTS. Six patients had closed TBI. at 4—penetrating. The extensive fractures of the cranial vault and/or skull bases are revealed at seven sufferers. The 6 patients had attributes of the compression of the brain. The penetrating damages of the chest were accompanied by a wound of pulmon at 5 patients. wound of heart—1. haemopneumothorax of a various degree—9 and haemothorax—1. The medical measures were directed on early liquidation of the disturbance of the external respiration. hemorrhage and struggle with a shock caused thoracic component of a trauma: adequate anesthesia. automatic controlled breathing. fluid and replacement therapy. aggressive therapy. The 6 patients was reanimation at receipt to hospital. water seal drainage of chest—8 patients. 5 sufferers is executed emergency laparotomy. 1—thoracophrenolaparotomy. 1—thoracotomy. Neurosurgical operation was executed for 6 patients. Four (40%) sufferers died. CONCLUSION. The patients with severe head injury and open damages of a chest need active surgical and neurosurgical treatment.

P554.

HEAD INJURED PATIENTS WHO TALK AND DETERIORATE: ANALYSIS OF 86 CASES REGISTERED ON THE JAPAN NEUROTRAUMA DATA BANK

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[Introduction] In order to clarify the clinical profile of head-injured patients who talk and deteriorate into coma. we reviewed 721 patients with head injuries who were registered on the Japan Neurotrauma Data Bank (Japan Society of Neurotraumatology) from 1998 to 2000. [Results] Eighty-six patients (12%) talked prior to deterioration. and 81 deteriorated into coma (Glasgow Coma Scale (GCS) <= 8 p). In all cases. CT scans revealed development of focal lesion(s) with a mass effect and resultant midline shift. Forty-three patients (50%) had a subdural hematoma. 25 (29%) had cerebral contusion/Intracerebral hematoma. and 18 (21%) had an epidural hematoma. The Glasgow Outcome Scale was GR in 21 (24%). MD in 11 (13%). SD in 13 (15%). VS in 5 (6%). and D in 36 (42%). The latent periods to deterioration were <= 3 hours in 59 (72%). 3–6 hours in 12 (15%). and > 6 hours in 11 (13%). demonstrating a shorter latency than those reported in previous studies. Sixty-four patients (74%) underwent surgery. i.e. evacuation of hematoma. and/or contusion necrotomy. The predictors for a poor outcome were a low GCS following deterioration. subdural hematoma. and being an elderly patient. In contrast. GCS during lucid intervals. and the length of time until deterioration or until operative intervention did not influence the final result. [Conclusion] A majority of cases (87%) showed deterioration within 6 hours post trauma. caused by a progressive mass effect. Deterioration into a low GCS resulted in a poor outcome. so that early operative intervention is strongly recommended prior to the inevitable deterioration.

P555.

SURGICAL COMPLICATIONS OF DECOMPRESSIVE CRANIECTOMY FOR HEAD INJURY

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Background: although in the last decade many published papers showed a renewed interest for this ancient surgical procedure and a debate is still open. In our knowledge few authors mentioned about complications. Patients and methods: we reviewed and analysed clinical and TC data of all comatose head injured patients admitted in our Department in the period 1996–2001 and operated on for decompressive craniectomy and checked any surgical complication also in the post discharge period till one year. Results: 35 patients were included . age ranged from 14 to 69 years with a mean age of 36.6. The initial mean Glasgow Coma Scale on admission was 5. Decompressive craniectomy was not performed routinely but on surgeon judgment instead of simple debridement or lobectomy also performed in our Institute. Bone was stored and frozen at −80. Within 12 hours 9 patients (26%) had epidural or subdural collection far from the craniectomy and 8 re-operated . Bone flap was reversed at variable time but 5 patients needed shunt after reverse for clinical and radiological signs of comunicant hydrocephalus and all showed a degree of clinical inprovement. Discussion and conclusion: in our knowledge this is the first report of a group of surgical complications after decompressive craniectomy for head injury and this should be weighted in the decision-making process. Beneath the number of patients is small. our experience suggests that early CT after decompression is needed and time of reverse bone flap and its influence on cerebrospinal fluid dinamics(1) should be investigated. REFERENCES: (1) Czosnyka and coll.: Post traumatic hydrocephalus: influence of craniectomy on the CSF circultion. Letter to the editor. J Neurol Neurosurg Psychiatric 2000:68:248–256.

P556.

EARLY EDEMA FORMATION IN CEREBRAL CONTUSION: ULTRA-EARLY STUDY (<24 HOURS POST-TRAUMA) WITH DIFFUSION MRI AND ADC MAPPING

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In the previous studies. we have reported that heterogeneous mechanisms exist in early edema formation in cerebral contusion. and cytotoxic edema plays an important role within 48 hrs following injury. It is remains unclear. however when edema begins to develop following injury. In order to determine the time course of edema development. diffusion imaging and ADC (apparent diffusion coefficient) mapping are performed in 10 patients within 24 hours post-trauma with cerebral contusion using a 1.0T echo planar MRI. Within 3 hours post-trauma. diffusion MRI showed no remarkable changes. and the ADC values were within normal limit (ADC ratio (contused/normal brain) = 1.00+/-0.21. (mean+/-SD)). At 6 hours post-trauma. diffusion images demonstrated a low intensity core in the contusion proper and a high intensity rim in the peripheral area of contusion. The ADC value increased in the contusion proper (ADC ratio = 1.26+/-0.13) and decreased in the peripheral area (ADC ratio = 0.58+/-0.19). These findings indicated that early cellular swelling in the peripheral area of contusion begins within 6 hours following injury. This delayed occurrence of contusion-induced cellular swelling suggests that the CBF dose not decrease to ischemic level immediately following injury.

P557.

DOES THE USE OF JUGULAR BULB OXYGEN SATURATION IMPROVE THE PROGNOSIS IN HEAD INJURED PATIENTS?

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Introduction: To use jugular bulb oxygen saturation (SjO2) determination helps in the proper management of head injury (HI) patients. It is widely used and could have a beneficial effect on mortality. but its efficacy has not been proved. Objective: A.- To identify among HI patients with intracranial pressure (ICP) monitored those where the SjO2 is more frequently used. B.- To determine if the use of SjO2 improves hospital mortality. Material and Methods: Of the 370 HI patients admitted consecutively to ICUs in 7 teaching hospitals in Catalonia between February 1998 and January 1999. we studied the cohort of 184 cases who underwent intracranial pressure monitoring. The patients were managed according to each hospital clinical practice. Demographic data were collected and patients were classified according to Glasgow Coma Score on admission (GCS) and cranial tomography scan (CT) image using both the Traumatic Coma Data Bank (TCDB) and a morphological classification. Variables related to hospital mortality were analysed. Univariate and multivariate analysis were performed in order to identify the groups of patients in which the SjO2 was used and its potential benefit. Results: The patients had mean age of 36.7±; 19 years. mean GCS 6.78±; 3.3 points. and hospital mortality was 29.3% (54 / 184 cases). The SjO2 was used in 96 patients (52.2%). was more frequently used in younger patients (34 ± 18 vs 40 ± 20 years. $p < 0.05$). with lower GCS (6.3 ± 3.4 vs 7.3 ± 3.2 points. $p < 0.05$). in those with at least one episode of ICP > 25 mmHg during 10 minutes (72% vs 37%. $p < 0.001$) and in those with CT classification TCDB III or IV versus TCDB I or II (65% vs 43%. $p < 0.05$). We did not find any significant differences in its use related to pupillary reactivity. subarachnoid hemorrhage. morphological lesion on CT. or hospital. The bivariate analysis did not identify any group of patients in which the use of SjO2 reduced the hospital mortality. When logistic regression analysis adjusted for variables statistically significant related with hospital mortality (age. GCS. pupillary reactivity. ICP >25 mmHg and CT lesion) was performed. the use of SjO2 did not show any benefit. Conclusions: In our area the SjO2 catheter is widely used. It is more frequently used in younger patients with lower GCS. Its use in clinical practice does not appear to provide benefits in relationship to hospital mortality.

P558.

DIFFUSE AXONAL INJURY FOLLOWING FLUID PERCUSSION TRAUMATIC BRAIN INJURY IN THE RAT: CHARACTERIZATION AND CORRELATION BETWEEN ELECTROPHYSIOLOGICAL AND HISTOLOGICAL FEATURES.

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Diffuse axonal injury(DAI) is associated with poor outcome following traumatic brain injury(TBI). While the temporal pattern of histopathological changes involved in posttraumatic axonal injury has been well described. there has been no clear correlation between these observations and the functional status of axons in cerebral white matter. We sought to quantify the functional deterioration of axons in a major cerebral white matter tract following TBI and correlate this with histological and molecular markers of injury. Adult male rats underwent central fluid percussion-induced TBI. Compound action potentials(CAPs) were recorded in the corpus callosum at 3h, 1d, 3d, 7d, 2wks and 4wks following injury and compared to shams(n = 5/group). Brains were harvested in shams. 3d. 7d. 2wks and 4wks(n = 4/group), sectioned, and stained for APP, injured myelin and TUNEL, and positively stained cells in the corpus callosum were counted. Corpus callosum CAPs in sham. mild and moderate injury were 1.11 ± 0.10mV. 0.82 ± 0.11mV and 0.49 ± 0.08mV respectively. After moderate injury, the CAPs were 0.55 ± 0.08 at 3h. 0.61 ± 0.08 at 1d. 0.60 ± 0.09 at 3d. 0.95 ± 0.12 at 7d. 0.62 ± 0.21 at 2wks. and 0.59 ± 0.08 at 4wks. APP expression was significantly increased at 3d, 7d, 2wks and 4wks, peaking at 7d. Injured myelin staining was also increased at these time points with a peak at 4wks. TUNEL counts were significantly higher at 3d, 7d and 2wks, peaking at 7d. APP and injured myelin deposition was distributed caudally and laterally in the corpus callosum, while apoptotic cells were located in the central portion. This study is the first to report an evaluation of the degree and temporal pattern of axonal dysfunction following TBI. Reversibility was seen between 3d and 7d electrophysiologically but not histologically. This highlights the value and importance of combined functional and morphological approaches in the evaluation of experimental TBI, especially DAI.

P559.

DELAYED TRAUMATIC INTRACEREBRAL HEMATOMA AND COAGULOPATHY IN THE PATIENTS DIAGNOSED WITH A TRAUMATIC SUBARACHNOID HEMORRHAGE

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The detection of delayed traumatic intracerebral hematoma (DTICH) has increased steadily with improved imaging. However. the pathogenesis of DTICH has not been clearly elucidated. It has long been recognized that a traumatic insult to brain tissue may result in substantive coagulation abnormalities. The present study was carried out in an attempt to find out the association of coagulopathy and the development of DTICH in patients diagnosed with a traumatic subarachnoid hemorrhage (TSAH). Sixty-three patients were diagnosed as having TSAH from the initial CT scans obtained within 2 hours after trauma. On admission. peripheral blood samples for coagulation studies. including platelet. thrombin time. prothrombin time. activated partial thromboplastin time. fibrinogen. serum fibrinogen degradation product (FDP) were taken within 6 hours after injury. All patients had subsequent CT scans performed within 24 hours of admission. Thirty (47.6%) of 63 patients exhibited radiological evidence of DTICH on their subsequent CT scans. There was a significant correlation between the increased value of serum FDP (>40 micrograms/ml) and the development of DTICH. We observed that the origin of the hematoma might be caused by those radiographically unidentifiable parenchymal lesions often found with TSAH on the initial CT scan. We conclude that a clotting study at the time of admission is of value in predicting the occurrence of DTICH associated with TSAH.

P560.

PRELIMINARY REPORT: TRAUMATIC COMA DATA BANK PROJECT IN JAPAN

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An epidemiologic research was conducted on medical treatment for traumatic head injury in Japan from January. 1998 to June. 2000. Sudden death by accident" including severe head injury is the third major cause of death in our country. National Police Agency announced the numbers of death by traffic accident as 10.000 per year. According to Ministry of Health. Labour and Welfare. traffic death reaches 13.000. Severe head injury is supposed to be a majority of them. Unfortunately these statistics include little information from medical facilities and we don't have any comprehensive data that describes situation around traffic death or severe head injury from a medical point of view. Ten medical emergency centers took part in the traumatic coma data bank project. Patients with severe head injury were eligible for entry with a Glasgow Coma Scale (GCS) Score of 8 or less. Children under 6 years old were excluded. We made an original data sheet with 376 items containing information about characteristics of the injury. prehospital treatment. diagnosis. treatment and follow-up information concerning outcome. This time. we studied 721 cases. classified into two groups; 442 cases of motor vehicle accident and 270 cases of non-motor vehicle accident. While focal brain injuries occupy a majority in the non-motor vehicle accident group. diffuse brain injuries surpass in number in motor vehicle accident group. We are still in the preliminary stage for this clinical study. However. we hope that our project will explain in part the actual circumstances of severe head injuries in Japan and that our report will be a significant data to be used in international comparative survey.

P561.

LACK OF INTERLEUKIN-1 TYPE 1 RECEPTOR DOES NOT IMPROVE WHITE MATTER AXONAL DYSFUNCTION FOLLOWING TRAUMATIC BRAIN INJURY

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Following traumatic brain injury(TBI). the acute response involves the production of cytokines. including interleukin-1beta. which contributes to secondary injury mechanisms. The action of IL-1beta are mediated by the IL-1R1 receptor. We have previously characterized axonal electrophysiological dysfunction in white matter following TBI in the rat. In this study. we adapted this novel functional assessment method to the mouse and sought to determine whether transgenic mice lacking IL-1R1 had diminished axonal dysfunction following TBI. Adult male mice were injured with moderate central fluid percussion injury. Compound action potentials(CAPs) were recorded in the corpus callosum of brain slices isolated at 1d and 7d after injury and compared to shams (n = 5/group). IL-1R1-deficient mice were injured in the same fashion and corpus callosum CAPs were recorded at 1d post-injury and compared to wild-type control littermates (n = 5/group). In a second set of experiments CAPs were recorded after a 30min period of ischemia in vitro. 24 hours after in vivo TBI. Following moderate fluid percussion TBI. corpus callosum CAP amplitude was reduced to 0.68±; 0.08 mV at 1d and 0.54±; 0.04 mV at 7d in injured mice compared to 1.40±; 0.15 mV in shams. At 1d following injury. CAP amplitude in IL-1R1-deficient mice was 0.58±; 0.04 mV compared to 0.76±; 0.03 mV in controls. Following a 30min period of ischemia in vitro. CAP in IL-1R1-deficient mice previously injured with fluid percussion in vivo recovered to 36.7±; 9.3% of baseline compared to 46.4±; 9.1% in controls. Our results indicate that white matter axonal dysfunction is measurable in the mouse following TBI. We also showed that mice deficient in the IL-1R1 receptor do not show any improvement in white matter axonal conduction following TBI and secondary ischemia. and may actually have a worse outcome.

P562.

THE X-CHROMOSOME-LINKED INHIBITOR OF APOPTOSIS (XIAP) PREVENTS CELL DEATH IN THE 158N IMMORTALIZED OLIGODENDROGLIAL CELL LINE

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Apoptotic cell death is a fundamental biological process involved in the normal development of the nervous system. However. apoptosis contributes to cell death in certain neurodegenerative diseases as well as following traumatic spinal cord injury. head trauma. and ischemia/stroke. The apoptotic program is executed by caspases. which are regulated. in part. by the inhibitor of apoptosis (IAP) family of proteins. The X-chromosome-linked IAP (XIAP) has been reported to reduce cell death in response to a variety of apoptotic stimuli. In this study. we examined whether transient overexpression of XIAP protected an oligodendroglial cell line (158N) from apoptotic cell death induced by staurosporine (STS) or dopamine (DA) treatment. 158N cells were transfected with either pCMV-Myc-XIAP or control pCMV-Myc plasmid. At 48 hr post-transfection. western blotting and immunocytochemical staining showed robust XIAP overexpression in pCMV-Myc-XIAP transfected cells relative to non-transfected or pCMV-Myc transfected cells. Subsequently. similar groups of 158N cells were treated with STS (100nM) or DA (300μM) and cell viability was determined using the MTT and Live/Dead assays. As expected. STS treatment for 4 hr or DA treatment overnight resulted in significant cell death in non-transfected and pCMV-Myc transfected cells. In contrast. there was significant survival of cells transfected with pCMV-Myc-XIAP. These results show that XIAP overexpression in vitro protects cells from apoptotic cell death and suggests a therapeutic role for XIAP overexpression on oligodendroglial survival following an insult in vivo. Sponsored by NS40015 and KSCHIRT (JES).

P563.

SECONDARY COMPLICATIONS IN ELDERLY INDIVIDUALS WITH ACUTE TRAUMATIC SPINAL CORD INJURY

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Relatively little has been reported regarding secondary complications in the elderly (age>60 y.) after spinal cord injury (SCI). This study was undertaken to clarify this critical issue. given the increasing prevalence of SCI in the elderly. Data were analyzed using ANOVA. Student-t and Chi-square tests. Complications occurring in the acute stage of SCI (≤60 days) in 78 consecutive patients with traumatic SCI admitted during a three-year period were analyzed. There were 32 elderly (14 F. 18 M; mean age 74.3 y.). and 46 younger (9 F. 37 M; mean age 39.2 y.) individuals. The severity and level of SCI were similar in both groups (P = 0.21; P = 0.73). Medical comorbidities were more frequent among elderly patients (84.4% vs. 39.1%; P < 0.01). Secondary complications were also significantly higher in geriatric patients (56.3% vs. 23.9%; P = 0.01). There was a trend. which did not achieve significance. for increased mortality in the elderly (elderly: 12.5% vs. 2.2%; P = 0.15). The most common secondary complications in elderly individuals were: infection (56.3%). psychiatric disorders (28.1%) and cardiovascular disturbances (15.6%). Early autonomic dysreflexia (5%) was observed only in younger individuals. The geriatric population is more susceptible to secondary complications after acute SCI. Greater awareness by clinicians is essential to minimize secondary complications after SCI and to improve quality of life for elderly individuals (Supported: Heart & Stroke Foundation of Ontario).

P564.

AGE-DEPENDENCY ON DEVELOPMENT OF NEUROPATHIC PAIN BEHAVIOR FOLLOWING SPINAL CORD INJURY IN RAT

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Spinal cord injury (SCI) often leads to chronic central pain (CCP) syndromes such as allodynia and hyperalgesia. Although. several experimental animal models for CCP exist, little is known about the effect of age on the development of CCP following SCI. In this study, we evaluated behavioral outcomes to mechanical and thermal stimuli using three different ages of Sprague-Dawley rats following SCI. SCI was induced by hemisection of the spinal cord at T13. Behavioral outcomes were measured by paw withdrawal frequency (PWF) in response to 10 applications of mechanical stimuli (von Frey filament) and paw withdrawal latency (PWL) to radiant heat stimuli on both the forelimbs and hindlimbs. In forelimbs, young rats (164.6 ± 5.46 g. 40 days) displayed increased PWF to mechanical stimuli (4.17, 9.48 mN) compared to adult rats (273.2 ± 8.09 g. 60 days) on both sides, whereas old rats (546 ± 12 g. 12 months) did not change. In addition, PWL of young and adult rats significantly decreased on both sides whereas PWL of old rats did not change. In hindlimbs, PWF of young rats significantly increased on both sides whereas adult and old rats did not change. Also, young rats (ipsilateral) and young and adult rats (contralateral) displayed significantly decreased PWL, but old rats did not change on either side. These results indicate that younger rats developed more robust neuropathic behaviors than older rats, indicating that age selection is important in animal models of CCP syndromes following SCI.

P565.

GENECHIP ANALYSIS AFTER ANEURYSM CLIP-INDUCED SPINAL CORD INJURY IN MOUSE: A COMPREHENSIVE STUDY OF CHANGES IN EXPRESSION OF GLUTAMATE RECEPTORS; APOPTOSIS-ASSOCIATED GENES; AND GENES RELATED TO OXIDATIVE STRESS

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Spinal cord injury (SCI) still remains one of the most devastating forms of trauma. During the last decades new biological techniques have greatly extended our understanding of molecular events during SCI. Microarray DNA chips are the newest tools for comprehensive gene expression studies. Although genechips have been used for the spinal cord injury models in rats. currently there is no information available on the mouse model of SCI. Current observations in our laboratory have shown alteration in glutamate receptor expression after SCI. Spinal cord injury is also associated with apoptosis in neurons as well as glial cells. There are also some reports on involvement of oxidative stress induced damages after SCI. In present study we have used customized 15.5k mouse cDNA microarrays to address the differential gene expression in a model of moderate SCI injury. We have investigated the following objectives: 1) Differential expression of glutamate receptors after SCI. 2) Temporal expression pattern of apoptotic related genes. and 3) To seek whether the expression of oxidative stress related genes is altered during SCI. An 8.3 g clip was used to induce moderate SCI at T7 level. Specific cDNAs for Glu-R1-7. Kainate 1-2. NMDAR1. R2A. R2B. R2C and R2D and R3A as well as metabotropic glutamate receptors mGluR1. 2. 3 and 5. Rel A. IkB and Glutathione reductase were prepared using One-step RT-PCR. The cDNAs were then printed on 15.5K mouse microarrays. Total RNA was extracted at 1. 6. 24. 48 hrs and 1. 2. and 6 weeks after SCI. Cy5 and Cy3 labeled cDNA probes were generated using indirect labeling method. . The results are currently being quantified and will be presented at the NINTS meeting. This study is the first application of genechips in mouse model of SCI. Funded by CIHR (MT-14459).

P566.

ALTERED DISTRIBUTION AND EXPRESSION OF KV1.1 AND KV1.2 K+ CHANNELS IN SPINAL CORD WHITE MATTER AFTER CLIP COMPRESSION SPINAL CORD INJURY: ACUTE AND CHRONIC IN VIVO STUDIES

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Following spinal cord injury (SCI) surviving white matter axons display axonal dysfunction due to demyelination. and show enhanced sensitivity to K+ channel blockers such as 4-aminopyridine (4-AP) and a-dendrotoxin (a-DTX). Studies from our laboratory have shown that this abnormal axonal function is associated with increased expression and altered distribution of Shaker voltage-gated K+ channel subunits Kv1.1 and Kv1.2 on spinal cord axons after chronic SCI (Nashmi et al., 2000). In normal PNS and CNS myelinated axons. Kv1.1 and Kv1.2 subunits are highly co-localized in the juxtaparanodal regions. whereas after SCI. they acquire a dispersed immunostaining pattern along the axons. In this study. we have investigated the temporal and spatial patterns of Kv1.1 and Kv1.2 subunits expression in spinal white matter at varying times after in vivo clip compression SCI at T7. Using western blot analysis. we found an increased expression of Kv1.1 and Kv1.2 between one to two weeks after injury. We also used confocal immunocytochemistry to study the distribution of Kv1.1 and Kv1.2 along the injured axons. In contrast to uninjured spinal axons with juxtaparanodal localization. Kv1.1 and Kv1.2 showed a markedly dispersed labeling along the internodes of injured axons. This redistribution of Kv1.1 and Kv1.2 occurs as early as 1 hr postinjury along some injured axons. and appeared to be more pronounced one week after injury and evolves over time. To seek for mechanisms involved in aberrant distribution of Kv1.1 and Kv1.2 after SCI. we examined the localization of Caspr (contactin associated protein) which is found in the paranodes and is believed to separate K+ and Na+ channels in myelinated axons. Our immunostainig indicated a more diffusely localization of Casper along injured spinal cord axons. These results suggest that redistribution of Caspr may be associated with aberrant localization of K+ channel subunits Kv1.1 and Kv1.2 on spinal cord axons after SCI. Funded by CIHR (MT-14459).

P567.

iNOS INHIBITION BY PHARMACOLOGICAL OR GENE THERAPEUTIC MEANS LEADS TO REDUCED BLOOD-BARRIER PERMEABILITY AND NEURONAL SURVIVAL AFTER SPINAL CORD INJURY (SCI).

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iNOS is a key mediator of inflammation. iNOS is expressed during CNS injury and is responsible for the formation of high levels of nitric oxide. due to both a stable mRNA transcript and its calcium-independent activity. The production of highly reactive and cytotoxic O2 species e.g. peroxynitrite. in turn leads to tissue damage. We have used an acute administration of iNOS antisense oligonucleotides (ASO) 3h after moderate contusive injury and the pharmacological inhibitors; 1400W (two injections i.v.. 3 and 9h) and aminoguanidine (two injections i.p.. 0 and 6h). to decrease the number of iNOS immunoreactive cells and iNOS activity at the site of a spinal cord contusive injury. Both iNOS ASO and pharmacological inhibition of iNOS reduced the degree of blood-brain-barrier-disruption (measured by calculating the area of plasma leakage of rat immunoglobulins). however. that mediated by iNOS ASO inhibition was much more pronounced and was comparative to an increased ablation of iNOS. Hypertrophic astrocytes and marked gliosis in the white matter were also present with iNOS inhibition. Hypertrophic astrocytes may allow these cells to better support vasculature integrity and aid in preventing leakage. We observed that only the dramatic inhibition of iNOS by ASO was able to reduce neuronal necrosis in the dorsal horn compared to controls. Neuronal necrosis was detected in vivo by examining if cell membrane disruption had occurred using intra sub-arachnoid infusion of propidium iodide before perfusion. Neutrophil accumulation within the injury site was not significantly reduced by any of the treatments. These novel findings report that an inhibition of iNOS acutely is beneficial in retarding SCI pathophysiological processes. (Supported by NIHNS38665 and The Miami Project).

P568.

NEUTROPHIL INFILTRATION AND HEME OXYGENASE-1 INDUCTION ARE EARLY PROGNOSTICATORS OF SPINAL CORD INJURY SEVERITY

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We have previously shown that tissue damage in the injured spinal cord is in part related to an early inflammatory response that involves neutrophil infiltration. activation of matrix metalloproteinase-9 (MMP-9). and induction of heme oxygenase-1 (HO-1). In this study. we subjected adult male mice to either a moderate or severe spinal cord contusion injury and determined the extent to which neutrophil infiltration. MMP-9 activity. and HO-1 induction reflect injury severity. To assess neutrophil infiltration. we developed a novel method for extraction and quantification of neutrophils in the spinal cord using flow cytometry. We found that neutrophil infiltration was significantly greater in the more severely injured group. In contrast. MMP-9 activity. defined by gelatin zymography. was similar within each of the injury groups. HO-1 induction. as determined by immunocytochemistry. appeared more robust with a greater axial distribution in the more severely injured group. Since it is also a marker of oxidative stress. we next determined the extent to which HO-1 induction correlated with patterns of blood flow using a lectin perfusion technique. In regions of no flow HO-1 was not induced. whereas endothelial induction occurred in low flow regions. and glial/endothelial induction was present in regions of relatively normal flow. Thus. cell specific HO-1 induction correlated with flow. We conclude that neutrophil infiltration and HO-1 induction can be used as early indicators of spinal cord injury severity. Moreover. cell-specific induction of HO-1 may serve as an index for defining relative vulnerability of cells to changes in the local microenvironment. Supported by NS 39278. NS39847. and the Dana Foundation.

P569.

A NOVEL APPROACH TO THE ONSET-MECHANISM OF CERVICAL SPONDYLOTIC MYELOPATHY: COMPUTER SIMULATIONS BASED ON MECHANICAL FEATURES OF THE SPINAL CORD.

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In cervical spondylotic myelopathy (CSM). there are four pathological and clinical features without adequate explanations: 1) the spinal cord is highly tolerant towards slowly progressive chronic compression. 2) the lateral corticospinal tract is more severely damaged than other white matter tracts. 3) the anterior spinal artery is almost never occluded. 4) symptoms often show step-wise progression. We previously found that (a) gray matter is more rigid although more fragile than white matter(J Neurotrauma 2001). (b) continuous compression of the spinal cord results in a gradual decrease in cord stress. (c) cord stress increases proportional to the speed of compression. In the present study. the four features were examined in 3 computer simulation models based on a). b) and c) mechanical features. Three models were simulated in vitro. A) Acute compression. B) Chronic compression(CSM). C) Acute on chronic compression(CSM). In the acute model. damage occurred in the gray matter and then the lateral corticospinal tract. In the chronic CSM model. gradual compression caused a gradual decrease in stress. the anterior funiculus did not show damage. and the anterior spinal artery was not severely compressed. In the acute on chronic compression model. cord stress increased in the gray matter and then in the lateral corticospinal tract. bulging of the ligamentum flavum repeatedly compressed the spinal cord. and CSM mechanical features were gradually aggravated. Thus. anterior and posterior acute compression easily damages the lateral corticospinal tract. and the decreased cord stress could also be related to high tolerance towards against slowly progressive chronic compression and the plasticity of the spinal cord.

P570.

ELECTROPHYSIOLOGY AND FUNCTIONAL IMAGING OF MATURE GLIAL CELLS IN SITU USING RAT SPINAL CORD SLICES

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Electrophysiological studies of functional properties of glial cells in spinal cord white matter have been mostly confined to cell cultures or acute slices of immature spinal cord of experimental animals (mice. rats). We have developed a viable acute in vitro 250 um-thick longitudinal slice preparation of mature rat spinal cord with preserved white matter compound action potentials. that allows for visual identification and patch clamp recording of white matter glial cells within their natural networks. The cells are visualized within the slices using infrared differential interference contrast videomicroscopy and approached with recording electrodes under visual control. The advantage of using longitudinal slices over more commonly used transverse slices of spinal cord is preserving the integrity of local white matter glial-axonal networks. which is especially important for oligodendrocytes which extend their processes for distances up to 250 um along the axons. Oligodendrocytes are easily identifiable by their close association with axons. which is further confirmed following intracellular injection of fluorescent dyes Lucifer Yellow or Alexa 350 that revealed long processes closely associated with axons. Astrocytes are identified by their characteristic stellar morphology and GFAP-positive immunostaining. Both oligodendrocytes and astrocytes (membrane resting potentials ranging between -42 mV and -66 mV. $n = 58$ and between -37 mV and -71 mV. $n = 35$. respectively) showed characteristic non-linear current-voltage relationships and pronounced voltage-dependent potassium currents activated at membrane voltages positive than -40 mV. AMPA/kainate agonists applied by microsuperfusion activated CNQX-sensitive inward currents (at -70 mV) in both types of cells. Our data represent the first electrophysiological recordings from mature spinal cord white matter astrocytes and oligodendrocytes in situ. The longitudinal slice preparation is viable for up to 8 hours in vitro and can be successfully used for both electrophysiological and fluorescence imaging studies of glial-axonal interactions in normal and post-injured spinal cord. Supported by CIHR and ONF.

P571.

NEUROPROTECTION AFTER ACUTE SPINAL CORD INJURY BY INHIBITION OF THE FAS APOPTOTIC PATHWAY

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Background: Recent evidence from our laboratory has shown an association between FAS receptor expression and apoptosis following acute spinal cord injury (SCI) (Casha et al Neuroscience 2001). Hypothesis: Administration of soluble Fas receptor (sFasR) in in vitro and in vivo models of SCI will result in significant neuroprotection. Approach: 1) To apply soluble Fas receptor to an in vitro organotypic slice culture model of SCI and demonstrate a decrease in total and apoptotic cell death. 2) To administer sFasR intrathecally for seven days (using an osmotic mini-pump) to the level of SCI and analyze effects of sFasR. Methods: Experiment 1. Using an organotypic slice model of injury. cell death was quantified using propidium iodide and sytox green. Experiment 2. In vivo SCI was induced using a clip compression injury model. With a mini-osmotic pump. sFasR was introduced to the intrathecal space at the level of injury. Immunoblots were used to identify NF 200. and caspase-3 activation. Results: The in vitro model of injury has shown decreased cell death with the administration of sFasR. In vivo preliminary results indicate decreasing caspase-3 activation. Conclusion: The Fas receptor appears to be a promising new neuroprotective target after SCI. Funding: Ontario Neurotrauma Foundation Studentship (AA); Christopher Reeve Paralysis Foundation Operating Grant. Ontario Ministry of Health Career Scientist Award and Krembil Chair in Neural Repair and Regeneration (MGF).

P572.

DEATH RECEPTOR EXPRESSION AFTER HUMAN CERVICAL SPINAL CORD INJURY: IMPLICATIONS FOR THE DEVELOPMENT OF NOVEL NEUROPROTECTIVE STRATEGIES.

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BACKGROUND: Despite recent advances. therapies for spinal cord injury (SCI) are minimally effective. Improved neuroprotective approaches are needed. Our laboratory has recently implicated the death receptors. Fas and p75NTR. as mediators of post-SCI apoptosis in experimental models. To determine if death receptor mediated apoptosis is relevant to human SCI. we have examined molecular mechanisms of cell death in injured human cervical spinal cord tissue. METHODS: Eight cases (2 females/6 males. mean age 53 years) with SCI (2 wks–2 yrs post-trauma) were examined. Using hematoxylin and eosin/luxol fast blue staining. the morphology of the injury epicenter and areas rostral and caudal to the lesion were assessed by light microscopy. Apoptotic cells were identified via TUNEL staining and activated caspase-3 immunohistochemistry. Death receptor expression was determined using antibodies against Fas and p75NTR. RESULTS: Apoptotic cell death in neurons and oligodendrocytes was a prominent feature of human SCI. An increase in TUNEL positive cells was noted as compared with age-matched controls ($p < 0.05$). Caspase-3 activation occurred after SCI. Fas and p75NTR death receptors were expressed in adult human spinal cord providing supportive evidence linking this mechanism with SCI. CONCLUSIONS: Our findings suggest that death receptor mediated apoptosis occurs after human SCI and that this mechanism is a clinically relevant target for neuroprotective strategies.

P573.

EVALUATION OF THE NEUROPROTECTIVE EFFECTS OF THE SODIUM CHANNEL BLOCKER RILUZOLE AND METHYLPREDNISOLONE IN A NOVEL ORGANOTYPIC SLICE CULTURE MODEL OF SPINAL CORD INJURY

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Until recently. methylprednisolone (MPS) was thought to be the standard of care for the acute treatment of spinal cord injury (SCI). However. concerns regarding the modest clinical benefits of MPS have resulted in the downgrading of this treatment to that of an option based on newly disseminated guidelines from the American Association of Neurological Surgeons/Congress of Neurological Surgeons. Based on this. further work to develop alternative or complimentary neuroprotective approaches to MPS are urgently required. Previously. we have shown that acute administration of riluzole (RIL). a sodium channel blocker. can mitigate secondary tissue loss. preserve axonal integrity. and enhance locomotor recovery in rodents with severe cervical SCI. In order to assess the neuroprotective effectiveness of RIL alone or in combination with MPS compared to MPS alone we evaluated mean cell death counts of propidium iodide (PI) labeled cells in an organotypic spinal cord slice injury model prepared from adult mice. Weight-drop injured slices were treated with 10 uM of RIL. MPS or RIL+MPS. 15 minutes post injury. Fluorescent. PI-labeled cells were imaged by confocal microscopy at 4. 24. and 48 hours after treatment. Mean PI cell counts from each treatment group were normalized against total cell death counts from corresponding treated slices to generate a percentage of cell death. Analysis by ANOVA indicated a significant effect of treatment ($F: 3.59$; $p = 0.025$) with RIL and RIL+MPS treated slices having a smaller percentage of labeled cells than those treated with MPS alone or those not treated with a drug. These findings suggest that RIL alone or in combination with MPS is an effective neuroprotective strategy for SCI. Further preclinical in vivo evaluation of the effective time window for RIL +/- MPS is warranted as a prelude to consideration for translation to clinical trials. Supported by the Ontario Neurotrauma Foundation.

P574.

AMPA RECEPTORS AFFECT THE DEVELOPMENT OF CHRONIC CENTRAL PAIN (CCP) AFTER SPINAL CORD INJURY (SCI)

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SCI results in CCP development in most patients. Cyclothiazide (CTZ). a positive allosteric modulator of AMPA receptors. potentiates the AMPA receptor by blocking desensitization whereas NBQX is a competitive antagonist. NBQX but not CTZ was hypothesized to improve behavioral outcomes. return glutamate receptor (GluR) subunit expression to control values. and protect neurons from excitotoxicity by blocking actions excitatory amino acids (EAA). To evaluate the effects of the agents on EAA release. samples were collected for HPLC analysis before. during and after SCI (injury at T10 with an NYU impactor; 12.5 mm drop. 10 gram rod of 2 mm diameter) with microdialysis fibers inserted into the cord. Injury was immediately followed by an epicenter injection of the agent. Neither NBQX (15 nmol) nor CTZ (7 nmol) significantly affected the release of EAA. However. injection of the same amounts of the agents altered behavior and protein expression. CTZ administration returned thermal forelimb withdrawal latencies to control at post contusion days (PCD) 7 and 14 ($p < 0.05$). NBQX also increased thermal forelimb withdrawal latencies. but much later (PCD 47 and 54. $p < 0.05$). Protein expression after SCI was evaluated by western blot analysis at PCD 7 and 28. NBQX but not CTZ returned the expression of GluR2 to near non-SCI levels. We propose that NBQX stabilizes the composition of the AMPA receptor to its usual form by retaining GluR2 expression near its control levels in the cord. thereby decreasing the calcium influx and reducing the debilitating effects seen in SCI.

P575.

TRANSPLANTATION OF HUMAN OLFACTORY ENSHEATHING CELLS IN THE INJURED ADULT RAT SPINAL CORD

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In the present study we investigate the ability of human olfactory ensheathing cells (hOECs) to facilitate axonal regeneration and behavioral recovery following moderate contusion injury in the adult rat. Human neuroepithelial tissue was obtained via biopsy. providing a source from which hOECs were then isolated. cultured. and amplified in vitro. Five to seven days following contusion injuries in adult female rats. single-cell hOEC suspensions were transplanted into the lesion site. The animals were monitored and behaviorally tested over a period of 6 weeks post-injury. Fluoro-ruby was then injected in the motor cortex to label the descending corticospinal tract (CST). while fluoro-emerald was injected in the sciatic nerve to visualize ascending tracts. Two weeks after labeling. the animals were perfused and the tissue analyzed for the spread of transplanted cells. the extent of axonal growth. the ability of transplanted cells to remyelinate. and the injury response of endogenous cells. Our data indicate that injured animals transplanted with hOECs exhibit anatomical and functional recovery. This work was supported by the Reeve-Irvine Research Center and the Roman Reed Fund. Dr. Vawter supported by William Lion Penzner Foundation.

P576.

GRAFTING GENETICALLY MODIFIED NEURAL STEM CELLS TO THE INJURED SPINAL CORD

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We have examined the effect of delivery of genetically modified adult neural stem cells (NSC) to the rat spinal cord subjected to impact injury. Cells harvested from adult rat spinal cords were grown to "neurospheres" in culture and transduced with Ngn2-IRES-eGFP 9 days after harvest. 20 female Sprague Dawley rats were subjected to a 12.5 mm impact injury imparted by a NYU impactor. One week after the injury. 12 rats received 80.000 Ngn2-IRES-eGFP NSC. 8 other rats received similar injections of vehicle alone. Cells that are clustered around the sites of injection 2 days after transplantation mature. migrate and integrate in the substance of the spinal cord and form interconnecting networks at 9 weeks. Initial primitive morphology is progressively replaced with more complex appearing cells with elongated processes extending from the perikarya as early as 2 weeks after transplantation with further maturation at 9 weeks. Immunohistochemical studies reveal striking absence of GFAP staining. RIP positive cells are observed at two weeks and at 9 weeks. The proportion of RIP positive cells is increased at 9 weeks compared with earlier time points. Taken together with the morphological observations of these cells. an oligodendrocytic differentiation may have occurred. Our preliminary results suggest that neurogenin-2 transfected NSC integrate and migrate in injured spinal cord and may differentiate along oligodendroglial lines. Detailed functional studies are currently underway.

P577.

THE SMALL GTPase RIT INCREASES AXONAL BRANCHING IN AN MEK-INDEPENDENT MANNER

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Successful neuronal regeneration following central nervous system (CNS) injury requires axonal sprouting. elongation. branching. and synapse formation. Important signaling molecules that regulate these events include the small nucleotide binding proteins of the Ras superfamily. Rit is a member of the Ras subfamily that regulates growth-stimulating pathways in NIH3T3 fibroblasts. where expression of a constitutively active mutant causes tumorigenic transformation without activating known MAP kinase cascades or PI3K/Akt pathways (Rusyn et al.. 2000. Oncogene 19: 4685). Here we investigate the role that Rit plays in neuronal regeneration using the SH-SY5Y human neuroblastoma cell line. Adenoviral expression of a constitutively active Rit (RitL79) induces robust neurite outgrowth. determined by measuring neurite initiation. elongation. and branching. Using phospho-specific antibodies. we determined that Rit79L activates ERK1. 2. but not Akt. whereas both proteins were activated by constitutively active Ras. Interestingly. the MEK inhibitor PD 098059 blocked Rit79L mediated increases in neurite initiation. but not branching. These data. obtained in human cells. support previous studies showing Rit activates ERK. but not Akt. in rat PC6 cells (Spencer et al.. 2002. J. Biol. Chem. 277: 21060). and newly identify a prominent function for Rit in axonal branching. Importantly. our results may identify Rit as a target for therapies to enhance multiple aspects of neuronal regeneration including axonal sprouting. elongation. and branching following CNS injury. [Supported by grants from NIH (EY10545 and DA12719 to DMS) and KSCHIRT (# 0-8. to DLH)].

P578.

ROLE OF CIRCULATING IGF-I IN FUNCTIONAL RECOVERY FROM SPINAL CORD INJURY UNDER NORMAL AND ENRICHED

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Physical activity (e.g. voluntary wheel running or enriched environment (EE)) has been shown to enhance return of locomotor function in spinal cord injured rats. A possible mediator of these beneficial effects is circulating IGF-I as uptake of this growth factor across the blood-brain barrier increases during exercise. In this study. animals were subjected to a moderate (12.5 gcm MASCIS) spinal cord contusion injury and return of locomotor function was scored using the BBB- and BBBsub-scores. ThoracoLumbar Height test. Gridwalk and CatWalk during the next 8 weeks. In the first experiment. IGF-I (14 ug/day for 28 days) was infused sc using osmotic minipumps. IGF-I treated animals regained better locomotor function than controls. In a second experiment. IGF-I antiserum or pre-immunesrum (20%. 6 ul/day for 28 days) was infused sc in animals housed in the EE or under standard conditions. EE housed animals recovered better locomotor function than control housed animals and under both housing conditions IGF-I antiserum diminished the level reached. significantly so with EE housing. Antiserum treatment did not completely block the beneficial effects of EE housing. We conclude that circulating IGF-I mediates the protective effects of enhanced physical activity. but not the effects EE housing exerts on central pattern generator function.

P579.

A NOVEL DELIVERY SYSTEM FOR TREATMENT OF SPINAL CORD INJURY

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A novel method of localized administration of therapeutic agents for spinal cord injury (SCI) is being investigated. The strategy consists of a polymeric drug solution that gels upon injection in the subarachnoid space (SAS). A spinal canal model was developed to test the safety of implanting the drug delivery system (DDS) in the SAS. In vitro results showed that intrathecal implantation of the DDS is safe. The release of bioactive EGF and FGF-2 from the DDS was monitored in vitro for 2 months. In vivo. the safety of the DDS was evaluated by injecting collagen or aCSF intrathecally in Sprague Dawley rats. both uninjured and injured. by 20g cord clip compression. Histologic analysis of the uninjured and injured cords showed the presence of collagen in the SAS up to 56d. Injection of collagen or aCSF in uninjured animals did not cause inflammation or astrocytosis. In injured animals. protoplasmic astrocytes were evident in both the collagen and aCSF groups. and the response to injury was not different between groups. There were no macrophages in uninjured animals. In the injured groups. collagen did not increase the macrophages compared to controls. BBB scores between groups in uninjured animals were similar. and injured animals injected with either collagen or aCSF also had similar BBB scores at 56d. Thus. an injectable DDS may allow drug delivery over a prolonged period of time and holds promise as a therapeutic strategy for treatment of SCI. Supported by: CIHR. Univ. of Toronto.

P580.

BRAIN DERIVED NEUROTROPHIC FACTOR INFUSION INTO THE MOTOR CORTEX PROMOTES SPROUTING OF INTACT CORTICOSPINAL FIBERS WITHIN THE CERVICAL SPINAL CORD

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After spinal cord injury. one approach to enhance functional recovery or compensation is to exploit intact axons. which extend past the point of injury. to sprout and connect to potential targets. We have previously shown that application of the neurotrophin Brain Derived Neurotrophic Factor (BDNF) to the sensorimotor cortex stimulates the expression of regeneration-associated genes (GAP-43. Ta1-tubulin). This treatment also results in enhanced sprouting of corticospinal fibers rostral to the site of thoracic injury (Hiebert et al.. 2002). In the present study. we investigate. whether infusion of BDNF into intact sensorimotor cortex induces sprouting of intact corticospinal fibers into denervated cervical spinal cord. A left unilateral pyramidal lesion of the corticospinal tract was performed. BDNF was infused (500 ng/ 0.5 ml/hr) into the contralateral intact sensorimotor cortex for 14 days after injury. On day 28. the in tact corticospinal tract was labeled with BDA. and the animals sacrificed on day 42. Digital images of the C6 spinal cord were taken from cross sections. and labeled axon profiles from the intact sensorimotor cortex were manually traced using Photoshop. The cumulative area covered by the digital pencil tracing was quantified. Results show a 3 fold increase in corticospinal axon profiles into the denervated half of the C6 spinal cord. Other levels of spinal cord are currently being analyzed. We are also studying the efficacy of a number of locomotor and precision tasks (i.e. gait analysis. swimming analysis. kinematic analysis. and food pellet reaching task) in our model of injury. as well as assessing functional compensation as a result of treatment. This project is supported by Rick Hansen Neurotrauma Initiative and BC Neurotrauma Fund.

P581.

AUTOLOGOUS ACTIVATED MACROPHAGE THERAPY SHOWS POTENTIAL AS A TREATMENT FOR ACUTE COMPLETE SPINAL CORD INJURY

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Introduction: Complete spinal cord injury is a devastating and until now. almost untreatable condition. Experiments showing CNS regeneration and partial functional recovery in rats (Rapalino et al. Nature Medicine 4:814–821. 1998) served as proof of principle. Safety and efficacy have been demonstrated in extensive preclinical experiments that continue to produce promising results. Methods: A phase I clinical trial is underway. involving 8 patients with acute complete spinal cord injury (ASIA A). The treatment consists of activating autologous macrophages derived from the patient's peripheral blood and administering them into the parenchyma of the spinal cord within 14 days of injury. The follow-up consists of neurological. electrophysiological. rehabilitation and quality of life assessments. The patient outcomes are compared to historical controls. Results: Eight patients have been followed for up to 30 months. depending on the date of treatment. In none of them short- or long-term side effects that could be linked to the experimental treatment were detected. Of these eight patients. the three patients that have been followed for more than six months exhibit partial recovery of sensory and motor function and have upgraded their ASIA classification from ASIA A to ASIA C. These clinical findings are also supported by electrophysiological examinations. Conclusions: Based on the interim results of this small sample. it is proposed that the Autologous Activated Macrophage Therapy can be an effective treatment for Complete Spinal Cord Injury.

P582.

APPLICATION OF ADENO-ASSOCIATED VIRUS EXPRESSING BRAIN DERIVED NEUROTROPHIC FACTOR TO RUBROSPINAL NEURONS AFTER ACUTE AND CHRONIC AXOTOMY

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Rat rubrospinal neurons (RSN) undergo massive atrophy after cervical spinal cord axotomy and this can be prevented by BDNF infusion into the vicinity of the red nucleus. This treatment was still effective when initiated one year after spinal cord injury. However. the infusion of BDNF produces significant inflammation and tissue damage. Therefore. we used a single micro-injection (glass capillary) of an adeno-associated-virus (AAV) expressing BDNF. driven by the chicken beta actin promoter. into the vicinity of the red nucleus at various times after a C3/4 hemisection (in male SD rats). We observed that mainly glial cells in and around the RN were infected. Tissue damage and inflammatory reaction to the needle was minimal. Group 1 received AAV-BDNF at the time of axotomy and showed a significant ($p < 0.02$ vs. control virus) prevention of RSN atrophy. The cell profile sizes were 91% of contralateral on day 14 and 73 % on day 21 respectively. In contrast. the control-virus treated cells displayed sizes of 62 % and 57 % of contralateral by 14 and 21 days. Group 2 received the AAV-BDNF virus injection 2 weeks after axotomy and was analyzed on day 21. Their cell sizes were found to be 65 % of contralateral. which is somewhat bigger than the values of the control group (56%). but did not reach statistical significance ($p = 0.07$). Group 3 was injected with AAV more than 6 months after axotomy and RSN size were found at 63 % of contralateral. which is not significantly ($p = 0.16$) bigger than in control-virus treated animals (56%). We are presently analyzing the expression of regeneration associated genes. such as GAP-43 and t-alpha-1-tubulin. Our data indicate that treatment with AAV-BDNF effectively prevents the atrophy of acutely injured RSN but has only small effects on the reversal of chronic atrophy. Supported by the BC-Neurotrauma Foundation.

P583.

RELATIONSHIP OF MITOCHONDRIAL DEPOLARIZATION TO GLIAL CELL DEATH AFTER IN VITRO NEUROTRAUMA

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Hypothesis: Recent evidence has shown that mitochondrial depolarization and dysfunction can precede neuronal cell death after toxic insults such as glutamate . oxygen glucose deprivation. and physical injury. Less certain is the role of mitochondrial depolarization in glial cell death in the spinal cord after injury. To address this issue we examined the mitochondrial potential in cells from spinal cord mixed glial/neuronal cultures that died after physical injury. Methods: Mixed glial/neuronal cultures were derived from 1 week old mouse pups. The cultures were then injured with a scratch from a pipette tip at 3–4 weeks after plating. Prior to injury the cells were loaded with the mitochondrial potential dye Rhodamine 123. The pre-injury and post-injury whole cell fluorescence levels were then measured using a 40X water immersion objective and epifluorescence microscopy. The maximum increase in fluorescence after injury was normalized and compared to uninjured cultures. Fluorescence levels of cells directly in the path of the pipette tip were not used due to concerns of dye leakage from ruptured cellular membranes. Cultures were also loaded throughout the experiment with propidium iodide to assess cell death. Results: The maximum change in fluorescence in the injured cells was found to be an 86.2 % increase. The maximum change for uninjured cells over a similar time period was found to be an 11.0 % increase. These results were found to be statistically significant. Studies are currently underway to examine the effect of blocking mitochondrial depolarization on preventing posttraumatic glial cell death. Conclusion: Spinal cord glial cells undergo significant mitochondrial depolarization prior to cell death. Given the evidence that glial-axonal signaling is critical to white matter integrity after SCI. these results could have important pathophysiological implications.

P584.

AMPA RECEPTOR EXPRESSION IN WHITE MATTER GLIA AND ASSOCIATION WITH APOPTOSIS AFTER ACUTE SPINAL CORD INJURY

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Increasing evidence suggest that AMPA receptors play a key role in mediating excitotoxic cell damage after acute spinal cord injury (SCI). In the present study we examined changes in AMPA receptor expression. the cellular distribution of these changes and the association between AMPA receptor expression and apoptosis after SCI. Immunohistochemistry and Western blot were used to examine the distribution of AMPA receptors in spinal cord white matter. A lack of GluR2 expression in white matter glia suggests that these cells are highly calcium permeable and susceptible to Ca2+ mediated secondary injury mechanisms. Quantification of AMPA receptor expressing cells in spinal cord white matter indicated a predominance of GluR3 expression in oligodendrocytes and proportionately greater GluR4 expression in astrocytes. A clip compression model of SCI was used to examine the changes in AMPA receptor expression in dorsal column white matter 1. 3. 7 and 14 days after injury. Quantitative analysis of GluR3 levels of expression indicate a significant decrease at 3 days post-injury compared to uninjured animals. followed by a recovery of expression by 2 weeks. GluR4 levels of expression followed a similar temporal pattern of expression. Gene message expression of GluR3 and GluR4 Flip/Flop mRNA splice variants exhibited a similar temporal pattern of expression that correlated with protein expression of the respective subunits. Quantification of TUNEL positive cells expressing GluR3 and GluR4 subunits indicated a significant increase at 1. 3 and 7 days after injury. A large decline in GluR3 expressing oligodendrocytes 3 days post-injury in association with TUNEL data suggest that this subunit may be involved with the early induction of apoptosis in white matter glia. An increase in GluR3 and GluR4 expression 7 and 14 days after injury correlates with the phenomenon of astrogliosis after CNS trauma. The effects of altered cell populations expressing AMPA receptors after SCI may have important implications with respect to plasticity and neurological recovery after SCI. Supported by CIHR/CNRP.

P585.

WHITE MATTER INJURY AND ACUTE INFLAMMATION IN ENDOTHELIN-1 INDUCED SPINAL CORD ISCHAEMIA

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Many spinal cord injury models rely on impact injury to simulate the effects of contusion of the spinal cord in man. Although physical injury is often the initiating event in spinal cord injury, the resulting lesion may involve ischaemia, excitotoxicity and the acute inflammatory response. We have developed a model of focal ischaemia in the rat spinal cord. which allows us to dissect the mechanisms involved without the effects of direct trauma. Microinjection of endothelin-1 (250nl ≡ 15pmol ET-1) into the spinal cord ventral grey matter results in an ischaemic lesion characterised by rapid (within 24 hours) neutrophil recruitment followed by a pronounced mononuclear phagocyte response at 3 days [1. 2]. APP positive axons and axonal end-bulbs are present in the white matter adjacent to the lesion site from 6 hours after microinjection. peaking at 24 hours. This suggests that the developing ischaemia in the ventral grey matter can have an effect on adjacent white matter structures. Longitudinal spinal sections show that the lesion develops rapidly rostrocaudally, so that motor neurons one vertebral level above or below the microinjection site are absent at 6 hours. Neutrophils and macrophages are also found at distances up to 7mm rostral or caudal to the microinjection site at later times. Laser-Doppler flowmetry has been used to observe the kinetics of the ET-1 induced ischaemia and the rostro-caudal extent of the hypoperfusion. These data may enable us to relate the extent of ischaemia to the observed white matter pathology. 1. Corkill. D.J., D.C. Anthony, and V.H. Perry. Contrasting inflammatory responses in endothelin-1 induced ischaemic lesions in rat brain and spinal cord. British Neuroscience Association Abstracts. 2001. 16: p. P12.07. 2. Corkill. D.J. and V.H. Perry. A model to dissociate the ischaemic and mechanical components of spinal cord injury. Journal of Neuroimmunology. 2001. 118(1): p. Pr 59.

P586.

IN VIVO EVIDENCE OF MEMBRANE DAMAGE FOLLOWING COMPRESSION OF ADULT GUINEA PIG SPINAL CORD

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Loss of membrane integrity plays an important role in the pathogenesis of traumatic spinal cord and brain injury. It is well established in in vitro studies that controlled mechanical insults inflict membrane damage. which correlates with functional loss and cell survival. Much less evidence. however. has been demonstrated in vivo with similar injuries. where significant secondary degeneration occurs. Current study constitutes an attempt to document the dynamics of the loss of membrane integrity following controlled compression at 1 hour. 1 day. 3 days. and 7 days post injury in a live animal model. Female adult guinea pigs were subjected to spinal cord compression with modified forceps. which crushed the cord from an original width of approximately 3.5 mm to 1.3 mm in a span of 2.3 mm along the longitudinal axis of the cord. Using an HRP-exclusion assay to examine the membrane integrity. we have found that membrane damage was evident 1 hour after the injury in the center. but not at 10 mm away from the compression area. However. at 1–7 days after compression. the membrane damage spreaded to outside of the original crushing site. Apparent tissue loss at gross level accompanied the membrane damage. In summary. membrane damage existed days after initial mechanical insults and spreaded to the neighboring uncrushed area. Therefore. the secondary tissue loss may be the result of retrograde degeneration of damaged axons and/or biochemical toxins released from the original site which can independently inflict further membrane damage.

P587.

NEURALIZATION OF MULTI-POTENTIAL STEM CELLS ISOLATED

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The surprising possibility has recently emerged that some non-neural adult tissues may possess stem cells capable of neural differentiation. Here, we aimed to more clearly define the neurogenic potential of recently identified multi-potential Skin-derived Precursors (SKPs) (Toma et al., Nature Cell Biol 3:9. 778–784). SKPs produce floating clusters of cells that are isolated when trypsinized rodent skin cells are grown in the presence of FGF-2 and EGF. Following serum-based differentiation on laminin/poly-D-lysine–coated slides, a sub-population of SKPs migrates out from the clusters and displays multiple characteristics of neural precursors: (i) expression of the neuroepithelial stem cell marker, nestin, (ii) maintenance in an undifferentiated state with FGF2, and (iii) FGF-2 withdrawal-induced differentiation into neural or neural crest progeny, including neurons, glia, and smooth muscle cells. In short term cultures (<10 passages), extensive neuronal differentiation was evidenced by the expression of both early and late pan-neuronal genes (ie. bIII-tubulin and MAP2), as well as appropriate neuronal morphology. In longer term cultures (10–50 passages), neuronal differentiation could be enhanced by modulation of serum concentrations or supplementation with specific neurogenic factors, including BMPs and retinoic acid. Together, these data identify skin as an easily accessible source of potentially transplantable neural precursors.

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VASCULAR TRAUMA: COMPLEX AND
CHALLENGING INJURIES, PART I

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CURRENT CONTROVERSIES IN SHOCK AND RESUSCITATION

Michael Orlinsky, MD, FACEP, William Shoemaker, MD, FACS,
Ernane D. Reis, MD, and Morris D. Kerstein, MD, FACS

To paraphrase Kirby in his introductory remarks at the Hyland Symposium in 1978, "There are some readers, no doubt, who feel the subject of colloid and crystalloid therapy . . . has been overly emphasized . . . and there can be little new information to add to an already voluminous body of literature."[108] Well, that subject and many other controversial issues have continued to be studied and written about, perhaps because the thirst for knowledge continues to grow as new technology leads to new findings, or perhaps what appeared initially to be simple questions were indeed far more complex than imagined. The aim of this article is not necessarily to resolve all the controversies, but rather to point out a select few, provide some understanding of current knowledge, and foster an ongoing interest by individual clinicians as to how best to care for their patients.

To better appreciate the issues, it is important first to emphasize some pathophysiologic concepts of hemorrhagic shock and see how they intertwine with newer, and particularly non-invasive, monitoring devices. Most health care providers, and no doubt many physicians, would define shock by the level of blood pressure and judge successful treatment on the reestablishment of a preconceived baseline value. This

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would be erroneous, not only because of all the false positive and false negative blood pressure values that can occur, but it would not really represent the problem, or the solution, of hemorrhagic shock. Indeed, a normal arterial blood pressure in the face of observed blood loss means that some organ somewhere is underperfused by vasoconstriction of its vascular bed. Oxygen is not stored in tissues, so underperfusion produces progressive hypoxia and proportional damage.[128]

Shock is typically recognized by nonspecific signs and subjective symptoms such as cold clammy skin, pallor, weak thready pulse, unstable vital signs, cyanosis, mottled skin, restlessness, and an altered level of consciousness. Unfortunately, these findings are imprecise, subjective, and observer-dependent; they are secondary effects of acute circulatory failure, not the principal physiologic problem. The lack of objective criteria and the inability to quantify these signs and symptoms have been major problems for understanding the physiology and for the development of optimal therapeutic goals. Nevertheless, shock is first recognized, routinely diagnosed, and often managed by these symptoms. Monitoring is used to recognize circulatory problems, to describe temporal physiologic patterns, to reinforce clinical opinions with physiologic analyses, to evaluate effectiveness of alternative therapies, and to predict and improve outcome.

CONVENTIONAL MONITORING, PHYSIOLOGY, AND THERAPEUTIC APPROACH TO SHOCK

Monitoring is frequently used to measure and evaluate mean arterial pressure (MAP), heart rate (HR), central venous pressure (CVP), hematocrit (Hct), urine output, and arterial oxygen tension (PaO_2). These traditionally monitored variables characterize circulatory failure, especially in advanced stages of shock. They are not measures of the adequacy of circulatory function or tissue perfusion in the early stages. They reflect secondary aspects of shock syndromes, not primary mechanisms. Moreover, the appearance of hypotension or other signs and symptoms of shock do not mark the beginning of circulatory failure, but rather represent the beginning of decompensation. Measurements begun after the appearance of hypotension reflect late effects, not early primary mechanisms. Hemodynamic and oxygen transport variables, not the secondary manifestations of the syndrome, should be used to evaluate circulatory function and shock.[21, 196, 197]

The current widely held therapeutic paradigm for hemorrhagic shock is to promptly give sufficient therapy to reduce the symptoms and normalize the vital signs, arterial blood gases, hematocrit, urine output, and other routine circulatory measures. The assumption is that normal values of these parameters are criteria of circulatory normalcy and that their attainment assures adequate resuscitation; however, the underlying low flow, inadequate tissue perfusion, and tissue hypoxia may remain unnoticed and untreated until organ failure appears. Blood

pressure early in the course of shock is not well correlated with blood flow.[250] In a study of patients with multiple injuries and head trauma, Scalea et al[184] noted that despite being normotensive and neither tachycardic nor oliguric, 80% of the patients had evidence of inadequate tissue perfusion based on elevated lactate levels or decreased venous oxygen saturation.

An appropriate basic assumption is that low flow, poor tissue perfusion, shock, and other circulatory dysfunctions can be recognized early by objective noninvasive criteria, and that more promptly delivered therapy might be more efficacious. Noninvasively monitored data may be used to titrate early fluid therapy to achieve optimal physiologic criteria to prevent development of lethal organ failures.

PATHOPHYSIOLOGY OF SHOCK

Tissue injury, pain, fear, and hypovolemia activate the sympathoadrenal axis, releasing epinephrine and norepinephrine from the adrenal medullae and sympathetic effector neurons.[199, 201, 202] Also, in response to hypovolemia, extracellular fluid is translocated from the interstial space into the intravascular compartment. Continued stress and the sympathoadrenal response activate the hypothalamic-hypophyseal-adrenal axis, stimulating the adrenals to secrete cortisol, which increases cardiac output and in part mediates the post-traumatic hypermetabolic state. The hypermetabolic state, which requires increased blood flow, makes tissues more susceptible to local ischemic events. [198, 199]

Cardiopulmonary catecholamine effects immediately after trauma include increased blood pressure, heart rate, cardiac contractility, minute ventilation, and peripheral vasomotor tone. Although these adaptive effects are often beneficial, especially in minor insults, exaggerated but uneven peripheral vasoconstriction leads to maldistributed microcirculatory flow with localized areas of hypoperfusion and tissue hypoxemia. The hypoxic, acidotic endothelium of poorly perfused capillaries activates macrophages and leukocytes and produces cytokines, platelet activating factor, eicosanoids, intravascular coagulation, and other immunochemical cascades. The activated macrophages and white blood cells produce oxygen–free radicals and local tissue destruction that mark the systemic inflammatory response syndrome (SIRS). With resuscitation and reperfusion of hypoxic capillaries, these activated cellular and immunochemical cascades are washed into the venous circulation and lead to SIRS, end-organ dysfunction, multiple organ failures, and death. Survivors have greater physiologic reserve capacity and the ability to generate increased flow and tissue perfusion needed to provide adequate tissue oxygenation in the presence of increased metabolic need. Differences between survivors' and nonsurvivors' hemodynamic patterns have motivated investigators to suggest aggressive fluid therapy titrated to reach optimal physiologic goals, defined by the survivors' patterns, as a strategy to improve patient outcome. Fluid therapy is titrated to main-

tain intravascular volume, improve tissue perfusion, and overcome regional circulatory deficiencies caused by uneven, maldistributed vasoconstriction.

Oxygen Transport as a Measure of Tissue Perfusion

The common denominator in early shock is inadequate oxygen delivery (DO_2), needed to meet normal or increased metabolic activity as measured by oxygen consumption (VO_2). An oxygen debt is said to accrue when the actual oxygen consumption permitted is less than that needed. (Recall that DO_2 is the amount of oxygen delivered to the tissues per minute, as described by the formula $DO_2 = CO \times CaO_2 \times 10$, where CO is the cardiac output, and CaO_2 is the arterial oxygen content. Also recall that VO_2 is the amount of oxygen consumed by tissues and is equal to the difference in O_2 delivered to tissues and the O_2 returning from tissues, denoted by $VO_2 = CO \times (CaO_2 - CmvO_2) \times 10$). Inadequate DO_2 is an early pathogenic event that precedes hypotension, limits metabolism, leads to oxygen debt, and increases mortality.[198] Increased CI (cardiac index = CO/body surface area) and DO_2 correlate well with survival, but failure of the body to develop adequate DO_2 and VO_2 responses is highly correlated with death.[21, 196–198, 250]

Inadequate VO_2 may be produced by combinations of low blood flow from hemorrhagic shock, maldistribution of microcirculatory flow from uneven vasoconstriction, and increased metabolic needs from trauma. The physiologic problem is the imbalance between the supply (DO_2) and demand (VO_2) of oxygen. Oxygen debt from an inadequate VO_2 secondary to an inadequate compensatory DO_2 response is the common denominator of most shock syndromes and a major determinant of outcome. Reduced VO_2 and O_2 debts were greater in patients who died.[198] Survivors compensated for tissue hypoxia by increasing CI by way of their neuroadrenal stress response, leading to an increase in DO_2, and VO_2. (Further compensation is provided, if need be, by the tissues extracting more oxygen from the blood, lowering venous oxygen saturation. The limits of compensation are reached when the mixed venous oxygen saturation reaches about 50%. Beyond this point, anaerobic metabolism ensues, leading to a build up of lactate and base deficit.) Nonsurvivors, who poorly compensated for their VO_2 deficiencies, developed lethal multiple organ failures.[27, 197, 198] The survivors' patterns of CI, DO_2, and VO_2 are assumed to represent the effects of the initiating stress and the body's successful compensatory response. The nonsurvivors' patterns are assumed to reflect the effects of severe illnesses and the body's inadequate compensatory responses with subsequent decompensations.

The bulk movement of oxygen is a useful measure of tissue perfusion and is a measure of overall circulatory function. DO_2 reflects perfusion to peripheral tissues; its early increase compensates for earlier inadequate tissue oxygenation after trauma. The temporal patterns of

DO_2 and VO_2 changes are more informative than a single set of measurements, as sequential changes provide a history of physiologic events that lead to shock and subsequent organ failures.

The Hemostatic Plug

With vascular trauma there is another physiological aspect that assumes great importance. Active bleeding from an injured vessel may be uncontrollable without surgical hemostasis or may stop spontaneously by vessel retraction, vasoconstriction, tamponade, or intra- or extraluminal thrombus formation, buying precious time for eventual surgical correction.[17] The hemostatic plug, which forms over several minutes, consists of platelet aggregates and fibrin mesh containing blood cells and other plasma components. According to La Place's law, Poiseuille's law, and the Bernoulli equation, factors that would tend to prevent plug formation and allow for continued blood loss or provide for leakage through and around the plug and allow for renewed bleeding include increased volume, increased blood pressure, vasodilation, and decreased blood viscosity secondary to hemodilution—all factors that are associated with fluid resuscitation.[17, 79, 171] However, given that fluid resuscitation leads to increased oxygen delivery to the tissues, and that ischemia/reperfusion injury leading to SIRS and multiple organ failure may occur earlier than thought, the issue of immediate (prehospital) emergency department (ED) versus delayed (intraoperative) fluid resuscitation becomes important.[57, 151] The fact that multiple organ failure is the most common cause of late death after injury, and with the exception of immediate mortality resulting from neurologic lesions or exsanguination, is the main cause of posttraumatic death, underscores the significance of the issue.[56, 207]

SURVIVOR/NONSURVIVOR PATTERNS

Early Hemodynamic and Oxygen Transport Patterns in Postoperative Patients

High-risk surgical patients may be used as a model for other etiologic types of shock, because time relationships are precisely documented in the chart after elective surgery.[197] Patterns of nonsurvivors of high-risk surgery consisted of reduced flow and oxygen transport in the intraoperative and immediate postoperative periods, while survivors had less intraoperative circulatory deficits and significantly increased flow and oxygen transport in the early postoperative period.[21, 197, 199] Survivors, compared with nonsurvivors, had: greater increases in CI and flow-related variables with lower CVP and wedge pressures, less pulmonary vasoconstriction (lower PVPI and MPAP), greater increases in DO_2 and VO_2 with lower oxygen extraction rates and normal blood

gases, greater hematocrit, blood volume, and red cell mass, and less pulmonary shunt (Qsp/Qt).[21, 196, 197, 199] These postoperative survivor and nonsurvivor patterns were consistent despite a wide variety of surgical illness and operations. The assumption was that early changes statistically related to survival represented the effects of surgery plus adequate physiologic compensations and, therefore, these values could be used as therapeutic goals, while early changes related to death reflected the overwhelming effects of surgery plus inadequate bodily compensations. These values may be used as early warning criteria for impending death.

Prospective Clinical Trials of Supranormal CI and DO_2 as Therapeutic Goals

The hypothesis was tested that increased CI and DO_2 represent compensations that have survival value when DO_2 increases and there is concomitant improvement in VO_2 indicative of improved tissue oxygenation.[14, 26, 27, 197, 247, 253] An early prospective clinical trial evaluated effectiveness of supranormal values as therapeutic goals to improve outcomes over a 7.5-year period. The initial clinical trial evaluated 252 high-risk surgical patients allocated to one of three services. Normal values were used as therapeutic goals for the control service and the supranormal values as goals for the protocol service. There were marked significant reductions in mortality in the protocol patients (19% versus 44%).[2] Subsequently, a randomized control trial was performed that preoperatively allocated patients to one of three groups: CVP catheter group with normal values as goals; pulmonary artery (PA) catheter control group with normal values as goals, and a PA-protocol group with supranormal values as goals. The result showed no significant differences in outcome of the CVP group compared with the PA-control group. Both used normal values as goals. Thus, if the intent is only to maintain normal values, the PA catheter has no real advantage over the CVP catheter. By contrast, the PA-protocol group had significantly reduced mortality compared with the PA-control group (4% versus 33%, $p < 0.02$), as well as fewer days on mechanical ventilation, fewer hospital days, fewer ICU days and reduced costs.[197]

Some recent reports have failed to confirm these observations. In an insightful meta-analysis, Boyd and Hayes[28] showed no outcome improvement in eight randomized studies done several days postoperatively after organ failures had developed, but reduced mortality in eight prospective randomized studies done early (i.e., 8 to 12 hours postoperatively). Moreover, these trials showed improved survival, reduced organ failure, and lower costs when optimal values of CI, DO_2, and VO_2 were used as early goals. The concept of survivors' supranormal values as optimal goals has been supported by a considerable number of studies. Edwards et al,[67] Yu et al,[253] and Boyd et al[26–28] reported improved outcome with optimal values in postoperative patients; Scalea et al,[185] Bishop et al,[20] Pasquale et al, [152] and Moore et al[138] have reported

improved outcome with supranormal values in trauma patients, and Boyd et al[26–28] reported improved outcome in preoperatively randomized optimization of high-risk surgical patients; Edwards et al[67] and Tuchschmidt et al[224] reported improved outcome in medical septic shock; the latter study was randomized. Creamer et al [55] demonstrated survival in 14 of 17 patients with cardiogenic shock after acute myocardial infarction when they were able to increase CI from 1.3 ± 0.5 to 2.6 ± 0.4 L/min · m^2. Edwards[67] also showed improved outcome in cardiac patients. All had some variations in their protocols, but each used supranormal values as early goals.

Bishop et al[20] showed that severely traumatized patients resuscitated to optimal values in less than 24 hours after injury had an 18% mortality, while those who reached optimal goals in more than 24 hours or failed to reach them had 38% mortality. Other investigators have confirmed the increased CI, DO_2, and VO_2 in survivors of septic shock.[165, 224]

Time Relationships in Shock

When monitoring is started early, survivors start with low flow but promptly develop compensatory hyperdynamic states, while nonsurvivors continue with low or relatively normal flow and poor tissue perfusion/oxygenation. These lead to organ failure, capillary leak, and finally death.[198] Time is the single most important outcome-related issue. When the aim in high–risk surgery was to achieve the optimal supranormal goals in the first 8 to 12 hours postoperatively, there was marked reduction in organ failure and mortality[26, 27, 197, 247]; however, optimization of DO_2 and VO_2 in the late stage after organ failure occurred did not improve mortality.[26] In Bishop's[20] study on trauma patients, those that were optimized when less than 24 hours had elapsed between the hospital admission and the time the goals were achieved, mortality was 18%; however when optimal goals were not achieved until after 24 hours after admission, mortality increased to 39%. In severe trauma patients, low incommensurate VO_2 responses to increased DO_2 in the first day after injury were associated with increased organ failure and mortality. By contrast, increased DO_2 associated with increased VO_2 favorably affected outcome. There was 92% survival when the optimal goals were achieved within 24 hours of ICU admission, but 93% mortality when achievement of the goals was delayed or not reached at all, and lactate levels did not return to satisfactory levels.[20, 27, 197]

NONINVASIVE CARDIAC OUTPUT MONITORING

The gold standard for evaluation of hemodynamic measurements has been by invasive PA balloon-tipped thermodilution (Swan-Ganz R) catheter. This technology is expensive, time consuming, and personnel intensive. Noninvasive alternatives to the thermodilution technique for

cardiac output estimation include thoracic electric bioimpedance, transthoracic (TTE) and transesophageal (TEE) Doppler echocardiography, the partial CO_2 rebreathing method, and the pulse contour method. Noninvasive monitoring allows calculation of the net cumulative deficits or excesses of each variable by integrating the area between continuously monitored values and normal or optimal values. The ideal cardiac output monitoring method is noninvasive, reproducible, inexpensive, continuously displayed, user friendly, in reasonable agreement with thermodilution, and acceptable to patients.

Thoracic Electric Bioimpedance Cardiac Output

In the impedance method, electrodes inject a small-amplitude (0.2–4.0 mA) alternating current at 40 to 100 kHz to produce an electrical field across the thorax from the base of the neck to the level of the xyphisternal junction. The electrical signals travel predominantly down the aorta, rather than through aerated alveoli. The changes in aortic flow throughout the cardiac cycle are correlated with changes in impedance (i.e., the apparent changes in resistance).[16,200] Wang et al[242–244] developed and improved an impedance system that was marketed as the IQ system (Wantagh, Inc., Bristol, PA). They used noninvasive disposable prewired hydrogen electrodes positioned on the skin and three EKG leads placed across the precordium and left shoulder, and a 100 kHz, 4 mA alternating current passed through the patient's thorax by the outer pairs of electrodes. The voltage was sensed by the inner pairs of electrodes.[16]

Comparisons and Limitations of Bioimpedance and Thermodilution Measurements

Noninvasive monitoring compares favorably with invasive thermodilution catheter monitoring; however, there is appreciable disparity in the presence of pulmonary edema, advanced acute respiratory disease syndrome (ARDS), congestive heart failure (CHF), and late-stage septic shock with capillary leak. Nonetheless, the comparison is considered sufficiently accurate to be useful clinically for making therapeutic decisions in over 90% of acute critically ill patients. Trends of bioimpedance cardiac outputs closely track changes in thermodilution cardiac output method.[200] The differences between thermodilution and impedance cardiac output estimations are more than offset by continuous on-line displays of data that allow instant recognition of abnormalities, calculation of the deficits of each monitored variable, and evaluation of therapeutic responses.[200]

In all monitoring and imaging techniques, motion, anxiety, restlessness, shivering, agitation, and hyperventilation may interfere with measurements and increase physiologic responses; however, it is less important in emergency conditions to have the same accuracy required in

stable ICU conditions, since the patient's own baseline measurements are often unknown, and optimal values for each patient may vary with comorbid conditions. In practice, 15% differences between invasive and noninvasive cardiac output estimations are acceptable when greater than 50% changes from the normal range are present. Thermodilution, however, also has appreciable inaccuracies in both high and low cardiac output ranges and especially when the patient has hypothermia, dysrhythmias, valsalva effects, motion artifacts, shivering, anxiety, and errors from injectate temperature calibration.

MULTIPLE NONINVASIVE MONITORING SYSTEMS

Multiple noninvasive physiologic monitoring systems are feasible as the initial screening system or as the "front end" of invasive monitoring during the resuscitation of acutely ill patients shortly after ED admission. The temporal patterns of cardiac function, pulmonary function, and tissue perfusion obtained by noninvasive monitoring systems compare reasonably well with the patterns of invasive monitoring. These noninvasive monitoring systems display early small changes that can be treated before they progress to life-threatening proportions. Early deficits are easily and effectively corrected, while late effects of shock after organ failure has developed may be irreversible. These noninvasive monitoring systems have been clinically evaluated in widely varying circumstances in a large series of severely ill patients to identify clinical conditions where the impedance methodology is appropriate.[200]

The ED is the primary entry point into medical care for many acutely ill patients, and this early period provides a crucial opportunity for early assessment and rapid therapeutic interventions that may affect outcome. A major dilemma is that shock is easily diagnosed in late stages when therapy is ineffective, but early diagnosis is difficult because shock is first recognized by imprecise signs and subjective symptoms. Noninvasive monitoring of circulatory dysfunction is an alternative approach that allows very early application in the ED, operating room, and hospital floors. The continuous on-line graphic displays of data allow prompt recognition of circulatory abnormalities and early therapeutic intervention and titration of therapy to optimal physiologic goals in acutely ill emergency patients where time factors are crucial.[200] Monitoring provides objective circulatory criteria that can replace clinical suspicion and guesswork with physiologic criteria related to outcome.

Clinical evaluations under worst case scenarios of emergency trauma cases in an inner city hospital have shown stable impedance signals and satisfactory agreement with simultaneous thermodilution cardiac output measurements. Simultaneous noninvasive measurements may be used to evaluate: cardiac function by blood pressure and noninvasive impedance, Doppler echo, or partial CO_2 rebreathing cardiac output systems; pulmonary functions by pulse oximetry estimation of arterial hemoglobin saturation; and tissue perfusion by $PtcO_2$, $PtcO_2$/

PaO_2 index, and $PtcCO_2$. These may be supplemented by invasively measured CI, DO_2, and VO_2 when available to validate noninvasive methods and to provide additional information such as wedge pressures. Baseline data sets, the low point (nadir) of the event, and the period of recovery immediately afterward describe the patterns of the interacting circulatory components: heart, lungs, and peripheral tissue perfusion/oxygenation functions. A microcomputer–based data acquisition system was developed to measure and record up to eight channels of data simultaneously and to display any two of them.[200]

Sequential Hemodynamic Patterns in Hemorrhagic Shock

Initial typical patterns of hemorrhagic shock as monitored noninvasively in the ED showed reduced flow (CI), MAP, and $PtcO_2$ with increased $PtcCO_2$; these effects were more pronounced in nonsurvivors. The initial $SapO_2$ and PaO_2 values were usually close to normal, while tissue perfusion, reflected by reduced $PtcO_2$ and increased $PtcCO_2$, rapidly declined with moderate degrees of hypovolemia. With severe hypovolemia, hyperpnea and tachypnea occurred usually with slightly reduced PaO_2 and pH values. With prolonged shock, poor tissue perfusion led to acidosis, base deficits, and increased lactate levels.

Rapid hemorrhage studied in the intensive care unit (ICU) by invasive pulmonary artery (PA) monitoring has demonstrated reduced MAP, CI, CVP, PAOP, stroke index, stroke work, mixed venous oxygen saturation (SvO_2), pH, hematocrit, DO_2, and VO_2 concomitant with increased systemic vascular resistance index (SVRI) and oxygen extraction ratio.[201] The initial compensatory responses included increased heart rate, which increased CI by neural and neurohormonal mechanisms; increased SVRI, which tended to maintain arterial pressures in the face of decreasing flow; and increased oxygen extraction ratios, which improved tissue oxygenation when blood flow had been reduced.

With prolonged hemorrhage, the shock pattern showed greater reductions in hematocrit and lesser reductions in MAP, CI, DO_2, and VO_2. The reduction of VO_2 was lower quantitatively but more prolonged than that occurring after rapid losses of comparable quantities of blood. After bleeding was stopped and blood volume restored with appropriate fluids, the survivors' recovery pattern usually consisted of normal or elevated values for CI, DO_2, and VO_2.[197–199] The smaller initial transient fall in cardiac index in survivors and the subsequent increase in flow and DO_2 were compensations to the initial low flow state. This is affected by the amount of injury, hypovolemia, preload therapy, and increased metabolic demand from the trauma itself. The combination of increased demand and abnormal tissue perfusion led to oxygen debt, multi-organ failure, and death.[198, 199]

Early Hemodynamic and Oxygen Transport Patterns After Trauma

Evaluations of circulatory dysfunction are often essential for decisions to operate in patients with blunt trauma. Moreover the timing of surgical operations may depend on clinical evaluation of the circulation and the temporal progression of clinical signs and symptoms. In the standard Advanced Trauma Life Support (ATLS) course, the degree of shock and amount of blood loss are estimated by the systolic and diastolic blood pressure, pulse pressure, and chance observations such as pallor, cold clammy skin, pulse rate, capillary refill, temperature, respiratory rate, and mental status. Neither blood pressure nor other signs and symptoms, however, are well correlated with blood flow or outcome.[195, 250]

Multiple noninvasive hemodynamic monitoring systems were used to prospectively evaluate circulatory patterns in 151 consecutively monitored severely injured patients beginning with admission to the ED in a university–run large urban county hospital. Noninvasive monitoring was feasible, easy–to–use, inexpensive, and safe during the resuscitation of emergency patients with severe trauma. Noninvasive systems provide continuously real–time displays of data from the ED to the operating room (OR), and to the ICU for early recognition of circulatory dysfunction in acute emergency conditions. The net cumulative deficit or excess of each monitored parameter was calculated by the area between normal values and the curve produced by the continuously monitored values for each variable in each patient. The deficits of cardiac, pulmonary, and tissue perfusion functions were analyzed in relation to survival by disciminant analysis and crossvalidated. The mean ($\pm$SEM) net cumulative excesses (+) or deficits (−) from normal in surviving versus nonsurviving patients, respectively, were: for cardiac index, $+81 \pm 52$ versus -232 ± 138 L/m^2 ($p<.037$); for MAP, -10 ± 13 versus -57 ± 24 mmHg.h ($p<0.078$); for arterial saturation, -1 ± 0.3 versus -8 ± 2.6%.h ($p<.006$); for tissue perfusion, $+313 \pm 88$ vs. -793 ± 175 torr.h ($p<.001$). Discriminant analysis classified 95% of the survivors and 62.5% of the nonsurvivors shortly after the initial resuscitation. Survival was predicted by discriminant analysis of the net cumulative deficits of flow, arterial hypoxemia, and tissue perfusion, which were more pronounced in the nonsurvivors.[202]

In summary, the goals of multicomponent noninvasive monitoring systems are to obtain comparable data to that of invasive monitoring, but continuously and in real time.

The feasibility of a multicomponent noninvasive monitoring system was demonstrated in the immediate postadmission period. Impedance cardiography can be applied like ECG electrodes in the ER, OR, ICU, hospital floors, or in doctor's offices and other prehospital settings. They are less labor-intensive, easier to operate, simpler, cheaper, and safer both on the patient and on the staff. The system allowed description of the time course of the major components of the circulation: total body blood flow reflecting cardiac function; arterial oxygenation by pulse

oximetry, reflecting pulmonary function; and $PtcO_2$ and $PtcCO_2$, reflecting tissue perfusion. The temporal patterns of these interacting noninvasive components were roughly comparable with the data obtained simultaneously with invasive monitoring.

Noninvasive systems may be used to characterize the physiology of surviving and nonsurviving patients beginning with ED admission. The data obtained with the three simultaneously monitored noninvasive systems—thoracic electric bioimpedance or other noninvasive measures of cardiac output, pulse oximetry, and transcutaneous O_2 tension instituted shortly after ED admission—were comparable with data from the invasive PA catheter when this became available. These three noninvasive systems provided evaluation of the cardiac, pulmonary, and tissue perfusion functions of the circulation.

When large volumes of fluids are required, CVP or PA catheters may be used to monitor venous pressures to avoid fluid overload. The noninvasive systems are less hazardous than invasive catheters for the patient in terms of catheter complications and for the staff in terms of exposure to hepatitis, HIV, and other infections. Continuous on-line noninvasively monitored data provide a means to calculate the net cumulative deficits or excess of each monitored variable. Discriminant analysis gives a more quantitative estimate of circulatory dysfunction and a powerful view of the rapidly changing circulatory dynamics, transcending the boundaries of old concepts of shock. Because noninvasive monitoring can provide the essential circulatory information in patients throughout the hospital and prehospital areas, the data may be used to describe survivor and nonsurvivor patterns, to predict outcome, to define therapeutic goals, to titrate therapy to achieve these goals, and eventually to improve outcome. This may change standard methods of managing acutely ill patients.

FLUID THERAPY

Based on previously discussed pathophysiologic concepts, it is apparent that the goals of therapy in vascular trauma are twofold: restore oxygen perfusion to the tissues and provide hemostasis through operative intervention. Oxygen perfusion is provided through proper ventilation, oxygenation, and fluid therapy.

The Proper Amount and Timing

Despite the aggressive approach to fluid resuscitation advocated by the Committee on Trauma of the American College of Surgeons, there is a history of wartime observation that urges caution.[3, 34, 35] In 1983, Aprahamian et al[5] looked at "scoop and run" patients versus those who were given prehospital care, including fluid resuscitation, by paramedics. Although there was little difference if the initial systolic pressure

was greater than 60 mmHg, fluids clearly increased survival from 15% to 60% in those patients with penetrating abdominal vascular trauma and systolic pressures less than 60 mmHg. On the other hand, a study of close to 7000 trauma patients did not show a survival benefit with early prehospital fluids, regardless of the injury severity score, and despite the fact that hypotension was associated with mortality.[105] The question could then be asked if there was something good about no fluids, or was there something bad about using fluids. The issue was taken to the laboratory where, in an uncontrolled hemorrhage model in swine, Bickell et al[17] showed that administrating no fluids was better than rapidly administrating lactated Ringer's (LR), which led to increased intraperitoneal hemorrhage and death. The problem was postulated to be a blow-out of the hemostatic plug caused by increased pressure and decreased blood viscosity. In an accompanying editorial, it was noted that despite the relatively enormous volume and rate given to the animals, too much fluid can be detrimental, and that surgical hemostasis is key for uncontrolled hemorrhage.[158] In the same animal model, Bickell et al[18] showed that hypertonic saline with dextran (HTS-D) led to more bleeding and death than no fluids, but LR was still worse. In similar animal model studies using normal saline (NS), controlled resuscitations at lower targeted pressures between aggressive fluid resuscitation and no resuscitation were investigated. Limited–to–moderate resuscitation was shown to be best.[110, 212] Dronen et al[65] helped elucidate the problem further when they compared controlled to uncontrolled blood loss, with and without resuscitation. The worst survival was with no resuscitation in either model of blood loss, but they obtained 100% survival with fluids in the controlled model versus 22% with fluids in the uncontrolled model. Again, it was proposed that in an uncontrolled model of vascular injury, the hemostatic plug could be rendered ineffective, and increased volume could cause increased mortality.

Perhaps the most noted study in this controversy was done by Bickell's group in 1994.[19] The study included 598 patients with penetrating torso injuries and systolic pressures less than 90 mmHg who received either standard prehospital/ED fluid resuscitation (immediate group) versus no fluids until they were in the operating room (delayed group). The delayed group had better survival, fewer complications, and shorter hospital stays. Again, the findings were attributed to accentuation of ongoing hemorrhage or hydraulic disruption of an effective thrombus, followed by a fatal secondary hemorrhage. The recommendation was to delay aggressive fluid resuscitation in hypotensive patients with penetrating torso injury until the time of operative intervention.

Studies that have occurred since Bickell's contribution basically arrive at the same conclusion, that moderate resuscitation is best for uncontrolled vascular injury, whether the end point is CI and oxygen delivery,[151] the observation period is longer,[37] the order of NS and blood is interchanged,[213] the repacement ratio of crystalloid to blood loss is altered,[171] regional blood flow in a venous uncontrolled hemorrhage

model is looked at,[207] crystalloids and hypertonic/colloid solutions are used,[32] or traumatic brain injury is present.[215]

There are two additional points that should be mentioned. Firstly, although in Smail's study[207] the no resuscitation group eventually "caught up" to the favored moderate resuscitation group, the time spent in a hypoperfused state can be deleterious to the subsequent ischemia/reperfusion injury.[31] Secondly, Owens[151] brings up a good point that concerning HTS, it is the animal studies where there is a bleeding problem,[18, 79] not in the clinical studies.[131, 231, 238]

Reduced morbidity and mortality might result if it could be determined who would benefit from early fluids and which patients would have increased bleeding and mortality. Concerning penetrating or vascular injury, the recommendation by Owens et al[151] seems pertinent: "A limited prehospital resuscitation regimen in which fluid is administered judiciously to maintain a level of cardiovascular function, less than normal, but above the level of progressive circulatory shock might offer the optimal approach."

Isotonic Crystalloids—Normal Saline or Lactated Ringers Solution

Which crystalloid is preferred for patients in hemorrhagic shock centers on three issues: acidosis, survival, and compatibility with blood. Although a nonanion gap acidosis is associated with NS, it is not the hyperchloremia that accompanies excess saline that leads to acidosis.[90] In fact, the so-called hyperchloremic acidosis is in reality a lactic acidosis caused by hypoperfusion. There is no apparent gap, because the severe depletion of serum albumin, an unmeasured anion, reduces the "normal" anion gap. It is not that NS causes the acidosis, but rather that LR improves it as the L-lactate isomer is metabolized by the liver and kidney to generate bicarbonate (HCO_3) and provide a buffer. The concern that it takes several hours to metabolize the lactate because of decreased hepatic metabolism in the shock state was not born out in Healey's study[90] when acidosis was improved despite large quantities of LR, leading to higher bicarbonate levels within 2 hours. Likewise, Coran et al[50] showed that the low pH in hypovolemic shock went further down with NS, but the pH returned toward normal with LR. Indeed, both Cervera et al[38] and Horton et al[97] showed that adequate resuscitation returns the pH to normal regardless of which crystalloid is used. Additionally, lactate levels are not elevated with LR.[50, 97, 221]

Two animal studies show an increased survival rate with LR.[90, 221]. In one study, the nonsurvivors were considerably more acidotic[90]; however, when Traverso et al[221] compared NS with LR and Plasmalyte A (Baxter Edwards Critical-Care, Irvine, CA), both bicarbonate precursors, Plasmalyte had the highest mortality, despite having pH, HCO_3, and base deficit levels equal to those of LR. Therefore, the acidosis may not be causative in the difference in mortality.

The issue of whether LR is compatible with blood may be the most

important. Storage of blood requires citrate-phosphate-dextrose (CPD) solution. The sodium citrate prevents blood from coagulating by chelating the calcium ion and disrupting the coagulation cascade. The theoretical problem with LR is that the calcium in LR will exceed the chelating capabilities of citrate in the stored blood and cause clot formation. Depending on the size of clot, infusion rates could be slower if clot blocks the blood filter or tubing, or clots could reach the circulation compromising the pulmonary capillaries. Lorenzo et al[122] studied whole blood and packed red blood cells (RBC) mixed with NS as control compared with LR with different amounts of calcium added. Despite a 1:1 ratio of blood to LR, there was no significant difference in infusion times, filter weight, or clot formation, except at the highest calcium level, which was considerably higher than the usual LR. In addition to the low concentration of calcium in LR, he felt the rapid rate of infusion, as would be used in emergent fluid resuscitation, prevented any clot formation. The recommendation from this study was to allow the use of LR in the rapid transfusion of packed RBCs. Cull et al[58] showed no difference between NS or LR mixed with PRBC at different flow rates with varying hematocrits; however, clotting did occur at a 1:1 ratio with LR, and at least a 2:1 blood to LR ratio was needed to avoid clotting. King et al[107] likewise saw clotting at less than a 2:1 ratio, but found no difference with NS or LR at a rate of 540 mL/hr. Ryden et al[180] felt that LR should not be used with blood, but they used slow infusion rates, and found that the slower the infusion and the warmer the ambient temperature, the more clots formed. It therefore appears that LR can be used with packed RBCs if the ratio of admixture is at least 1:1, preferably at 2:1, and the infusion rate is fast.

Although it appears that LR is the preferred choice of crystalloid, there is an exception, namely, traumatic brain injury (TBI). Although either crystalloid can be used as long as hypoosmolality does not develop, and rapid administration of large volumes are avoided, NS is preferred, because the 154 mmol/L of sodium is slighly hypertonic to the 130 mmol/L of sodium in LR.[160, 184] Lastly, the usual replacement ratio of 3:1 crystalloid to lost blood should probably be rethought as studies place the adequate ratio at 7:1 or 10:1 because of decreased colloid oncotic pressure secondary to decreased serum protein concentration from hemorrhage, capillary leaks, and crystalloid replacement.[38, 90]

Hypertonic Saline With or Without Dextran

Hypertonic saline (HTS) is usually supplied as 7.5% NaCl, often combined with a colloid, 6% dextran 70. Both in animal models and in clinical trials, it is most often used as an initial small volume bolus of 4 mL/kg or 250 mL. HTS works by shifting water into the plasma first from RBCs and the endothelium, then from the interstitial space and tissue cells.[132] This leads to its two most important attributes: a rapid but transient increase in blood volume to support and improve hemody-

namics, and a hemodilution and endothelial cell shrinkage that decrease capillary hydraulic pressure and improve tissue perfusion.

There are many animal studies and clinical trials that show HTS and HTS-D to be effective at expanding plasma, raising blood pressure, improving cardiac output, lowering systemic and pulmonary vascular resistance, lowering subsequent fluid and blood requirements, and improving oxygen delivery.[30, 114, 117, 119, 131, 141, 149, 238] Adding dextran to HTS prolongs the circulatory effect.[117] Improved regional tissue perfusion, decreased leucocyte-endothelial cell interaction, and decreased stickiness of leucocytes point to a potential decrease in ischemia/reperfusion injury and subsequent multiple organ failures.[51, 63, 114–117, 145] Adding dextran has been shown to increase regional blood flow.[114]

Although some studies do not show any benefit to patient survival,[30, 231] other studies report an increase in predicted survival rates with HTS[93, 230, 232] or an increased survival with HTS-D in patients destined for the operating room or having associated traumatic brain injury.[131, 238] Despite theoretical concerns, clinical use has shown HTS and HTS-D to be quite safe. Central pontine myelinolysis with HTS and bleeding, difficulty with cross-matching, or anaphylactoid reactions with dextran are rare to non-existent in the literature.[231, 238] Although animal studies have shown an exacerbation of bleeding in an uncontrolled hemorrhage model,[79, 111–113] this has not been a problem in patients.[231, 238]

Hypertonic saline with or without dextran seems effective without much downside in fluid resuscitation in hemorrhagic shock. Where small volume resuscitation is desirous, such as in the military or prehospital care setting, it certainly would represent a logical choice of therapy. Its hypertonic nature and small volume usage are particular advantages, in traumatic brain injury.

Hypertonic Saline in Hemorrhagic Shock and Traumatic Brain Injury

Head injury is the leading cause of traumatic death in the United States, and when combined with hypotension there is a doubling of mortality by creating a secondary ischemic injury.[25, 136, 142] Thus, aggressive and rapid resuscitation of systemic hemodynamics seems appropriate.[25, 192] Because it is the osmolality, not the oncotic pressure, that affects water movement in the brain, and the blood brain barrier is essentially impervious to sodium, HTS would seem a logical choice of fluid.[187, 256] HTS-D was also shown to inhibit leucocyte margination in the cerebral microcirculation thereby attenuating an inflammatory response thought responsible for secondary brain injury.[88] Additionally, concern still exists over excess volume resuscitation in traumatic brain injury, making HTS and its ability to provide small volume resuscitation a seemingly ideal choice for hypotension associated with hypovolemia. Several studies lend credence to the use of HTS in this subset of patients.

Several studies show that crystalloid infusion can be safely used in

TBI and that maintaining normovolemia with fluids does not lead to increased intracranial pressure (ICP).[44, 184, 187] In a porcine model, however, Bourguignon et al[25] showed that LR decreased cerebral oxygen delivery and increased ICP. In studies where hypotension was combined with TBI, HTS and HTS-D improved cerebral parameters[82, 192, 237, 240]; however, in one investigation, LR resuscitation led to increased ICP.[82] Patients with TBI without hypotension had their ICP successfully managed by adding 3% HTS or HTS-acetate to their treatment plan over several days.[106, 153, 163, 193, 205] As the concentration of serum sodium increased, the ICP decreased. Importantly, in animal studies where hypotension occurred without TBI and hypertonic fluid and crystalloids were used to achieve resuscitation, good cerebral parameters occurred with the hypertonic fluid, but the crystalloids led to increased ICP.[83, 159, 188] Even in comparison to mannitol, there are sufficient advantages such as avoidance of hypotension and renal failure that favor the use of HTS.[106, 153, 163] Lastly, in an animal model, HTS offers similar advantages to improvement in microcirculatory flow of the spinal cord.[225]

The Colloid Versus Crystalloid Controversy

Despite theoretical advantages for crystalloids such as the ability to replenish interstitial fluid loss, or for colloids such as the less likelihood of pulmonary edema,[239] the practical situation is that the greatest difference between the two choices is the relatively exhorbitant cost of the colloids.[2, 252] Because colloids have never proved superior to crystalloids for fluid resuscitation, the Consensus of the University Hospital Consortium favors crystalloid as the preferred fluid, except that colloids are appropriate to be used in conjunction with crystalloids when blood products are not immediately available when needed.[233] Habits die hard, however, as Yim et al[252] discovered when their survey based on the guidelines showed that colloids were used more often than expected, and only 24% of colloid use was considered appropriate.

Over the years, there have been studies showing one type of fluid superior to the other. Hankeln et al[85] showed the colloid hetastarch to have better cardiopulmonary parameters than LR, or Moss et al[139] said that human serum albumin (HSA) performed as well as LR. The problem with these and many other studies was that the number of patients in each study was quite small. To demonstrate a 10% difference in treatment effect between crystalloid and colloid resuscitation, assuming a 15% baseline mortality, a two-tailed alpha of 0.5 and a beta of 0.20, a randomized clinical trial would need to involve almost 6000 patients.[41] In large systematic reviews of all appropriate studies, there was no significant difference in overall mortality.[2, 41, 186] Choi et al[41] found no difference in length of stay or development of pulmonary edema, but there was a trend toward better survival in trauma patients with crystalloid use. These systematic reviews recommend crystalloid use given no significant difference except the much higher price for the colloids.

There are two areas of animal research involving hydroxyethyl starch (HES), of importance to multisystem organ failure. First, several studies have shown that the iron-chelator desferoxamine can be combined with HES and can attenuate the iron–dependent generation of toxic oxygen-derived radicals during reperfusion of ischemic tissue.[10, 11, 64, 176] Second, an animal study showed that HES itself can reduce reperfusion injury through several possible mechanisms, including decreasing the oxidant-generating enzyme, xanthine oxidase.[144]

The issue of pulmonary edema surfaces in most discussions on the fluid controversy. Rackow et al[164] showed more clinical pulmonary edema and much lower colloid oncotic pressures with NS compared with albumin, but Weaver et al[245] demonstrated a greater need for ventilatory support and worse oxygenation with albumin. Other studies showed no differences in pulmonary functions, pulmonary failure, or lung water, despite the fact that crystalloids reduced the colloid oncotic pressure.[70, 123, 220] Tranbaugh et al[220] said that the oncotic pressure is only 25% as important as any changes in hydrostatic pressure, and Lowe et al[123] said that although albumin crosses the capillary membrane, it is washed out of the pulmonary interstitium by the lymphatic system.

Lastly, albumin has been shown to have an increased risk of death in a systematic review of 30 studies with 1419 patients.[1] Albumin has been shown to have a negative inotropic effect,[59] reduced coagulation activity,[125] and anticoagulant properties.[1] It is recommended that the use of albumin be strictly reviewed and curtailed.[1] Otherwise, the colloids, which include modified gelatins, dextrans, and etherified starches, are fairly equivalent,[29] but in hemorrhagic shock clearly less preferred than crystalloids.

Oxygen Carriers

Given the values of moderate fluid resuscitation and small volume resuscitation described previously, coupled with HIV transmission in blood and the desire of the military for a rapidly available, easily storable resuscitation fluid, an oxygen carrier substitute fluid seems ideal.[213, 249] Unfortunately, early versions of such solutions had many problems such as a short half-life, high oxygen affinity, renal toxicity, and vasopressor effects.[210, 249] It was noted, however, that altering the characteristics of acellular solutions could lead to profound physiologic differences.[73] The goal of optimizing these properties to produce an efficacious product for shock resuscitation has led currently to nine products in various stages of development, hopefully some of which will be clinically available within a few years.[249] There are three classes of oxygen carriers: hemoglobin-based, perfluorocarbons, and liposome-encapsulated.

Hemoglobin–based oxygen carriers are solutions of free hemoglobin that are modified to prevent dissociation of the hemoglobin tetramer into dimers, thereby preventing renal toxicity. Additional modifications

have led to different groups within this class. Surface-modified hemoglobin (e.g., pyrodoxilated hemoglobin polyoxyethylene) is a conjugate of hemoglobin and larger molecules that will prolong intravascular retention (48 hours) and has retained a high oncotic pressure and viscosity, making them potent plasma expanders.[249] While able to restore hemodynamics, studies have shown a concern with oxygen transport and mucosal ischemia.[73, 148, 211] Intramolecular cross-linked hemoglobins (e.g., alpha-alpha Hb, DCLHb, HemAssist) include rHb1.1, manufactured using recombinant technology, providing for a reduced oxygen affinity and oxygen binding curve similar to normal human blood. Many studies show a restoration of mean arterial pressure, excellent tissue perfusion with uniform distribution, improvement in base deficit, and increased survival all equal or superior to other fluids.* Serious side effects including marked vasoactive properties and increased mortality have led to failed phase III clinical trials however.[81, 156, 157, 206, 249] Polymerized hemoglobins (e.g., PolyHeme [Northfield Laboratories, Chicago, IL], Hemopure [Biopure Corporation, Cambridge, MA], HBOC-201) have an oxygen affinity and oncotic pressure similar to those of human blood, increased molecular weight, leading to longer half-life, and few adverse effects in clinical trials.[76, 77, 81, 89, 130, 249]

Perfluorocarbons are carbon-fluorine compounds that are completely inert but can dissolve large amounts of gases.[210, 249] Because they are immiscible in water, they have to be emulsified. Instead of having a sigmoidal relationship like the modified hemoglobins and blood, pO_2 and O_2 content have a linear relationship requiring a relatively high paO_2 (FiO_2) to maximize oxygen transport. Nonetheless, perfluorocarbons exhibit excellent oxygen unloading.[210, 213] Other advantages include being well tolerated with mild flu-like symptoms and transient thrombocytopenia, low cost, long shelf life (>1 year); however, they do not expand the intravascular compartment as do the modified hemoglobins and must be used in small volume because of potential reticuloendothelial system overload.[84, 210, 249]

Liposome encapsulated hemoglobin (e.g., neo red cells [NRC]) offers the advantages of low viscosity and high oxygen transport ability,[226] but the high cost and complexity of the manufacturing process and the potential for RES overload have prevented large-scale development.[211, 226]

The main issue with this resuscitation modality is the vasoactivity caused by all the hemoglobin-based oxygen carriers, particularly with the intramolecular cross-linked hemoglobins. The predominant mechanism leading to vasoconstriction is the binding of hemoglobin to nitric oxide (NO), a key mediator responsible for the physiologic regulation of vasodilatory tone.[146, 147, 155–157, 167] Other mechanisms that may play a role include endothelin release and increased sensitivity to adrenergic receptors. The question is whether vasoconstriction is a good or bad effect. Nolte et al[146, 147] found that the vasoconstriction is short-lived (2 minutes) and is followed by a longer lasting alteration of vasomotion.

*References 48, 60, 119, 147, 155–157, 162, 190, 203, 229, 236

This modulation of vasomotion frequency and amplitude can interfere with microcirculatory flow distribution and velocity, leading to improved and homogeneous local tissue oxygen levels. Studies mentioned previously for the intramolecular cross-linked hemoglobins appear to support this contention. On the other hand, Spahn[210] found that if the patient has a decreased cardiac contractility and a normal or high mean arterial pressure, this will lead to a decreased cardiac output. Although in a healthy patient who sustains massive hemorrhage, the volume replacement, added oxygen transport, and a certain degree of vasopressor support would be helpful, in penetrating trauma, too high a pressure might lead to increased bleeding. Spahn also said that the vasoconstriction does not lead to a uniform distribution of flow. Interestingly, there is question as to what extent vasoconstriction happens in humans, as it appears to some extent to be species specific[47, 84]; however, Reah et al[167] have used cross-linked hemoglobin as a vasopressor in the medical ICU and have shown decreased norepinephrine requirements in septic shock and SIRS, although there was some decreased cardiac index and global oxygen delivery noted. Surface-modified hemoglobin has also been used with success in septic shock.[249] Additionally, the vasoconstrictive actions have been attenuated by using hypertonic sodium acetate or NO inhalers along with oxygen carriers.[156]

Recently, a new issue has been raised concerning mechanisms of oxygen transport. When modified hemoglobins are free in the plasma, they diffuse more readily in the tissue.[249] This facilitated diffusion of HbO_2 may, through an autoregulation, cause vasoconstriction as the body attempts to regulate (decrease in this case) the amount of oxygen in the tissue. Resolving this issue is important to future development of oxygen carriers.[249] If the primary mechanism of vasoconstriction is the binding of NO to heme, then to restrict that may prevent oxygen from combining, as the two molecules are similar in size. If the primary mechanism involves autoregulation, then decreasing facilitated diffusion by increasing the molecular size of the oxygen carrier, increasing solution viscosity, or altering oxygen affinity may be the answer. Another new direction involves combining superoxide dismutase and catalase to polyhemoglobin (polyhgb-SOD-catalase) to replace these enzymes normally present in RBCs. Without these enzymes, a worsened ischemia/reperfusion injury may occur; with these enzymes, there is an effective removal of oxygen radicals and peroxides, less iron release, and less methemoglobinemia.[40]

In summary, it appears that the reality of providing a plasma expander with oxygen transport capability that will not require typing and cross-matching and be readily available, have a long shelf-life, not transmit disease, exhibit no antigenicity, and be free of serious side effects is in the near future.

Allogenic Blood Transfusions

It would seem logical, based on the pathophysiology of hemorrhagic shock, to replace blood loss with some form of blood product. Without

blood, the risk of morbidity and mortality from hypovolemia increases with duration and degree.[128] Because humans are oxygen dependent, maintenance or restoration of oxygen delivery (DO_2) to the tissues is of primary importance in dealing with blood loss, and should be the goal of RBC transfusion.[78, 168] Recalling that DO_2 is proportional to cardiac output, hemoglobin, and oxygen saturation, it is reasonable to first increase oxygen delivery by providing the patient more oxygen and increasing the blood volume with crystalloid. Beyond a given limit for an individual patient, the addition of the oxygen carrying capacity of transfused RBCs or oxygen carriers will be needed to achieve a normal range DO_2.[78] It is not just the global DO_2, but the local DO_2 that is of concern. Indeed, a significant reduction in cardiac output leads to selective vasoconstriction, which may provide 100% flow to a vital organ like the brain, but may restrict flow to 30% at the gut level. As noted previously, the blood pressure and even the global DO_2 may be normal, but local areas may be quite underperfused with definite ischemic consequences, as Malone described from venous thrombosis and bronchopneumonia to ileus and wound dehiscence.[128]

Countering the reasons to give blood, there are many reasons for caution, some known, and others less well known. Well-known problems with transfusion include acute and delayed hemolytic reactions and disease transmission. As Schreiber et al[189] pointed out, however, with modern screening techniques, the rates of infection transmission are low and essentially occur during window periods: HIV 1/493 000, HTLV 1/641 000, hepatitis C virus 1/103 000, and hepatitis B virus 1/63 000. With the use of more sensitive screening methods like p24 antigen, DNA, or RNA polymerase-chain-reaction (PCR) testing, the rates are expected to be even lower. The lesser known problems with blood transfusions are related to the fact that whole blood, and questionably to some extent other RBC prepared or stored products, cause immunosuppression.[103, 104, 143, 150] Whole blood transfusions lead to post-operative infections,[66, 91, 102, 103, 134, 143] but by eliminating leukocytes[102, 103] or by using autologous blood,[91, 134] the increase can be controlled. The immunosuppression caused by whole blood transfusions is also implicated in tumor recurrence[143, 150] and an increased risk of first developing cancer.[22] Perhaps most importantly for hemorrhagic shock, blood has been shown to be an independent risk factor in the development of multisystem organ failure,[57, 66, 127, 183] as blood leads to an imbalance between proinflammatory and antiinflammatory mediators.[7, 23, 104] As a result, some say to use blood fairly quickly[128]; others are more cautious.[168]

As expected, there is no definite agreed upon number or "trigger" when blood transfusion should be given. For years, a hemoglobin of 10 g/dL or hematocrit of 30% (the "10/30 rule") was considered the trigger, but in 1988, the National Institutes of Health Consensus Conference[168] acknowledged that 7/21 would be more appropriate; however, clinical judgment should be the key ingredient along with the patient's duration of anemia, extent of operation, existing blood volume, potential for massive blood loss, and preexisting comorbid conditions. Importantly, conference members felt, as did Greenburg,[78] that certain laboratory

values should be considered that not suprisingly reflect oxygenation of the tissues: oxygen tension, oxygen extraction ratio, cardiac output, and lactate levels.

Given the potential problems with blood transfusions, future directions should include development of clinical monitors to better measure tissue perfusion, develop predictors to trigger transfusions, continue to modify and test oxygen carriers, and consider the use of autologous blood.

Autologous (Shed) Blood Transfusion

Autologous blood transfusions are relatively commonplace in many hospitals for a variety of elective operations and may represent an ideal fluid replacement in hemorrhagic shock caused by vascular trauma. Use of autologous blood would avoid the problems of transfusion reactions, disease transmission, and immunomodulation seen with allogenic blood.[109, 121, 223, 227] It would provide a source of fresh blood that is rapidly available, normothermic, has physiologic oxygen affinity, and would be acceptable to Jehovah's Witnesses. The autologous blood used in trauma patients is not predonated, but rather it is shed blood immediately returned to the patient. This concept is somewhat controversial because of potential clotting mechanism abnormalities and the reinfusion of potentially contaminated blood.[96] Likewise, it has not been used that frequently, because it is often difficult to know beforehand how much blood will be needed for a patient, or if there is bowel contamination that will discourage its use. Over the past several years many studies have helped put these problems in perspective and delineate the advantages and disadvantages of the two techniques to accomplish the transfusion: cell washing with centrifugation (CWC) and reinfusion after filtration (RAF).

Cell washing with centrifugation involves aspiration of shed blood through a machine that washes and centrifuges the blood to produce RBC concentrates with a hematocrit of 55% to 60%, and that is relatively free of plasma-free hemoglobin (free hemoglobin can precipitate in renal tubules leading to acute renal failure), procoagulants (can lead to DIC), bacteria, and malignant cells.[121, 223] Unfortunately, platelets and plasma proteins also are essentially removed, which can lead to a dilutional coagulopathy. The washing process itself can lead to hemolysis (and increase plasma-free hemoglobin) and the "salvaged blood syndrome" because of retained platelet-leukocyte deposits on the centrifuge bowl that produce procoagulant leukotactic substances and a DIC picture. What washing accomplishes with free hemoglobin levels in a given patient is variable, up to 10-fold.[109]

Although shed blood makes up only a percentage of total transfused blood in most patients, and it is difficult to determine which of the many causes of coagulopathies is responsible for bleeding in a given patient, Horst et al[96] found that 31% of patients had moderate–to–severe

prolongation of their PT and PTT, and the more shed blood received, the more abnormal the lab values. In the study, more than 15 units of intraoperative shed blood (220 mL per unit) led to coagulopathy, but with bowel injury, greater than 10 units was sufficient. Another study corroborated the amount at 3500 mL.[194] Tawes et al,[217] however, felt that despite some bleeding and minor clotting disorders, the DIC/ARDS problem was rare (0.05%) and usually resulted from other causes when it did occur. In fact, they felt most of the problems were technical and were caused by the dilutional effect of removing platelets and clotting factors during red cell washing, failure to properly wash out all particulate and soluble procoagulants, and inadvertent reinfusion of residual heparin. Use of fine screen filters, liberal heparinization, and high volume washing of RBCs was thought to eliminate the problem.

A second major concern is that plasma-free hemoglobin can lead to renal problems. It is assumed that the washing process removes free hemoglobin, and it is also known that free hemoglobin is found in banked blood up to 100 mg/dL without causing a problem, but Klodell[109] showed a correlation between total free hemoglobin and renal dysfunction (as defined as an increase in creatinine of 1 mg/dL over baseline) and felt that a free hemoglobin level should be determined beyond which shed blood may not be safe. Godet et al[75] showed that greater than five units of shed blood contributed to renal failure.

Using unwashed shed blood that is immediately reinfused through filtration (RAF) has advantages of a more efficient, more economical, more rapid, and less work-intensive set up. Whole blood is returned to the patient that still retains its platelets and proteins, but carries the concern of more procoagulant activity and free hemoglobin available to cause adverse reactions.[121, 223] Direct comparison studies of CWC versus RAF revealed that although free hemoglobin was higher in RAF, there were no renal consequences; that PT and PTT were elevated in both groups, but without coagulopathy, that platelets were decreased but without bleeding in either group, and that the lab abnormalities almost always returned to normal within 1 day.[121, 223] Importantly, a much higher percentage of shed blood was returned to the patient with RAF, and given the apparent safety, ease of use, and cost of operation, made the use of unwashed, directly reinfused blood more attractive than the expensive cell washed and centrifuged technique.

Two remaining issues needing further study include immunomodulatory effects and the use of shed blood from the abdominal cavity in trauma. There have been several studies that give conflicting results on whether washing affects inflammatory mediators,[6, 12, 100, 118, 204] but one interesting study in guinea pigs showed that autologous blood transiently caused greater myocardial damage than dextran, independent of the hemorrhage per se, and related to activated leukocytes.[133] The use of shed blood from the abdominal cavity is controversial. Some authors say to avoid; others report no major septic events.[74, 101, 218] Smith et al[208] found an association between shock, mortality, and contamination that was decreased by antibiotics, and Boudreaux et al[24] stated that cell

washing decreased, but did not eliminate bacteria. In Horst's study,[96] although bowel injury led to a quicker DIC picture, there was no increased infection rate.

SEVERE SHOCK AND RESUSCITATION COMPLICATIONS

Survivors of vascular trauma associated with hypovolemic shock can develop severe dysfunction of organ systems and long-term sequelae. Some of these complications are direct consequences of end-organ ischemia; others are caused by resuscitative interventions.[161] The most dreaded consequence of hypovolemic shock is brain damage; the most common is renal dysfunction. In severely injured patients, the leading cause of death is multiorgan failure, which occurs in at least 10% of patients with an Injury Severity Score (ISS)[179] greater than 20.[169] Resuscitation often requires massive transfusions of blood products, which may lead to fluid overload and electrolyte imbalance, pulmonary insufficiency, and coagulopathy. Development of multiorgan failure is influenced by severity, type, and distribution of injury, and duration of shock. Transfusions also contribute to a higher incidence of sepsis after trauma.[62] When circulation is reestablished after either prolonged shock or repair of major vascular injuries, reperfusion-reoxygenation injury is another concern.[86] Although almost any organ can be damaged permanently during shock and resuscitation, the main concern should be protecting the brain, heart, lungs, and kidneys; other organs rarely have dysfunction in the absence of damage to any of these four vital organs.

Critical in determining the prognosis of shock patients is the preexisting general health of the patient. Young and previously healthy patients have much more favorable outcomes than those with advanced age ("low functional reserve"), or comorbidities such as diabetes, atherosclerosis, renal insufficiency, or cirrhosis.[140, 251] Added risk can result from illicit drug or ethanol intoxication—present in a substantial proportion of trauma victims—that can impair both diagnosis and treatment, and alter the physiologic response to trauma.[209]

Although early resuscitation has been shown to reduce multiorgan failure in extensive burns and other hypovolemic conditions, fluids should be administered in concert with hemostatic maneuvers in hemorrhagic shock.[19, 174] All sources of significant bleeding should be controlled before effective blood volume is reestablished. Early surgical intervention is most effective in preventing the potentially disastrous consequences of hemorrhagic shock.

Central Nervous System Damage

The normal brain can recover fully from severe hypoxia sustained for approximately 4 minutes, or even longer periods, especially if some

cerebral circulation is maintained.[4, 182] In the presence of associated conditions, however, permanent cerebral damage tends to occur after shorter periods of ischemia. Patients with atherosclerosis of the carotid, vertebrobasilar, or intracerebral arteries may develop focal deficits after otherwise successful resuscitation from shock. Experimental data support the concept that preexisting cerebrovascular disease influences the response to hemorrhagic shock, potentiating alterations of cerebral microcirculation and increasing vascular resistance.[172] Similarly, ethanol intoxication can impair normal regulatory mechanisms and potentiate metabolic changes, thereby contributing to secondary brain injury during hypotension.[255] Traumatic brain injury can be exacerbated by hypotension as a result of complex local phenomena that also includes loss of physiologic compensatory mechanisms.[39]

To minimize secondary insult, management of patients with head trauma and hemorrhagic shock should ensure adequate cerebral perfusion by maintaining arterial pressure at a satisfactory level, which often requires continuous monitoring of both cerebral and arterial pressures. Hyperventilation is an effective means of reducing brain edema; however, prophylactic hyperventilation of patients with head injuries worsens outcome, presumably by exacerbating cerebral hypoxia. Studies in pigs have shown oxygen tension in the uninjured brain increases with hypoventilation and decreases with hyperventilation under continuous hemorrhage, suggesting hypercapnia may be beneficial in instances of cerebral hypoxia secondary to hemorrhagic shock.[129] Data from experimental animal studies indicate controlled hypothermic cardiac arrest (core temperature of 10°C) can help preserve the viability of brain tissue during hemorrhagic shock.[36] Although initial clinical studies showed induction of hypothermia to be beneficial in brain injury, a larger, multicenter trial demonstrated that hypothermia has no effect when compared with normothermia.[45]

Severe hypoxia-ischemia may result in brain death, characterized by unresponsiveness, absence of respiration and all reflexes (including brainstem), and an isoelectric electroencephalogram. Lesser degrees of extensive bilateral cortical damage may lead to coma followed by a "vegetative state," characterized by open eyelids, Babinski sign, posturing, and periods of autonomic overactivity.[254] Extensive multifocal or diffuse cortical infarcts are common histologic findings of anoxic-ischemic brain damage. "Watershed" infarcts can occur at territories between the major cerebral arteries (often involving the hippocampus) and can lead to persistent memory deficits and weakness of proximal muscle. Delayed postanoxic encephalopathy, progressive neurologic deterioration, coma, and death may occur rarely and are attributed to diffuse brain demyelination.

Spinal cord ischemia leading to paraplegia is caused most commonly by trauma or surgery of the thoracic aorta, but also has been reported rarely in association with acute hypotension and prolonged cardiopulmonary resuscitation.[98] A case of occlusion of the anterior cervical spinal artery (with paralysis of the diaphragm) after cardiopul-

monary arrest has been reported.[154] Nevertheless, in the trauma setting, mechanical compression of the spinal cord remains the major cause of paraplegia.

Myocardial Ischemia and Heart Failure

The heart can be resuscitated after 30 minutes of experimental isolation.[69] Young and previously healthy patients rarely develop heart failure after trauma resuscitation, except in instances of prolonged shock or cardiac arrest. Therefore, unless cardiac disease is preexisting, heart failure is rarely the cause of persistent shock after trauma. Myocardial infarction is the most common cause of posttraumatic heart failure; other potential causes include preexisting cardiomyopathy (e.g., diabetic, viral), cardiac contusion, and tamponade. In multiorgan failure, the highest mortality rate occurs with a combination of respiratory and heart dysfunctions.[169]

Heart failure—the inability of the heart to eject enough blood for the metabolic requirements of tissues—can be induced by a number of factors during hemorrhage and resuscitation.[248] Although anemia should increase cardiac output, with hematocrits below 20%, oxygen delivery may decrease, as blood velocity exceeds the capacity for adequate exchange at the capillary level. Hyperkalemia, hypocalcemia, hypophosphatemia, and hypermagnesemia all can impair cardiac contractility and rhythm. Prolonged shock can allow platelet aggregates to form in the coronary microcirculation, contributing to myocardial ischemia and dysfunction. Resuscitation, particularly with blood products, can induce injury to the myocardium independent of the degree of hemorrhage.[133] Severe brain damage can contribute to myocardial depression and irreversible shock. Whereas mild hypoxia increases sympathetic tone and cardiac contractility, severe hypoxia impairs myocardial function; severe acidosis (pH <7.0) also impairs contractility. Cardiac failure after hemorrhage also has been attributed to "myocardial depressant factors," most likely derived from the pancreas in response to ischemia.[170] Hypothermia (<35°C) is known to severely impair cardiac responsiveness. Patients with renal or liver failure are more susceptible to becoming fluid overloaded, which can precipitate heart failure. Vasopressors usually increase systemic vascular resistance, reducing the cardiac output, tissue perfusion, and oxygen delivery.[72] Conversely, maneuvers that increase the preload and reduce afterload should help in achieving optimal myocardial oxygen consumption during resuscitation.

Persistent hypotension after trauma usually is due to an active bleeding source or inadequate fluid resuscitation. Given patients with coronary artery disease are at high risk of acute coronary events during hemorrhagic shock, persistent hypotension after adequate fluid resuscitation should raise suspicion of myocardial infarction, particularly in diabetic patients, those in coma, and those unable to speak because of

intubation or other factors. Decrease in variability of heart rate with respiratory cycles is a subtle sign of myocardial infarction.[246]

Lastly, the role of vasopressin in shock has been determined to be not only a potent splanchnic vasoconstrictor, but also a key humoral factor in the maintenance of post reinfusion blood pressure.[92] Recently, it has been shown in an animal study that in hypovolemic cardiac arrest, it was vasopressin, not high-dose epinephrine, that resulted in sustained vital organ perfusion, less metabolic acidosis, and prolonged survival. Clinical evaluation of vasopressin during hypovolemic cardiac arrest may be warranted.[235]

Acute Renal Failure

As part of the response to hemorrhage and hypotension, blood flow to the kidneys—approximately 25% of the cardiac output—is reduced to help preserve effective blood volume. In addition, renin is released from the juxtaglomerular apparatus to cleave circulating α-2 globulin, generating angiotensin I, which, in turn, is transformed in the lungs (by angiotensin-converting enzyme) to angiotensin II, a potent vasoconstrictor. Hypotension and increased blood osmolality during shock also can activate the release of antidiuretic hormone by neurohypophysis. Loss of more than 15% of blood volume, however, usually exceeds the autoregulatory capacity of the kidneys. Renal blood flow is further reduced and redistributed; sodium and water retention mechanisms become impaired. If hypovolemia is profound or persistent, acute renal failure caused by acute tubular necrosis may ensue; however, with adequate resuscitation, the incidence of renal failure requiring dialysis should be less than 5%, even after severe trauma.[169] Maintaining the urine output above 2 mL/hour without diuretics should protect the kidneys during shock resuscitation.[126] The main factors predisposing to renal failure after trauma are advanced age, chronic hypertension, and preexisting renal insufficiency. Early renal failure after shock has been associated with myoglobinuria and hypotension during the resuscitation phase, whereas late renal failure correlates with development of sepsis and drug toxicity.[94]

Both ventilatory support and general anesthesia can impair autoregulatory renal responses and reduce renal perfusion. Nonsteroidal antiinflammatory drugs can aggravate renal vasoconstriction. Aortic cross clamping is a known risk factor for renal failure after trauma. Radiocontrast iodinated contrast media used routinely may alter renal hemodynamics or cause direct tubular toxicity; therefore, except in rare situations, contrast studies should be done only when urine output and renal function tests are normal. Myoglobinuria and hemoglobinuria can cause renal tubular damage, especially in the presence of concomitant hypotension and acidosis. Prophylaxis and treatment of this complication are based on urine alkalinization and maintaining high urine output through volume replacement and administration of sodium bicarbonate and

mannitol or loop diuretics. Myoglobin-induced renal failure is usually associated with crush, electrical, or reperfusion injury.[172] Gastric stress ulcerations and platelet dysfunction also can occur as a result of renal failure.[53]

Renal vasoconstriction subsides slowly after adequate resuscitation, and oliguria may persist despite normalization of blood volume. Diuretics should not be used under these circumstances. Low-dose dopamine has been advocated; however, a small randomized, double-blind, placebo-controlled trial showed low-dose dopamine offers no advantage to normovolemic patients after elective abdominal aortic surgery, patients with acute oliguric renal failure not included.[8] Recent evidence suggests a potential for use of concomitant infusion of low-dose dopamine and norepinephrine.[95] Anuria (urine volume <50 mL/day) after trauma is usually caused by mechanical obstruction. A third of critically ill patients may have glomerular or tubular damage despite normal routine renal function tests.[61]

Hyperkalemia is the most common life-threatening result of renal failure; it requires immediate treatment, including potassium antagonist (10% calcium gluconate), correction of acidosis (sodium bicarbonate), stabilization of cardiac membranes (glucose-insulin infusion), and use of cation exchange resins (Kayexalate, Sanofi Winthrop, New York, NY).

Hemodialysis is indicated for fluid overload with pulmonary edema, refractory hyperkalemia, severe metabolic acidosis, and uremic pericarditis or encephalopathy. Dialysis may not be effective in correcting platelet dysfunction.[53] Although controversial, early dialysis may benefit the hemodynamically stable patient with severe renal impairment by allowing adequate nutritional support and facilitating administration of fluids. Side effects of hemodialysis include hypoxemia, hypotension, bleeding, infection, and increased intracranial pressure. In addition, use of heparin during hemodialysis may increase the risk of bleeding in conditions such as pelvic fracture and retroperitoneal hematoma, even when protamine is administered into the inflow conduit. Hemodialysis without anticoagulation has the potential limitation of activating the coagulation system and causing greater fibrin deposition on dialyzer membranes.[175] Forms of dialysis used in critically ill patients include intermittent hemodialysis (IHD), continuous arteriovenous hemofiltration (CAVH), continuous venovenous hemofiltration (CVVH), continuous renal replacement therapy (CRRT), and slow low-efficient daily dialysis (SLEDD). CRRT and SLEDD appear to be advantageous over IHD because of hemodynamic stability, correction of hypervolemia, and better solute removal. A retrospective study of 100 patients showed that early institution of CCRT improves survival of trauma patients who develop acute renal failure.[71] Although a relatively new method, SLEDD is less expensive than CRRT and allows better mobilization of the patient.[228] Patients with acute renal failure treated with CAVH for a mean period of 10 days had a complication rate of 10%, mostly associated with the vascular access in the femoral artery.[94] Although CVVH has

been associated with an increased likelihood of death, this appears to be related to severity of illness and not the treatment choice itself.[191]

Early resuscitation can shift renal failure from the oliguric to the non-oliguric form. Non-oliguric renal failure (urine output >400 mL/day plus azotemia) is associated with a 20% mortality rate, whereas the oliguric form has a mortality rate of more than 50%.[94] Although early fluid resuscitation may reduce the incidence of renal dysfunction and mortality in burn victims,[42] achieving hemostasis should have the highest priority in hemorrhagic shock.[19]

Lung Injury

Causes of pulmonary insufficiency (PCO_2 >45 mm Hg; PO_2 <60 mm Hg; normal or low pH) after trauma include atelectasis, lung contusion, pneumonia, thromboembolism (noted in approximately 14% of autopsies from trauma),[49] and acute respiratory distress syndrome (ARDS).[13] Respiratory failure is most common in patients with an ISS greater than 16, shock on admission, and age of more than 55 years. Head injury can contribute to respiratory failure because of increased risk of aspiration, hypoventilation, atelectasis, and need for ventilatory support (barotrauma). In addition, a form of "neurogenic" pulmonary edema has been described. Fat embolism should be suspected when cerebral and respiratory dysfunction, thrombocytopenia, and increased alveolo-arterial gradient are associated with pelvic or long-bone fractures.

Acute respiratory distress syndrome is characterized by hypoxemia resistant to increases in fraction of inspired oxygen (FiO_2).[13, 222] ARDS occurs most commonly in patients with peritonitis. The pathophysiology involves recruitment of leukocytes to capillary beds in the lungs. Subsequent local release of multiple inflammatory and vasoactive substances, including oxygen–free radicals, proteases (e.g., elastase, collagenase), arachidonic-acid metabolites that increase platelet aggregation, and complement activation, leads to increased capillary permeability, edema, further inflammation, and thrombotic occlusion of capillaries. Although shock itself can cause ischemia of pulmonary tissues, with impairment of ciliary activity and surfactant production, hemorrhage alone rarely causes ARDS, which usually requires an "inflammatory state." Measurement of extravascular lung water in 16 severely traumatized patients did not correlate with hemorrhagic shock, massive transfusion, and crystalloid resuscitation; post-traumatic elevations in lung water, capillary hydrostatic pressure, and capillary permeability correlated with lung contusion or sepsis.[219] Delayed-onset pulmonary insufficiency, however, has been demonstrated experimentally in non-human primates resuscitated from hemorrhagic shock.[178] Although excess fluid aggravates ARDS, the role of fluid overload and massive transfusions as primary causes of pulmonary failure is controversial.

In one study, pulmonary complications were noted in approximately 10% of more than 3000 trauma victims.[15, 45, 248] The lung is the most

frequent organ involved in multiorgan failure and is usually the first organ to fail after injury.[169] Respiratory failure has the highest mortality rate compared with failure of other organ systems.

Use of extracorporeal life support (ECLS) has been advocated in adult trauma patients with multiple injuries and severe pulmonary failure. In 30 patients who had an estimated mortality risk greater than 80%, early institution of ECLS was associated with improved oxygen delivery, diminished ventilator-induced lung injury, and improved survival.[135] Preclinical studies suggest resuscitation with hemoglobin solutions (pyridoxilated hemoglobin polyoxyethylene conjugates) can lead to vasoconstriction and elevated arterial and regional PCO_2.[73]

The mortality rate of ARDS remains approximately 35% despite modern intensive care. Increased pulmonary hypertension indicates a poor prognosis in patients with trauma and respiratory failure.

Adrenal Insufficiency

Acute adrenal insufficiency secondary to bilateral adrenal hemorrhage or infarction has been described in trauma patients (adrenal apoplexy).[43] Most cases of adrenal insufficiency after shock, however, are not associated with adrenal apoplexy. A state of "relative" adrenal failure may occur during phases of shock and resuscitation, particularly in elderly patients, because of the increased demand for adrenocortical hormones. Some patients may have subclinical adrenal hypofunction. In addition, it has been postulated that negative regulation of adrenal function may be caused by a sustained increase in plasma corticosterone levels because of decreased hepatic 11-HSD activity following trauma and severe hemorrhage.[241]

Adrenal failure has been reported in 19% of critically ill patients who are hemodynamically unstable and on vasopressors.[173] Adrenal hypofunction, as assessed by plasma cortisol, may occur in approximately 50% of critically ill patients.[181] In unselected patients in the ICU, however, incidences as low as 1% have been reported.[9] Therefore, screening may be indicated only for patients with prolonged stays in the ICU and ages of more than 55 years.[9]

Mesenteric Ischemia

Nonocclusive mesenteric ischemia is a low-flow state that may result from hypovolemic hypotension in the trauma patient. Adequate cardiac resuscitation usually causes this entity to be extremely uncommon. Associated low-flow states may occur, however, and include cardiac failure, cardiac arrhythmia, myocardial infarction, shock, hypovolemia, aortic valvular insufficiency, and use of certain inotropic drugs. Vasoconstriction to such states is a compensatory mechanism designed to improve perfusion to vital tissues such as the brain, heart, and

kidneys. Vasoconstriction remains fixed until reversed by vasodilating drugs. Angiographic studies reflect spasm of segmental branches of the superior mesenteric artery; the spasm is diffuse, focal or concentric. Spastic areas are uniformly smooth. Clinical manifestations of this non-occlusive low-flow phenomena are the same as those mentioned when one discusses the response of the intestine to ischemia, no matter what mechanism. Trauma to the abdomen can result in direct injury to the stomach, duodenum, small bowel, and colon, resulting in hemorrhage or perforation. This setting, however, focuses on the sequelae of hypovolemic hypotension. Complications may present weeks to months following discharge in the patient who survives. The compound problem of a direct injury to the abdomen and GI track, with superimposed hypovolemic hypotension, presents a complex situation. Blunt trauma causes visceral or mesenteric injury and minor contusions. It may produce significant hematomas, or even perforations. Sometimes these injuries are severe enough to include mucosal injury, but the intact serosa prevents early peritoneal soilage. Given the compound problem of hypotension superimposed on abdominal trauma, the subsequent ischemic perforation will result in infection and fistula formation.

Nonoperative management of mesenteric insufficiency requires a diagnosis to determine whether visceral organ injury is present. Patients perceived to be stable undergo diagnostic tests, such as the computed tomography (CT) scan, for a definition of that injury. Differentiating between a mesenteric injury and bowel perforation can usually be ascertained by repeated physical examinations in the conscious patient. Peritoneal lavage may be optional. A negative celiotomy may occur, but it should be infrequent based on frequent clinical and CT examinations. Mesenteric ischemia with colitis is not an acute event, although it may occur during the ensuing 24 to 72 hours. The diagnosis is more often obtained through sigmoidoscopy. This procedure may be diagnostic in nearly 50% of the patients.

The trauma patient is not immune to non-trauma GI disorders, but that is not the thrust of this section. The issue of GI hemorrhage may be due originally to hypotension and hypovolemia with stress ulcerations or, subsequently, may cause hypotension hypovolemia with hematemeses and melena. Rarely, Dieulafoy's syndrome may cause massive post-traumatic GI hemorrhage. The patient who is hypovolemic and, subsequently, continues to bleed from either an intraabdominal or extra-abdominal source will have some sequelae, such as ischemic colitis, following preresuscitation of hypotension and hypoperfusion. Bloody diarrhea may result. Exacerbation of preexisting peptic ulcer disease is possible, but hemorrhage of the stress ulceration following multiorgan failure is more common, and occurs in the patient with a prolonged ICU course.

Acute gastric and/or duodenal ulceration may follow the stress of hypovolemic hypotension.[33] Perforation may occur, but is less frequent than bleeding. In response to hypovolemic hypotension, the signs that may present include blood from the nasogastric tube, melena, and shock. As an extreme, one may see peritonitis, abdominal distension, and a

septic course. Diagnosis is usually through endoscopy, but for perforation and adverse events further in the GI track, CT scan is appropriate.[120] Hemorrhage from a duodenal stress ulceration is usually a later manifestation and not acute following hypovolemic hypotension; nevertheless, the treatment should involve H_2 blockers and antacids, gastric lavage, and others. The stress insult, hypotension, should be removed. The outcome of mesenteric ischemia is, at worst, perforation, but more likely a stricture of a segment of bowel. It does not necessarily require acute surgical intervention.

Hepatic Dysfunction

Hepatic dysfunction following major trauma is a relatively common adverse event. The epidemiologic setting of severe automobile accidents suggests this is a predisposing factor. Many accidents also are associated with alcohol and drug abuse; they have their own impact on direct trauma superimposed on the hypotension the body incurs. The primary adverse effect on the liver through trauma is shock and the need for anesthesia, massive blood transfusions, and prolonged operations. Among the clinical problems is the element of jaundice after the trauma. The jaundice one identifies is, more likely than not, caused by several issues: shock liver, benign postoperative cholestasis, hepatic venous congestion, increased pigment load, sepsis, drug-induced, and major hepatic resection.[124]

The insult to the liver through hypovolemic hypotension and resultant hypoxia is secondary to hypotension, the most significant factor contributing to development of jaundice and liver dysfunction. Only occasionally does the investigation of posttraumatic jaundice lead to a single etiologic factor. More likely than not, multiple causes are listed as contributing to postoperative jaundice in the trauma patient with hypotension and hypovolemia.[216] This patient usually has received multiple blood transfusions, prolonged operation and episodes of hypoxia. Shock alone could lead to hepatic mitochondrial swelling and centrilobular necrosis. In the typical patient, one sees the bilirubin as high as 5 to 20 mg percent within 1 week of the surgical intervention, or within 1 week of hypovolemic hypotension. The serum glutamic oxaloacetic transaminase (SGOT) may range from 100 to 500 U/mL, and the alkaline phosphatase may be elevated no less than two to three times normal. Treatment of the shock liver consists of supportive measures and avoidance of those drugs that adversely affect hepatic mechanisms.

A second type of liver injury associated with shock resuscitation, hypovolemic hypotension, and associated hypoxia is benign postoperative cholestasis. Patients with this condition have essentially the picture of obstructive liver chemistry with elevated bilirubins and markedly elevated alkaline phosphatase. The SGOT level is usually less than 200, and the majority of patients recover.

Another type of hypoxic hypovolemic liver damage is related to

prolonged venous congestion of the liver. Often referred to as 'cardiac jaundice,' it occurs with a pure cardiac defect that may be seen in the patient with hemorrhagic hypovolemia. One clearly sees the centrilobular necrosis and hemorrhage from elevated venous pressure and anoxia. Hypervolemia is a postresuscitation phase, and massive crystalloid infusion may contribute to the hepatic edema seen in this condition.[54]

In the immediate period following hypovolemic hypotension, one also can identify overt jaundice following a hemolytic reaction; however, this is uncommon without hepatic parenchymal dysfunction. The usual response to intravascular hemolysis is a decrease in haptoglobin level, the appearance of free hemoglobin in the blood and urine, and an increased unconjugated (indirect) bilirubin. The unconjugated bilirubin is strongly protein-bound and limited to the vascular space. It does not appear in the interstitial fluid and is not filtered by the kidney. Massive hemolysis associated with hypovolemic hypotension may saturate the reticuloendothelial system and bilirubin excretory system, allowing conjugated bilirubin to reflux into the blood. Conjugated bilirubin is more soluble and less strongly protein-bound than unconjugated bilirubin and, therefore, diffuses easily into the interstitial fluid and may appear in the urine. Posttraumatic hemolysis results from destruction of transfused red blood cells, which have a shortened lifespan. They also may occur because of adverse interdrug reactions, with hemolysis as a result. Among those drugs that precipitate hemolysis are aspirin, sulfonamides, or nitrofurantoin. The latter is particularly noticeable in glucose-6-phosphate-deficient patients. Major hepatic resections in the presence of hypovolemic hypotension and profound shock are associated with jaundice. The cellular mass in this case has been decreased and, therefore, the response of the liver—"shock liver"—is more dramatic.

The manifestations of hypovolemic hypotension as regards hepatic dysfunction include: jaundice, encephalopathy, hepatomegaly, and acholic stools. The laboratory assessment should include enzymes, bilirubin, prothrombin time, ammonia, and assessment of albumin.

Management is one of resuscitation: volume resuscitation to restore hepatic viability, with adequate blood flow and metabolism. Ultimately, it will be necessary to determine whether any subsequent liver disorder has become hepatocellular or obstructive. Sepsis may follow the damage to the liver, and one has to be alert to this possibility. Subsequently, all medications and anesthetics must be identified carefully before they have an adverse impact on hepatic metabolism. One must address the role of hemolysis when jaundice appears, and whatever it takes for adequate oxygenation, including protection of pulmonary function, is necessary. The patient must be supported with adequate oxygen delivery through this adverse period.

Finally, hyperalimentation formulas have to be adjusted, depending on the hepatic metabolism and capacity of filtering. One must consider reducing aromatic amino acids and supplying extra arginine and branch-chain amino acids in patients with liver dysfunction. It is suggested that these alterations minimize adverse impact on liver function tests.

The management of hepatic encephalopathy requires eradicating blood from the GI tract if hemorrhage has been present, removing any drug that adversely affects the liver, and minimizing protein intake with the focus on branch-chain amino acids. To remove the nitrogenous load from the GI track, one should consider enemas or cathartics. The use of neomycin may decrease urease-producing bacteria. Lactulose appears to minimize encephalopathy. Finally, one must again prevent a septic focus from occurring.

In summary, the principles of treatment for liver injury include control of bleeding, debridement of devitalized tissue, and establishment of appropriate drainage. The liver injury will further compound and compromise liver function in the patient with hypovolemic hypotension.

Pancreatitis

Pancreatitis is uncommon in the trauma patient, and is usually related to direct injury to the pancreas.[15, 46] During hypovolemic hypotension, however, the pancreas can be subjected to an ischemic event. Pancreatitis can then manifest, with fever and/or an elevated white-blood-cell count, in addition to abdominal tenderness and ileus. Pleural effusions may occur.

The diagnosis is based on elevated serum amylase or elevated urine amylase. There also may be an elevated serum lipase. Abdominal x-rays suggest an ileus and a colon cut-off sign. CT will confirm an edematous pancreas. Treatment is nothing by mouth, nasogastric suction, and cardiovascular support with adequate resuscitation and intravascular volume. Future sequelae would require monitoring for abscess, pseudocyst, respiratory failure, hypocalcemia, and hemorrhage from an eroded artery. The effect of pancreatitis on the lung is well established, causing adult respiratory distress syndrome (ARDS) with destruction of surfactant by release of phospholipase A.

Ischemic pancreatitis is an extremely uncommon adverse event; when it occurs, the physician will be treating other problems in addition to the pancreatitis.

Coagulopathy

The hemostatic balance between coagulation and fibrinolysis requires adequate pH, temperature, and blood composition of cells and proteins. The observed decrease in levels of clotting factors early after severe hemorrhage is attributed to consumption and plasma dilution from resuscitation. Massive transfusions of blood, or colloid or crystalloid solutions, can lead to so-called "dilutional coagulopathy." During later phases, enhanced hepatic synthesis, appropriate replacement, and other factors contribute to restoration of clotting factors.[87] Hypothermia and metabolic acidosis usually precede the development of coagulopa-

thy in severely injured patients requiring massive transfusion.[52] Hypothermia-related coagulopathy requires both rewarming and clotting-factor repletion.[80] A consumptive coagulopathy that develops within hours after blunt brain injury also has been described.[99] Bleeding abnormalities in renal failure may be associated with suboptimal binding of the von Willebrand factor to platelet membranes, acquired storage-pool deficiency, and anemia.[53] In management of uremic bleeding in the trauma patient, cryoprecipitate and desmopressin may be useful because of their short onset of action. Synthetic colloids, such as hydroxyethyl starch (HES), can be effective in restoring intravascular volume. Despite their antiplatelet properties, if used below upper-dose limits, these solutions can improve microcirculation and are safe for coagulation, reticuloendothelial, and renal functions.[166]

In general, transfusion of more than 10 units of packed RBCs results in thrombocytopenia, low fibrinogen, and prolonged prothrombin time; more than 20 units cause coagulation defects in 70% of patients.[68] Patients with ISS greater than 25 who receive 6 or more units of blood represent a high-risk group for development of multiple organ failure.[57] Disseminated intravascular coagulation is often associated with multiple organ failure and the systemic inflammatory response syndrome. Staged laparotomy may be indicated in select patients with refractory coagulopathy after trauma.[137]

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NEW APPROACHES TO TRAUMA MANAGEMENT USING SEVERITY OF ILLNESS AND OUTCOME PREDICTION BASED ON NONINVASIVE HEMODYNAMIC MONITORING

William C. Shoemaker, MD, FACS

Hemodynamic bedside monitoring by pulmonary artery catheterization (PAC) has been considered by many as the gold standard for critically ill patients, but its usefulness has been challenged,[9-11, 22] particularly in the late stages of illness after the onset of organ failures. Meta-analyses by Boyd and Hayes[7] and Kern and Shoemaker[12] showed no outcome improvements in seven randomized studies of patients who entered the ICU after organ failure or sepsis had occurred, but outcome was significantly improved in seven other randomized studies when PAC-directed therapy was given early or prophylactically.[12, 13, 27] Because time may be important in the initial resuscitation and management of emergency patients, noninvasive monitoring is a useful alternative approach to identify and correct hemodynamic deficiencies at the earliest possible time. Previous studies have documented satisfactory correlation between thermodilution and bioimpedance cardiac output values for trauma patients in the emergency department (ED), surgical suite (OR), and under ICU conditions.[20]

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In the present study, hemodynamic monitoring of severe trauma or high-risk surgical patients was reviewed, beginning in the ED, continuing in the radiology department, the OR, and then in the ICU. The author studied patients immediately after trauma or high-risk surgery because time factors are important and the early time course of circulatory events could be monitored.[16, 17] Many other studies showed the importance of optimal goals in the early period after injury or surgery.[4–6, 8, 14, 15, 20] Continuous visual displays of monitored data described rapidly changing patterns during unstable emergency conditions. Second, the author time-integrated the differences between the monitored curve and normal values or "optimal" goals derived from the patterns observed of previous series of survivors of acute severe trauma or operations. Third, the author then calculated the net cumulative excesses or deficits of each monitored variable for each patient and for the survivors and nonsurvivors.[21] Fourth, the author reviewed studies of discriminant analysis to predict outcome.[21] Finally, the author reviewed a stochastic control program that evaluates acute severely injured emergency patients throughout the course of acute illness and provides a decision support system.[1–3, 19] Acute injury was studied because the onset of illness occurred immediately before admission, and the course of circulatory events could be monitored from the time of ED admission beginning in the ED and continuing until hemodynamic stability was achieved.

MATERIALS AND METHODS

Noninvasive Hemodynamic Methods

Noninvasive hemodynamic monitoring of acute trauma patients was able to quantify hemodynamic deficits at the earliest possible time. Noninvasive hemodynamic monitoring systems consisted of a bioimpedance method for estimating cardiac output, together with pulse oximetry to reflect pulmonary function, transcutaneous oxygen tension to reflect tissue perfusion, and blood pressure to reflect the overall circulatory status.[20, 23–26] These continuously monitored noninvasive measurements were used to prospectively evaluate circulatory patterns in severely injured patients, beginning with admission to the ED in a university-run county hospital.

Calculation of Net Cumulative Deficit of Hemodynamic Variables

The area between the fluctuating monitored variables and either the normal values for blood pressure, $SapO_2$, and $PtcO_2/FiO_2$, or optimal value for cardiac index were calculated. This area was integrated over time to calculate the net cumulative amount of deficit or excess of each

monitored variable. The net cumulative deficit or excess for each variable was calculated in each individual patient and for survivor and nonsurvivor groups. For example, given a normal mean arterial pressure (MAP) of 85 mm Hg, in a patient whose MAP averaged 60 mm Hg for 2 hours before resuscitation, the calculated deficit is ([85–60] × 2) or −50 mm Hg · h.[21]

Outcome Prediction by Discriminant Analysis

There were significantly greater calculated deficits of cardiac index, pulse oximetry, and transcutaneous O_2 in the nonsurvivors than in survivors during the period of monitoring (see Table 4). These three variables and the Glasgow Coma Scale (GCS) score, having moderate levels of significance with outcome, were selected for the stepwise discriminant analysis.[21]

Database for Stochastic Analysis and Control Program

Databases for acutely injured patients have been developed to describe primary injuries, covariates, hemodynamic patterns by invasive and noninvasive methods, and outcomes, including survival or death, organ failure, other complications, hospital days, and ICU days. Noninvasive monitoring usually was begun in the ED, and the patient was followed to the OR, radiology department, and ICU. Invasive PAC was instituted when clinically indicated, usually after the patient arrived in the ICU. The time and place of monitoring, time of operations, times of ICU admission and discharge, and time of hospital discharge or death were recorded relative to time elapsed after admission.

These databases include the following 30 covariates:

Age
Gender
Estimated blood loss in the preoperative, intraoperative, and postoperative periods
Blunt truncal trauma
Penetrating truncal trauma
Nontruncal (extremity) injury
Spinal cord injury
Blunt cardiac injury
Penetrating cardiac injury
Pulmonary contusion
Pelvic fracture
Long bone fractures
Head injury
Brain death

Early stage (< 12 hours)
Middle stage (12–24 hours)
Late stage (> 24 hours) or after organ failure
Cardiac insufficiency (reduced cardiac reserve capacity determined by responses to standardized doses of transfusions, and fluid challenges)
Bacterial contamination, sepsis, or systemic immune response system
Respiratory dysfunction or failure immediately before the present acute illness
Preillness renal insufficiency or failure
Preillness hepatic failure
Nutritional insufficiency or failure
Uncontrolled diabetes
Preillness essential hypertension
Cardiac injury (blunt or penetrating)
Cardiac arrest
Pregnancy
GCS score
The injury severity score

Method for Stochastic Analysis Based on a Trauma Database

Bayard et al[1–3] developed a stochastic analysis and control program to determine individual patients' survival probabilities (SP), based on the patient's state and patients in the database with very similar states. The patient's "state" is defined by the primary diagnosis, 30 covariates, and hemodynamic variables. By "similar" is meant a group of patients, referred to as "nearest neighbors," with the same diagnosis who share the same set of specified covariates and have very similar hemodynamic patterns to the patient under study. Mathematically, the stochastic analysis is defined as policy iteration with respect to conventional therapeutic policy used for each patient as the database was developed. It is motivated by methods of machine learning[1, 3] and methods of dynamic programming for stochastic control.[1, 2] A therapeutic decision support program was designed to use the database of therapeutic responses to evaluate the relative effectiveness of various therapies by the responses of each therapy for the patient's nearest neighbors.[19]

The state of the patient at any time is defined in terms of primary diagnosis, the covariates, and the hemodynamic measurements. A state vector, $x(t)$, at time t is defined in terms of the various hemodynamic measurements, their derivatives, and their integrals. Assume that there are L different types of measurements taken on a given patient (e.g., cardiac index, blood pressure, pulse oximetry, and transcutaneous O_2 and CO_2 tensions). Specifically, for each measurement type, denoted as

y_l, define the state vector as a concatenation of the value y_l itself, its first and second derivatives y'_l, y''_l, and its first integral $\int y_l dt$, as follows:

$$x(t_k) = \left[y_l(t_k), y'_l(t_k), y''_l(t_k), \int_0^{t_1} y_l\,dt, \ldots, \ldots, \ldots, y_L(t_k), y'_L(t_k), y''_L(t_k), \int_0^{t_1} y_L\,dt \right]^T$$

that is, for L different measurement types there will be $4L$ states. In practice, the derivatives and integrals are approximated by finite differences and sums of the time-ordered data of the database.

RESULTS

Noninvasive Monitoring from the Time of Admission

The mean estimated blood loss, which reflects preoperative and intraoperative hemorrhage, measured at the end of surgery, was 2970 ± 3856 (SD) mL in survivors and 6263 ± 5540 mL in the nonsurvivors. In the present series, 22 patients had massive blood loss (> 5000 mL). Vigorous attempts were made to replace these losses at the time of surgery and in the immediate postoperative period.[19, 21] Noninvasive monitoring systems were found to be feasible in acutely ill ED patients for early description of temporal hemodynamic patterns and to provide quantitative calculation of the total amount of deficit or excess accumulated by each monitored variable. Table 1 lists the mean values ± SEM of cardiac index, MAP, $SapO_2$, and $PtcO_2/FiO_2$ for survivors and nonsurvivors averaged throughout the observation period. The CI, $SapO_2$, and $PtcO_2/FiO_2$ values of the survivors were significantly greater than the values of those who died. Nonsurvivors' $SapO_2$ values were significantly lower than the survivors' values, but these differences were not clinically important; when $SapO_2$ reductions occurred, they were

Table 1. NONINVASIVE HEMODYNAMIC VALUES FOR SURVIVORS AND NONSURVIVORS

Variable	Normal or Optimal Value	Survivors (N=103) Mean ± SEM	Nonsurvivors (N=48) Mean ± SEM	P value
CI, L/min/m²	4.0	4.14 ± 0.02	3.87 ± 0.03	< 0.001
MAP, mm Hg	85	88 ± 0.37	80 ± 0.69	0.066
$SapO_2$, %	98	99 ± 0.05	96 ± 0.26	< 0.001
$PtcO_2/FiO_2$, torr	200	206 ± 2.9	93 ± 2.6	< 0.001

Normal values for MAP, $SapO_2$ and $PtcO_2/FiO_2$, or "optimal" value for cardiac index. Mean ± SEM for cardiac index (CI), mean arterial pressure (MAP), $SapO_2$ arterial hemoglobin saturation by pulse oximetry ($SapO_2$), and transcutaneous oxygen tension indexed to FiO_2($PtcO_2/FiO_2$) calculated for the monitored period, and P values for differences between survivors' and nonsurvivors' values. *From* Shoemaker WC, Wo CCJ, Chan L, et al: Outcome prediction of emergency patients by noninvasive hemodynamic monitoring. Chest 120:523–528, 2001; with permission.

rapidly corrected by intubation, mechanical ventilation, or increased FiO_2. MAP values of survivors tended to be higher than those of nonsurvivors ($P = .066$). Correlation between simultaneous thermodilution and bioimpedance cardiac output measurements in the present series was $r = .91$, $r^2 = .83$; bias and precision were -0.30 ± 1.10 L/min/m^2.[21]

Net Cumulative Amount of Deficit or Excess in Monitored Variables

The net cumulative deficits of flow and tissue perfusion measured during the initial resuscitation period were greater in nonsurvivors than in survivors; these differences were correlated with outcome. Flow calculations, measured as volume per unit of time, are L/min/m^2. When multiplied by monitored time in minutes this gives, as units, L/m^2 for cardiac index or L for cardiac output. The units for MAP, $SapO_2$, and $PtcO_2/FiO_2$ are mm Hg · h, % · h, and torr · h, respectively. For example, during the monitoring period, the survivors' cardiac index averaged 81 L/m^2 more than the "optimal" 4.0 L/min/m^2, empirically determined from the plateau of high values of survivors within the first 24 hours of admission.[5, 6, 16, 17, 20, 21] This was equivalent to 140 L of cardiac output per patient over the monitored period. During the monitoring period of those who died, the cardiac index averaged 232 L/m^2 less than optimal, and the cardiac output averaged 402 L per patient less than optimal. The difference between survivors and nonsurvivors was 542 L, using 4.0 L/min/m^2 as the therapeutic goal (Table 2).

Outcome Prediction by Discriminant Analysis

Based on the classification function generated for cardiac index, pulse oximetry, GCS, and transcutaneous O_2 variables, the discriminant

Table 2. MEAN NET CUMULATIVE DEFICITS OR EXCESSES OF MONITORED VALUES OF SURVIVORS AND NONSURVIVORS THROUGHOUT THE PERIOD OF OBSERVATION

	Survivors		Nonsurvivors		
Variable	Mean	SEM	Mean	SEM	*P* Value
CI, L/m^2	+81	52	−232	138	< 0.007
MAP, mm Hg · h	−10	13	−57	24	0.078
$SapO_2$,% · h	−1	0.3	−8	2.6	< 0.006
$PtcO_2/FiO_2$, torr · h	+313	87	−793	175	< 0.001

Net cumulative deficits or excesses expressed as mean values ± SEM for cardiac index (CI), arterial hemoglobin saturation by pulse oximetry ($SapO_2$), and transcutaneous oxygen tension indexed to FiO_2 ($PtcO_2/FiO_2$) for the monitored period; cumulative deficits are shown for mean arterial pressure (MAP); *P* values are for differences between survivors' and nonsurvivors' values. Note the pronounced differences in the net cumulative deficits and excesses of cardiac index and $PtcO_2/FiO_2$ of the survivors versus the nonsurvivors. *From* Shoemaker WC, Wo CCJ, Chan L, et al: Outcome prediction of emergency patients by noninvasive hemodynamic monitoring. Chest 120:523–528, 2001; with permission.

Table 3. STEPWISE DISCRIMINANT ANALYSIS

Step Entered	Partial R^2	Prob > F	Cumulative R^2
1. Cumulative $PtcO_2/FiO_2$	0.210	.0001	0.2099
2. GCS	0.188	.0001	0.3581
3. Cumulative $SapO_2$	0.053	.0047	0.3921
4. Cumulative CI	0.031	.0336	0.4107

Classification of survivors, Z > 2.36; where Z = 0.0011 (cumulative $PtcO_2/FiO_2$) + 0.3300 (GCS) + 0.0656 (cumulative $SapO_2$) + 0.0423 (cumulative CI). *From* Shoemaker WC, Wo CCJ, Chan L, et al: Outcome prediction of emergency patients by noninvasive hemodynamic monitoring. Chest 120:523–528, 2001, with permission.

function, Z, was derived: Z = 0.0011a + 0.3300b + 0.0656c + 0.0423d; where *a* represents cumulative $PtcO_2/FiO_2$ values, *b* represents GCS score, *c* represents cumulative $SapO_2$ values, and *d* represents cumulative cardiac index values. Table 3 summarizes the relative influence of each variable with respect to outcome. Ninety-five percent of the survivors and 62% of the nonsurvivors were correctly classified in the first 24 hours postadmission (Table 4). There were 23 of 151 (15.2%) misclassifications. Five of the 35 patients predicted to die in the first 24 hours subsequently improved and lived.[21]

Outcome Prediction by Stochastic Analysis

A major assumption in the present approach is that circulatory deficiencies that ultimately lead to shock, organ failure, and death can be identified early by noninvasive monitoring, and the SP may be predicted by stochastic analysis of dynamic patterns. The SP is roughly equivalent to severity of illness. That is, the patient with a 90% likelihood of death is severely ill, whereas the patient with an estimated survival outcome of 90% may not be very ill. The proposed mathematical representation of the circulation defines the patient's state by specific diagnos-

Table 4. CLASSIFICATION SUMMARY FOR THE SERIES (N = 151)

	Predicted to Die		Predicted to Live		Total	
	N	(Row %)	N	(Row %)	N	(Col %)
Actual Outcome						
Died	30	62.5	18	37.5	48	31.8
Lived	5	4.9	98	95.1	103	68.2
Total (%)	35	23.2	116	76.8	151	100.0

Misclassification: 23/151 = 15.2%

Classification summary of those predicted to live and those predicted to die, based on the net cumulative deficits of monitored variables. Row %, the percentage of patients in that row. Col %, the percentage of patients in that column. Note: 95% of those predicted to live after initial resuscitation did live, while 62% of those predicted to die did not survive. *From* Shoemaker WC, Wo CCJ, Chan L, et al: Outcome prediction of emergency patients by noninvasive hemodynamic monitoring. Chest 120:523–528, 2001, with permission.

tic categories, covariates, hemodynamic variables, their derivatives, and their integrals in a multidimension grid. Since the database contains over 9000 time lines, each of which may represent a patient's state, there are many choices available for selection of the nearest neighbors. A patient's SP for a given state x is denoted by $S(x)$, which is calculated by first extracting the 40 nearest neighbor states of patients having the same diagnosis and covariates as well as hemodynamic values that are closest to the given patients' values. The SP is then calculated as the fraction of these nearest neighbors who survived with this treatment. The SP also may serve as a measure of the patient's severity of illness.[21] The average difference between a given patient's variables and the nearest neighbors' variable was between 0.1 and 0.2 of the SD. This indicates that, given a large database, one did not have to deviate far from the patient's state to find an adequate number of similar neighbors.

This approach was tested in the extenuating and sometimes chaotic circumstances of severely traumatized ED patients in a large inner city public hospital. Under these circumstances, the SP was found to closely track changes at each point during the initial observation period and the SP correctly predicted outcome in each patient before the end of the monitoring period, which was usually 12 to 24 hours after injury. Early clinical diagnosis and physiologic assessment are essential because they allow therapy to be initiated sooner, as earlier therapy may improve outcome in emergencies in which time is crucial.[4, 8, 13, 15–17, 20, 28] Moreover, the stochastic analysis and control program provided an independent mathematical tool to evaluate therapeutic responses objectively. The calculated SP of those who survived averages 75% or higher throughout the observation period. It was below 60% for nonsurvivors during the first 24-hour period of observation. Noninvasive systems in the early postadmission period provide continuously monitored, real-time displays of data from the ED to the OR, and to the ICU for early recognition of circulatory dysfunction in acute emergency trauma conditions. Initial studies showed that the differences between the measured SP values and the SP values predicted from the previous stage averaged $7 \pm 6\%$, indicating satisfactory consistency and reproducibility.[19]

Stochastic Control as a Decision Support System

The stochastic analysis of dynamic patterns also may be used for optimal decision making with a feedback control that, on the average, has proven to work best for similar patients in similar conditions recorded in the database, that is, nearest neighbors.

DISCUSSION

The advantages of this noninvasive monitoring system include technical convenience and the continuous display of data, allowing for calculation of the amount of deficit or excess of each variable from the time-integrated area under the curve. The area under the curve gives

arithmetic solutions to replace subjective evaluation of irregular curves and provides estimates of cardiac, pulmonary, and tissue perfusion functions. The high early cardiac index values in survivors suggest that there may have been less hypovolemia or better physiologic compensations. This concept is reinforced by the greater $PtcO_2/FiO_2$ net cumulative excesses, which suggest better tissue oxygenation in the survivors' initial stages. These preliminary studies need to be independently evaluated in larger series of trauma patients.

The hypothesis underlying this approach is that circulatory deficiencies that ultimately lead to shock, organ failure, and death may be identified early by noninvasive monitoring, even in the extenuating circumstances of severely traumatized ED patients in a large inner city public hospital. Earlier diagnosis of circulatory deficits allows therapy to be initiated sooner; earlier therapy is likely to improve outcome in emergencies in which time is crucial. More importantly, noninvasive monitoring, which is easy, inexpensive, fast, safe, and sensitive,[16, 17] provides similar information to that of the PAC, except for pulmonary artery occlusion pressures. Discriminant analysis and stochastic control programs provide mathematical basis for outcome prediction. Future prospective clinical trials at other institutions are needed to validate this approach and its cost-effectiveness. Because the essence of tissue perfusion is an adequate supply of oxygenated blood to the tissues, perfusion is inferred from the direct measurement of skin oxygenation using the Clark polarographic method for oxygen tension.[23, 24, 26] Although the skin is not representative of all tissues, it is the largest organ and the first organ to be affected by the adrenomedullary stress response. $PtcO_2$ provides early warning in acutely ill emergency patients; it tracks Vo_2 in acute clinical shock episodes and in the physiologic course of experimental hemorrhagic shock, as well as cardiac and respiratory failure, cardiac arrest, and CPR in acute surgical conditions; tissue perfusion was related to outcome.[19–21, 23, 24]

In the present study, the author used discriminant analysis to analyze the data of variables with P values $< .2$ to limit the number of variables for analysis. Interrelated or poorly conditioned variables having a common term, such as the combination of cardiac index and oxygen delivery, were avoided to minimize statistical problems of discriminant analysis. This does not mean that the more conventional variables, such as tachycardia, hypotension, acidosis, skin color, lactate levels, or mental status, are not useful at times when they occur. Obviously, when they are abnormal, they are extremely useful and important; however, criteria of the present study focused on early noninvasive hemodynamic variables in the immediate postadmission period that most consistently separated survivors and nonsurvivors.

SUMMARY

The mathematical model satisfactorily predicted outcome in acute emergencies based on noninvasively monitored flow, pressure, pulse

oximetry, tissue perfusion values, and their cumulative deficits. A decision support system provided information on the relative effectiveness of various therapeutic modalities based on the responses of patients with very similar states.

The concept that hypovolemia and oxygen debt is an early primary problem that plays an important role in low flow and poor tissue perfusion states is supported by direct observation of massive hemorrhage, estimated blood loss of hemoperitoneum and hemothorax at the time of surgery, and prior studies in the literature that documented blood volume deficits in posttraumatic and postoperative patients who subsequently developed organ failures and death.[18]

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Meta-analysis of hemodynamic optimization in high-risk patients*

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Objective: The aim of this evidence-based report was to review pertinent randomized controlled studies that describe hemodynamic goals in acute, critically ill patients and to evaluate outcome of resuscitation therapy in association with physiologic, clinical, and therapeutic influences.

Methods: MEDLINE was the source of randomized controlled studies written in English. The inclusion criteria were acutely ill, high-risk elective surgery, trauma, and septic patients. The goals of therapy were to resuscitate to either normal or supranormal values; the latter were described as a cardiac index of >4.5 $L \cdot min^{-1} \cdot m^{-2}$, pulmonary artery occlusion pressure of <18 mm Hg, oxygen delivery of >600 $mL \cdot min^{-1} \cdot m^{-2}$, and oxygen consumption of >170 $mL \cdot min^{-1} \cdot m^{-2}$. The outcome criterion was survival or death. We found 21 randomized clinical trials described in 20 articles. The studies were divided into groups based on the time that goals were implemented (i.e., "early," 8 to 12 hrs postoperatively or before organ failure, vs. "late," or after onset of organ failure) and the severity of illness, determined by the control group mortality as >20% (12 studies) or <15% (nine studies).

Results: In severely ill patients (control mortalities group >20%), six studies had a 23% mortality difference ($p < .05$) between the control and protocol groups with early optimization, but seven studies optimized after the development of organ failure did not have significantly improved mortality. Moreover, outcome was not significantly improved in less severely ill patients (control mortalities group <15%) and normal values as goals or when therapy did not improve oxygen delivery.

Conclusion: Review of 21 randomized controlled trials with various approaches to treatment revealed statistically significant mortality reductions, with hemodynamic optimization, when patients with acute critical illness were treated early to achieve optimal goals before the development of organ failure, when there were control group mortalities of >20% and when therapy produced differences in oxygen delivery between the control and protocol groups. (Crit Care Med 2002; 30:1686–1692)

KEY WORDS: noninvasive hemodynamic monitoring; bioimpedance cardiac output; thermodilution cardiac output; pulse oximetry; transcutaneous oxygen and CO_2 monitoring; trauma; high-risk surgery; acute septic shock; therapeutic hemodynamic goals; organ failure

Goal-directed studies with the pulmonary artery catheter (PAC) are highly controversial. Many studies showed no advantage of the PAC in cardiac and other medical conditions or in postoperative patients admitted to the ICU after organ failures had developed (1–5). However, other investigators (6–21) reported that early, optimally increased cardiac index (CI) and oxygen delivery ($\dot{D}o_2$) <12 hrs after surgery or 24 hrs after trauma were associated with improved survival. However, a evidence-based meta-analysis by Heyland et al. (22) showed that the attainment of supranormal hemodynamic goals did not significantly reduce mortality in critically ill patients. Recently, two consensus conferences also found insufficient evidence to fully determine whether PAC-guided therapy significantly alters outcome, but they did not consider time factors; by mixing early and late studies together, they concluded there were no significant differences in optimizing hemodynamic variables (23–25). In an insightful meta-analysis, Boyd (18) found no outcome improvement in seven prospective randomized studies of patients who entered the ICU after organ failure or sepsis had occurred (4, 5, 9, 11, 25, 26), but they noted significant outcome improvement in six other randomized studies when PAC-directed therapy was given early or prophylactically, that is, before organ failure or sepsis occurred (6, 7, 12, 14, 16, 17). Two recent studies also showed improved outcome with early goal-directed therapy (19, 20), suggesting that early optimization of $\dot{D}o_2$ and oxygen consumption values in high-risk surgical patients improves outcome. If, in some clinical circumstances, the hemodynamic values of survivors may be compensatory responses that have survival values, it is important to identify clinical conditions that may be appropriate for this type of goal-directed therapy. Second, it may be even more important to define therapeutic goals relative to the primary diagnosis and age; the presence of diabetes, hypertension, chronic cardiac and respiratory illnesses, and other comorbid conditions; and the severity of illness, timing of therapy, dose ranges, and other limitations of this approach.

Evidence-based studies have become the standard for testing important therapeutic questions, but evaluation of a therapeutic intervention should be clearly related to the central scientific idea defined by the research plan. As a prerequisite for clinical trial evaluation, important aspects of experimental study designs should be considered, including: definition of diagnostic categories; timing and dose of the therapeutic modality being evaluated; the patients' age, sex, and se-

*See also p. 1909.

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Supported, in part, by grants RR-11526 and GM-65619 from the National Institutes of Health.

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verity of illness; the presence of significant co-morbid conditions; and the clinical setting (Shoemaker WC, Bayard DS, Botnen A, et al., unpublished observations) (27, 28).

Clearly, lack of comparability of studies because of differences in the experimental design may preclude meaningful meta-analysis. Sweeping conclusions can hardly be justified by amassing many studies with large numbers of patients when the design features of the studies are not appropriately considered. Major questions include: In goal-directed therapy, are there outcome differences in the use of normal values compared with the supranormal values of survivors? What roles are played by time factors, various associated clinical conditions such as organ failures, mortalities of the control groups, and differences in therapy between control and protocol arms? Is there a single optimal hemodynamic goal for all critically ill patients, or does this depend on age, severity of illness, physiologic reserve capacities, organ failures, and other co-morbid conditions?

The present study reviewed 21 randomized clinical trials described in 20 articles to evaluate various influences that may contribute to outcome. Inclusion criteria of this meta-analysis were randomized clinical trials of high-risk elective surgery, trauma, and acute medical sepsis. We evaluated the definition of optimal therapy, time of optimization, age, types of illness, and severity of illness. The latter, for example, was defined by the mortality rate of the control group. The differences in mortality rates in the control and protocol groups were the main criteria for evaluation of therapeutic goals in various clinical circumstances, including acute illness, high-risk surgery, or trauma vs. chronic medical illnesses, the time that the therapeutic goals were implemented during the course of acute illness, and the presence or absence of organ failures. Hemodynamic values were used to evaluate the extent or aggressiveness of therapy to achieve the targeted protocol goals compared with the same therapy given to achieve the normal control goals. The differences between control and protocol groups were principally CI and $\dot{D}o_2$ because these have been reported to differentiate early survivor from nonsurvivor patterns (6–8, 27).

METHODS

A search strategy was developed with the assistance of a research librarian. The database for references was MEDLINE, and the search was limited to include only references in English. The study design included randomized clinical trials of supranormal CI, pulmonary arterial occlusion pressure of <18 mm Hg, and $\dot{D}o_2$ and oxygen consumption indexed as therapeutic goals. The search terms that identified the most acceptable references were supranormal oxygen, resuscitation endpoints, cardiac output, oxygen delivery, oxygen consumption, survival and nonsurvival, and hemodynamics. The search identified 72 references; 52 of these were rejected after screening because of irrelevant interventions, patient populations, or outcome definitions.

Three inclusion criteria were used to define the patient populations, therapeutic goals, and interventions. These were: 1) critically ill patients after high-risk elective surgery, severe trauma, and septic shock; 2) therapeutic goals for resuscitation and subsequent management were either normal hemodynamic values or supranormal values observed in previous series of survivors and specified as a CI of >4.5 $L \cdot min^{-1} \cdot m^{-2}$, pulmonary arterial occlusion pressure of <18 mm Hg, $\dot{D}o_2$ of >600 $mL \cdot min^{-1} \cdot m^{-2}$, or oxygen consumption of >170 $mL \cdot min^{-1} \cdot m^{-2}$ (6–8, 18, 27); and 3) initial intervention was fluid therapy, and if hemodynamic targets were not achieved, inotropes were then added. Twenty references, with 21 studies, were reviewed and accepted for meta-analysis (Table 1). Experimental designs of the studies revealed at least four different categories of patients or therapeutic regimens. These included normal vs. supranormal therapeutic goals, early vs. late administration of therapy to achieve the stated goals, and differences in severity of illness determined by the control group mortality. Late was arbitrarily defined as >12 hrs after surgery, 24 hrs after injury, or after occurrence of an organ failure.

We used the following characteristics to evaluate the quality of these randomized studies. An optimum randomization process may have included a third party, a table of random numbers, or a computer-generated list to assign impaneled subjects to either the treatment or control arm. The assignment to a treatment arm was "concealed" if a third party or sealed envelopes were employed to assign subjects to the treatment or control arm. The process was "blinded" when both the investigators and the subjects were not aware of the patients' assignment to the control or protocol arm. Finally, the withdrawal or dropout analysis was adequate if the investigators identified the number of subjects excluded, provided an explanation for exclusion, and provided the number remaining for evaluation. If the authors did not describe these processes, it was assumed that they did not employ the preferred method, and the study design was not considered optimal. The minimum criterion for inclusion was proper randomization. If the processes for concealment, blinding, or withdrawal or dropout were not described or verified by direct communication, these design components were scored as "not clear."

All studies reviewed were randomized. There were 15 studies (4–7, 9–11, 13, 15, 17, 19, 20, 29–32) on high-risk elective surgical patients, five of these included medical patients, and two of these studies also included trauma patients. Four studied only trauma patients (14, 16, 25, 29), and two studied septic (medical) patients (12, 25). Two studies were blinded to the investigators in terms of the fluid management; the other studies were not blinded. Table 1 lists the characteristics for each study.

The general variance-based method was used to calculate the summary statistic for the meta-analysis (33). The effect size calculated was the rate difference between the protocol group and the control group. The summary statistic was the rate difference between the groups. This method is based on the fixed-effects model. A significant p value was <.05.

RESULTS

The results are expressed as the mortality rate difference and confidence limits, which are twice the SD. The mortality rate differences between control and protocol groups in the series as a whole varied from −0.35 to 0.2. The average mortality rate difference for all 21 studies was $-0.05 + 0.02$, indicating statistically significant improvement with the protocol groups for the series as a whole ($p <$ .05). Table 1 lists the studies compiled from the literature in which either normal values or the optimal therapy, defined as CI > 4.5 $L \cdot min^{-1} \cdot m^{-2}$ and $\dot{D}o_2 >$ 600 $mL \cdot min^{-1} \cdot m^{-2}$, was given to the protocol groups, and their mortalities were compared with their corresponding control groups given standard therapy. In seven studies, the values of the protocol groups reached the proscribed therapeutic goals in the allotted time frame.

Figure 1 illustrates the values of the 14 randomized studies whose control group mortalities were >20%. Seven early studies whose optimal therapy was completed before organ failure occurred had marked and significant overall reduction in the mortality rate of $-0.23 + 0.07$ ($p <$ .05). Of the seven late studies of patients who had organ failure before initiation of the studies, the overall mortality rate difference was $0.01 + 0.06$, indicating no significant improvement with therapy. In these seven studies, only the study of Yu et al. (11) of patients aged

Table 1. Evidence for 21 studies and 20 articles

Author (Reference) No.; Yr	Diagnostic Group (%)	Study Design[a] Average Age per Group, yrs	Purpose
I. Control groups with mortality rates >20%			
A. Goals to supranormal values after organ failure			
Alia et al. (24) n = 63; 1999	Surgical Medical	Y, Y, N 66; 72	To evaluate the effects of increased oxygen delivery on morbidity and mortality in patients with severe sepsis or septic shock
Yu et al. (10) n = 66; 1998	Surgical	Y, N, N 63; 63	To determine whether treatment to a $\dot{D}o_2$ of ≥600 mL·min·m^{-2} in patients unable to mount this $\dot{D}o_2$ response affects mortality
Yu et al. (10) n = 39; 1998	Surgical	Y, N, N 81; 83	To determine whether treatment to a $\dot{D}o_2$ of ≥600 mL·min·m^{-2} in patients unable to mount this $\dot{D}o_2$ response affects mortality
Gattinoni et al. (5) n = 762; 1995	Trauma Surgical Medical	Y, Y, N 60; 62; 61	To determine whether targeting hemodynamic treatment to achieve either supranormal values for the cardiac index or normal values for $S\bar{v}o_2$ would improve morbidity and mortality among critically ill patients
Hayes et al. (4) n = 109; 1994	Surgical (40) Medical (60)	Y, N, N 62; 64	To examine the effects of treatment intended to increase the cardiac index and oxygen delivery and consumption to the previously reported median values in survivors
Yu et al. (9) n = 72; 1993	Surgical (85) Medical (15)	Y, N, N 58; 57	To evaluate the effect of increased $\dot{D}o_2I$ to >600 mL·min·m^{-2} on the morbidity and mortality of patients with sepsis, septic shock, hypovolemic shock, and ARDS
B. Goals to supranormal values before organ failure			
Lobo et al. (20) n = 42; 2000	Surgical	Y, Y, N 63; 63	To evaluate the effect of therapy aimed at achieving maximized oxygen transport values during the operation and in the first 24-hr postoperative period on outcome in a more homogeneous set of high-risk surgical patients
Wilson et al. (19) n = 138; 1999	Surgical	Y, N, N 72; 70; 72	To determine whether preoperative optimization of oxygen delivery improves outcome after major elective surgery
Bishop et al. (16) n = 115; 1995	Trauma	Y, N, N 34; 29	To test prospectively supranormal values of CI, $\dot{D}o_2I$, $\dot{V}o_2$ as resuscitation goals to improve outcome
Boyd et al. (7) n = 107; 1993	Surgical	Y, Y, N 69; 73	To assess the effect of a deliberate perioperative increase in oxygen delivery on mortality and morbidity in patients who are at high risk of both after surgery
Tuchschmidt et al. (12) n = 70; 1992	Medical	Y, N, N 49; 53	To prospectively evaluate the therapeutic effect of augmenting cardiac output and therefore oxygen delivery on mortality in patients with septic shock
Shoemaker et al. (6) n = 70; 1988	Trauma (13) Surgical (87)	Y, Y, N 56; 53; 55	To test the hypothesis that the physiologic pattern empirically defined by the survivors may be the appropriate therapeutic goals for high-risk critically ill postoperative patients
Schultz et al. (14) n = 70; 1985	Trauma	Y, N, N, Y 78; 67	To determine whether treatment of preoperative and postoperative hemodynamic variables improves outcome after hip surgery
II. Control groups with mortality rates <15%			
A. Goals to supranormal values			
Velmahos et al. (29) n = 75; 2000	Trauma	Y, Y, N 62; 64	To evaluate the effect of early optimization in the survival of severely injured patients
Ueno et al. (15) n = 34; 1998	Hepatectomy for cirrhosis	Y, NC, N 61; 58	To evaluate the response to therapy aimed at achieving supranormal cardiac and oxygen transport variables in patients with cirrhosis
Durham et al. (25) n = 60; 1996	Trauma (93) Medical (7)	Y, Y, N 35; 35	To test the hypothesis that the use of supranormal values for $\dot{V}o_2I/\dot{D}o_2$ as end points for resuscitation results in improved outcomes
B. Goals to normal values			
Valentine et al. (31) n = 126; 1998	Aortic surgery	Y, Y, N 64; 63	To evaluate the routine use of PAC in patients who undergo aortic surgery
Bender et al. (32) n = 104; 1997	Aortic and limb revascularization	Y, N, N ND; ND	To determine whether the preoperative placement of a pulmonary artery catheter with optimization of hemodynamics results in improved outcomes
Ziegler et al. (30) n = 72; 1997	Aortic and limb salvage surgery	Y, N, N 67; 64	To evaluate the effect of preoperative optimization of hemodynamic variables on outcome in patients undergoing aortic reconstruction of limb salvage procedures
Mythen and Webb (18) n = 60; 1995	CABG or valve surgery	Y, Y, N 64; 63	To test the hypothesis that perioperative plasma volume expansion would preserve gut mucosal perfusion during elective cardiac surgery
Berlauk et al. (13) n = 89; 1991	Peripheral vascular surgery	Y, N, N 68; 62; 66	To answer the question in patients with peripheral vascular surgery, does the use of a PA catheter to optimize LVF improve outcome?

OF, organ failure; PAOP, pulmonary artery occlusion pressure; CI, cardiac index; $\dot{D}o_2$, delivery of oxygen index; ICU, intensive care unit; $S\bar{v}o_2$, mixed venous oxygen saturation; ARDS, acute respiratory distress syndrome; PAC, pulmonary artery catheters; $\dot{V}o_2$, oxygen consumption index; CABG, coronary artery bypass graft; CVP, central venous pressure; pHi, gastric intramucosal pH; PA, pulmonary artery; LVF, left ventricular function.

[a]Study design: randomized, concealed, and blinded were described as Y = yes, N = no, NC = not clear, and ND = no data.

Table 1. (Continued)

Optimize 1) Before, 2) During, 3) After Surgery or After OF	Targets (Protocol Group)	Outcomes PAOP 12–18 CI > 4.5 $\dot{D}o_2I$ > 600	Mortality Protocol/ Control (%)
Admission to ICU with Diagnosis of Sepsis and OF	PAOP 14–16 $\dot{D}o_2I$ > 600	Yes, No, No	23/31 (74) 21/32 (66)
No, No, OF	PAOP 15–18 (both groups) $\dot{D}o_2I$ > 600	ND, ND, No	9/43 (21) 12/23 (52)
No, No, OF	PAOP 15–18 (both groups) $\dot{D}o_2I$ > 600	ND, ND, No	12/21 (57) 11/18 (61)
No, No, OF	PAOP < 18 (all groups) CI > 4.5, $\dot{D}o_2I$ > 600	Yes, Yes, Yes	123/253 (49) 21/252 (48)
No, No, OF	PAOP NS (both groups) CI > 4.5, $\dot{D}o_2I$ > 600	ND, Yes, Yes	25/50 (50) 15/50 (30)
No, No, OF	PAOP 15–18 (both groups) $\dot{D}o_2I$ > 600	ND, ND, Yes	12/35 (34) 11/32 (34)
Yes, Yes, Yes	PAOP 12–16 CI > 4.5, $\dot{D}o_2I$ > 600	No, No, Yes	3/19 (16) 6/18 (33)
Yes, Yes, Yes	PAOP > 12 (PAC group 1) $\dot{D}o_2I$ > 60	Yes, No, No	2/46 (4) 17/46 (37)
No, No, Yes	PAOP < 18 (group 1) CI > 4.5, $\dot{D}o_2I$ > 670	ND, Yes, Yes	9/50 (18) 24/65 (37)
Yes, Yes, Yes	PAOP ≥ 12–14 (both groups) CI > ↑ to plateau, $\dot{D}o_2I$ > 600	Yes, No, Yes	3/53 (6) 12/54 (22)
Admission to ICU within 4 hrs of Diagnosis of Sepsis	PAOP > 15 (both groups) CI > 6	Yes, Yes, Yes	13/26 (50) 18/25 (72)
No, No, Yes	PAOP < 18 CI > 4.5, $\dot{D}o_2I$ > 600	No, No, Yes	1/28 (4) 18/60 (38)
Yes, NC, Yes	PAOP ND CI 3–3.5	No, No, ND	1/35 (3) 10/35 (29)
Yes, Yes, Yes	PAOP ND CI > 4.5, $\dot{D}o_2I$ > 600	ND, ND, Yes	6/40 (15) 4/35 (11)
Yes, No, Yes	PAOP 9–18 CI > 4.5, $\dot{D}o_2I$ > 600	Yes, Yes, Yes	0/16 (0) 2/18 (11)
No, No, OF	PAOP < 18 (both groups) $\dot{D}o_2I$ > 600	Yes, Yes, Yes	3/27 (11) 3/31 (10)
Yes, Yes, Yes	PAOP 8–15 CI > 2.8	ND	3/60 (5) 1/60 (2)
Yes, Yes, Yes	PAOP 8–14 CI ≥ 2.8	ND	1/51 (2) 1/53 (2)
Yes, No, Yes	PAOP ≥ 12	ND	3/32 (9) 2/40 (5)
No, Yes, Yes	CVP; pHi	ND	0/30 (0) 1/30 (3)
Yes, No, No	PAOP 8–15 CI > 2.8	ND	1/68 (1.5) 2/21 (10)

50–75 yrs had improved outcome with optimized therapeutic goals.

Figure 1 also illustrates mortality rate differences in three groups of studies with control group mortalities of <15% or normal values for therapeutic goals. The first group consisted of two studies with control group mortalities of 10% and 11%. One study (26) consisted of patients with organ failures before therapy, and the second study (27), which excluded patients who died within 24 hrs of admission, had a control group mortality of 11% and a protocol mortality of 15%, but there was no difference in $\dot{D}o_2$ between the control and protocol groups. The latter suggested that the treatment of control patients were similar to that of the protocol patients. If there were no differences in therapy, no outcome differences should be expected, and none were found. Neither of these studies showed significant differences in the mortality rates between the control and protocol groups; the combined rate difference of these two studies was 0.03 ± 0.11 ($p > .05$). The fourth group in Figure 1 studied partial hepatectomy in cirrhotic patients who had an 11% control group mortality but a protocol group mortality of only 0% (16). The rate difference of -0.11 did not reach statistical significance, probably because the sample size was only 34 patients. The last group consisted of five studies that used normal values as goals and had control mortalities of <11%. Their subtotal rate difference was -0.01 ± 0.03 ($p > .05$). The three groups (ten studies) had control group mortalities that were <15%, with a mean of 7.1%, suggesting that these patients were not as severely ill as the first two study groups whose mean control mortality was 42.1% (Fig. 1 and Table 1). In high mortality series, fewer patients are needed to show improved outcome with different therapeutic goals.

DISCUSSION

Hemodynamic bedside monitoring by PACs has been considered by many as a standard for circulatory evaluation of critically ill patients, but its usefulness has recently been seriously questioned (1–5, 22–25), particularly in the late stages of illness after onset of multiple organ failures (23–25). The present review showed significantly improved outcome in randomized studies when PAC goal-directed therapy was administered early or prophylactically in patients who

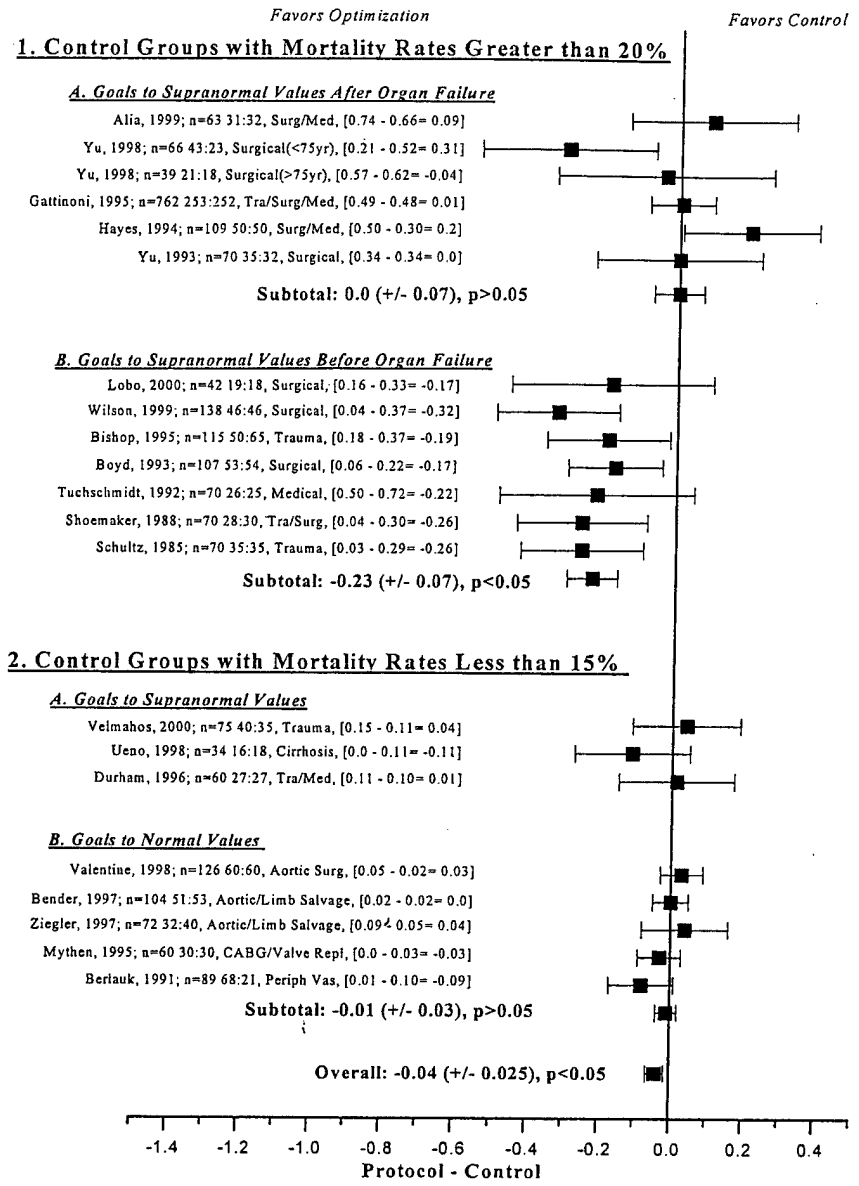

Figure 1. Mortality differences between protocol and control groups with control group mortality of >20% (*upper section*) and <15% (*lower section*). Therapeutic goals are specified as supranormal or normal hemodynamic values. Note for each study the first author's name, date of publication, number or randomized subjects, number of subjects evaluated as protocol vs. control groups, populations, mortality of the protocol patients minus control mortality, and the difference between protocol and control mortalities. *Surg*, surgery; *Med*, medical; *Tra*, trauma.

were optimized preoperatively and maintained in the intraoperative and immediate postoperative period.

Early studies using invasive ICU monitoring in randomized trials reported that increased CI and $\dot{D}o_2$ to values characteristic of survivors of high-risk surgery in the immediate postoperative period improved outcome (6). At the initial stage in the development of this concept, it was realized that the survivors of acute critical illnesses had a wide range of higher-than-normal hemodynamic values (6, 8, 10, 18, 19, 31, 34). Because it is not possible to test a range of values, the mean or median values were arbitrarily chosen as cutoff points, not to establish a set of optimal values but to test the hypothesis that critically ill patients have high metabolic rates and therefore require greater than normal hemodynamics and oxygen transport to sustain the increased body metabolism after trauma, surgery, or sepsis. Hemodynamic goals of surviving patients were proposed as a first approximation to optimal goals for the immediate postoperative period of high-risk surgical patients. These proposed optimal therapeutic goals were not intended to be generally applied to all patients at all times because metabolic requirements are affected by age, sepsis, blood loss, preexisting cardiac and pulmonary insufficiency, and other co-morbid conditions (10, 35). Ultimately, optimal goals may be calculated for each individual patient on the basis of his or her diagnosis, co-morbid conditions, past hemodynamic deficits, and temporal stage. This is presently approached by using discriminate analysis (27) and stochastic control programs (28).

In the initial randomized trial of supranormal hemodynamic values, the mortality was decreased, but more importantly, the prevalence of organ failures was reduced from 31 cases in the control group to 1 case in the protocol group (6). Moreover, in a series of postoperative patients invasively monitored before the diagnosis of ARDS, the nonsurvivors' CI values were in the normal range; the survivors who developed ARDS had CI values that were significantly elevated (4 $L \cdot min^{-1} \cdot m^{-2}$) but less than the values of survivors who did not develop ARDS (4.5 $L \cdot min^{-1} \cdot m^{-2}$) (34, 38, 39). Before the onset of ARDS, the mean pulmonary artery occlusion pressures were within acceptable limits for critically ill postoperative patients and none had a pulmonary arterial occlusion pressure of >18 mm Hg. Bishop et al. (16, 39) reported that supranormal goals within 24 hrs of the injury reduced the prevalence of ARDS and other organ failures after severe trauma; they reduced mortality from 39% to 18% ($p < .05$) and reduced prevalence of organ failure from 105 in 65 control patients (1.62 ± 0.28 organ failures per patient) to 37 in 50 protocol patients (0.74 ± 0.28 organ failures per patient) ($p < .05$). Less than optimal values in the early stage may lead to inadequate total blood flow and uneven microcirculatory blood flow from uneven vasoconstriction of the adrenomedullary stress response (8, 34, 38–42. Local hypoxia and acidosis of the capillary endothelium from uneven capillary blood flow is known to stimulate the systemic inflammatory response system and lead to organ failure (41, 42).

The definition of early as opposed to late studies is necessarily arbitrary. Cutoff points for the patient to reach the designated goals were: the first 12 hrs postoperatively in elective surgery, 24 hrs after injury in trauma patients, and before the onset of an organ failure. When

sepsis was the primary diagnosis, we accepted the definition of "early septic shock" proposed by Tuchschmidt et al. (12), which was within 4 hrs of the time of diagnosis. However, when sepsis was a complication of elective high-risk surgery, as in the studies of Yu et al. (9–11), it was arbitrarily designated as an organ failure or dysfunction and therefore classified as late. Of these three published articles (9–11), the 1998 publication that was a continuation of their earlier studies seems to include 47 of the 50 subjects that were evaluated in the 1995 article. Therefore, to avoid redundancy, the 1995 study was not included in this meta-analysis. In the 1993 study, Yu et al. (9) demonstrated that when both the protocol and control groups were aggressively hydrated to a pulmonary artery occlusion pressure of 15–18 mm Hg, the difference in the mortality rates was insignificant. In the interim study (1995), Yu et al. (11) observed that when the subjects in both the protocol and control groups who generated a $\dot{D}o_2$ of $\geq$600 mL·min^{-1}·m^{-2} after fluid resuscitation were excluded from the study, the mortality rate of the remaining protocol subjects was significantly less than the remaining control subjects. This difference was associated with the administration of inotropes and vasoactive drugs given to the protocol group to achieve a $\dot{D}o_2$ of $\geq$600 mL·min^{-1}·m^{-2}. In the 1998 study, Yu et al. (10) evaluated the larger series of patients randomized to protocol and control groups, and stratified the groups according to age: $\leq$75 yrs (50–75 yrs of age) and >75 yrs. All subjects who achieved a $\dot{D}o_2$ of $\geq$600 mL·min^{-1}·m^{-2} after fluid resuscitation were excluded. The mortality rate of the protocol group of the subjects aged $\leq$75 yrs was significantly less than the control group. However, the mortality rate in the protocol and control groups of subjects aged >75 yrs was not different ($p > .05$). These findings suggest that the subjects aged >75 yrs did not effectively respond, in terms of outcome, to aggressive vasoactive drugs or inotropes.

In the study of Wilson et al. (19), patients undergoing major elective surgery were randomized into three groups; two groups of 42 patients received invasively monitored fluid and either adrenaline or dopexamine to increase $\dot{D}o_2$, whereas the third group of 42 patients received routine postoperative care and served as the control. Only 3 of 92 patients (3%) in the optimized groups died, whereas 8 of 46 patients (17%) in the control group died ($p < .007$). The length of stay of the dopexamine group was significantly reduced compared with both the adrenaline group ($p = .02$) and the control group ($p = .009$). The authors concluded that because of the low doses of inotropes, fluid optimization was a major contributor to improved $\dot{D}o_2$ and improved outcome in their patients (19).

Three randomized trials not included in this meta-analysis deserve mention. In a study by Takala et al. (36) of postoperative patients with 13% control mortality that used relatively normal values as goals, patients were initially brought into the normal hemodynamic range, and then two dosage levels of dopexamine were tested in randomized trials, but the outcome was not significantly improved. Sinclair et al. (37) studied length of hospital stay in patients with proximal femoral fractures after optimizing stroke volume with repeated colloid fluid challenges measured by esophageal Doppler ultrasonography. They demonstrated significantly reduced hospital stay, but there was insignificant reduction in mortality because of only two deaths in the control group and one death in the protocol group. Polenen et al. (43) used mixed venous oxygen saturation and lactate levels as criteria for adequacy of resuscitation immediately postoperatively in 403 cardiac surgical patients. The median hospital stay was shorter in the protocol group (6 vs.7 days, $p < .05$), and morbidity was significantly less at the time of hospital discharge (1.1% vs. 6.1%, $p < .01$), but mortality was very low and not significantly affected by the study.

Low control mortalities suggest that the patients were not very ill and therefore may not respond as clearly to increased hemodynamics and, at the same time, may require much larger numbers of patients to show statistical significance. In the studies of Mythen et al. (17) and Ueno et al. (15), the protocol patients given therapy to achieve optimal goals had 0% mortalities, but because of the small number of patients, statistical significance was not achieved. Moreover, in the study of Berlauk et al. (13), the mortality was reduced from 9.5% in the control group to 1.5% in the optimized group, which was not significant; however, the number of complications were significantly reduced.

Similarly, if the control and protocol patients were treated in a similar manner, no differences in outcome should be expected. In the study of Velmahos et al. (29), the difference in $\dot{D}o_2$ between control and protocol patients was not statistically significant because the treatment of control and protocol patients were not different, and therefore, the mortality was, not unexpectedly, not different.

We conclude that increased CI and $\dot{D}o_2$ with pulmonary arterial occlusion pressure of <18 mm Hg should be considered as goals of therapy. When implemented early and aggressively, this reduces mortality and the prevalence of organ failures in acute postoperative and posttrauma conditions. Goal-directed therapy to achieve optimal goals is ineffective in the late stages after onset of organ failure because no amount of extra oxygen will restore irreversible oxygen debts, failed organs, or dead cells. In the late stage of acute illness after organ failure has occurred, aggressive therapy directed toward achieving the survivors' supranormal values is futile. When oxygen debt is no longer reversible, increased oxygen transport is not effective. Moreover, it is difficult to demonstrate significant changes after optimization when there are no significant differences between therapy given to the control and protocol groups. That is, there must be significant differences in the type of therapy or the amount of therapy given to expect significant outcome improvement. Furthermore, outcome differences may be extremely difficult to demonstrate when the patient population is not very ill, as indicated by control mortalities of <15%. Finally, no effect should be expected in chronic medical conditions in which physiologic compensations have already had their maximum effect.

ACKNOWLEDGMENTS

We thank Anahit Chirishyan, Dawn Hou, Jason Madamba, Hyo Park, Krystin Huynh, Don Lee, and Janet Nelson for contributions to this project.

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NONINVASIVE HEMODYNAMIC MONITORING OF EMERGENCY PATIENTS FOR OUTCOME PREDICTION BY A STOCHASTIC CONTROL PROGRAM:

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Supported in part by NIH grants RR-11526, and GM-65619, and DOD BAA99-1

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ABSTRACT

Background: Time is an important factor in the resuscitation and management of critically ill emergency patients. Early noninvasive monitoring with an outcome predictor to identify and correct cardiac, pulmonary, and tissue perfusion deficiencies may be a useful alternative approach to invasive pulmonary artery thermodilution catheters.

Objectives: The first aim was to present a stochastic control model, developed for noninvasive monitoring to predict survival outcome in acute life-threatening critical illness. The second aim was to evaluate the reliability of this stochastic model to track progress throughout the course of acute illnesses. The third aim was to explore the feasibility of a decision support program to evaluate the relative effectiveness of various therapies given to patients in similar clinical-hemodynamic states.

Methods: A stochastic control and decision support program was applied in 177 monitored severely injured patients to monitor the survival probabilities at the initial resuscitation on admission to the emergency department (ED) and at subsequent interval during the hospital stay in a county teaching hospital. The program provides a continuous readout of the survival probabilities based on the patient's diagnostic category, covariates, and noninvasive hemodynamic levels including cardiac output, pulse oximetry, transcutaneous oxygen and carbon dioxide tensions, and mean arterial blood pressure. The percentage of correct predictions was used to measure the reliability of the model. A therapeutic decision support program was used to measure the relative effectiveness of various alternative therapies.

Results: The cardiac index, mean arterial pressure, arterial saturation, transcutaneous oxygen and carbon dioxide tensions were higher in survivors than in nonsurvivors: a) in the initial resuscitation, b) before and after the probability nadir, and c) during emergency surgery. The calculated Survival Probability (SP) of those who survived averaged 75% or higher throughout first 24 hour observation period. It was below 60% for nonsurvivors during this period. Misclassifications, excluding brain dead patients and those who died suddenly in the OR of uncontrolled hemorrhage, were 16/165 or 9.7%. However, the APACHE scores of survivors and nonsurvivors were not significantly different during the first four ICU days in these patients. Noninvasive systems provided continuously monitored real time displays of data for the earliest recognition of circulatory dysfunction from the emergency department (ED) to the operating room (OR), and to the intensive care unit (ICU). A decision support system measured the relative effectiveness of various therapeutic modalities based on the responses of patients with very similar states.

Conclusions: The mathematical model satisfactorily measured survival probabilities and severity of illness in acute emergencies based on noninvasively monitored values of blood flow, blood pressure, oxygen transport in blood, and tissue oxygenation. Stochastic analysis of hemodynamic patterns provided a feasible therapeutic decision support system.

INTRODUCTION

Invasive pulmonary artery (PA) thermodilution (Swan-Ganz) catheters provide the maximum circulatory data at the bedside, but require intensive care unit (ICU) conditions. However, when invasive monitoring is started late in the course of illness after onset of organ failures, goal-oriented studies showed no advantage of the PA catheter (1-7). Moreover, reviews of trauma patients with shock revealed that delays in correcting circulatory deficiencies have led to organ failures and death (8-15). Since time is an important factor in resuscitation and management of critically ill emergency patients, early noninvasive monitoring with an outcome predictor may be a useful approach to identify and correct hemodynamic deficiencies as early as possible (9-23). Previous studies have demonstrated early outcome prediction with discriminant function (21). Similar to early diagnosis and therapy for cancer, early diagnosis and therapy for circulatory problems is more cost-effective than therapy delayed until late stages (8-11,15,18,21).

Recently, Bayard et al (24) developed a mathematical model that used a large database of noninvasively monitored hemodynamic variables, to provide outcome prediction as well as therapeutic decision support for acute critically ill patients. The aims of the present study were to evaluate the accuracy of this mathematic model to predict survival outcome, to explore the possible use of this program to track progress as an objective measure of severity of illness, and to develop a decision support system based on responses to various therapies given to patients in similar states. The present preliminary report focuses its application on acute severely injured emergency patients under worst-case conditions. Acute injury also was selected for study because the onset of illness occurred shortly before admission and the course of circulatory events could be monitored from the time of admission (8,13,21). Moreover, it is likely that mistakes occur more frequently during periods of acute life-threatening crises.

MATERIALS AND METHODS

Clinical Series

We studied 177 severely injured patients by noninvasive hemodynamic monitoring that reflect cardiac, respiratory, and tissue perfusion functions together with a new stochastic analysis and control program based on clinical and hemodynamic values. In general, emergency patients with major blunt or penetrating truncal trauma or significant risk of mortality or morbidity were selected for monitoring shortly after emergency department (ED) admission. Monitoring was usually begun in the ED and the patients were followed to the radiology suite, when these studies were indicated, to the operating room (OR), and then to the ICU. The protocol was approved by the Institution's Review Board.

Noninvasive Hemodynamic Monitoring

Noninvasive hemodynamic monitoring is continuous real time display of cardiac, pulmonary, and tissue perfusion functions in critically ill patients. The data was down-loaded at 30-second intervals, averaged over 5-minute periods, and then entered into the database. During the first 24-hour period, the survival probabilities were averaged over 2-hour periods.

Cardiac Output

An improved thoracic bioelectric impedance device (Yantagh Inc., Bristol, PA) was applied shortly after arrival in the ED. The noninvasive disposable prewired hydrogen electrodes were positioned on the skin and three EKG leads were placed across the precordium and left shoulder (26,27). A 100 kHz, 4 mA alternating current was passed through the patient's thorax by the outer pairs of electrodes and the voltage was sensed by the inner pairs of electrodes which captured the baseline impedance (Zo), the first derivative of the impedance waveform (dZ/dt), and the EKG. The EKG and bioimpedance signals were filtered with an all-integer-coefficient technology to decrease computations and signal processing time. The signal processing algorithm used a time-frequency distribution (modified Wigner Distribution) analysis that increased signal-to-noise ratios (26,27).

Previous studies have documented satisfactory correlations between thermodilution and bioimpedance cardiac output values for trauma patients in the ED, OR, and ICU conditions (25). Pulmonary artery catheters were placed when indicated by clinical criteria.

Limitations of the impedance method include faulty electrode placement, motion artifacts, restlessness, shivering, pulmonary edema, pleural effusion, valvular heart disease, dysrhythmias, and electrical leaks from other instruments using the same circuit. These are usually apparent from inspection of the impedance waveform and by previously described criteria: baseline impedance, Zo, >15ohms and the peak impedance signal, dZ/dt_{max}, >0.3ohms (21).

Pulse Oximetry

Routine pulse oximetry (Nellcor, Pleasanton, Ca) was used to assess continuously arterial oxygen saturation ($SapO_2$). Values were observed and recorded at the time of the cardiac index measurements. Appreciable or sudden changes in these values were also noted and confirmed by in vitro arterial oxygen saturation obtained by the standard blood gas analysis (25).

Transcutaneous Oxygen Tension

Conventional transcutaneous oxygen tension measurements were continuously monitored throughout the observation period. This technology uses the same Clark polarographic oxygen electrode routinely employed in standard blood gas measurements (28-31). The oxygen tensions were measured in a representative area of the skin surface heated to 44^0C to increase diffusion of oxygen across the stratum corneum and to avoid vasoconstriction in the local area of the skin being measured (28,29). Previous studies demonstrated the capacity of transcutaneous oxygen tensions to reflect tissue oxygen tension (25,28-32). Transcutaneous oxygen tension ($PtcO_2$) has been shown to reflect the delivery of oxygen to the local area of skin; it also paralleled the mixed venous oxygen tension except under terminal conditions where peripheral shunting led to high mixed venous hemoglobin saturation (SvO_2) values (28). While oxygen tension of a segment of the skin does not reflect the state of oxygenation of all tissues and organs, it has the advantage of being the most sensitive early warning tissue of the adrenomedullary stress response; vasoconstriction of the skin is an early stress response of hypovolemia and other shock syndromes (28-32). Transcutaneous oxygen tension was indexed to the FiO_2 to give a $PtcO_2/FiO_2$ ratio, because changes of the inspired oxygen produce marked $PtcO_2$ changes (21,29). Limitations of the transcutaneous methods are the thermal environment must be reasonably constant, marked changes in room temperature from drafts or open windows must be avoided; the electrode must be changed to a nearby thoracic or shoulder site and be re-calibrated at 4 to 6-hour intervals to avoid first degree skin burns.

Trauma Patient Database for Stochastic Analysis and Display Program

Database for acutely injured patients was developed to describe primary injuries, selected covariates hemodynamic patterns by invasive and noninvasive methods, and outcomes, including survival or death, organ failures, other complications, hospital days, and ICU days. Diagnostic categories included: truncal and nontruncal trauma, head injury, brain death. The database also included: age, gender, the presence of sepsis or systemic immune response system (SIRS), APACHE scores, Glasgow coma score, and injury severity score (ISS). Noninvasive monitoring was usually begun in the ED and the patient was followed to the OR, radiology department, and ICU. Invasive pulmonary artery catheterization (PAC) was instituted when clinically indicated after the patient arrived in the ICU. The time and place of monitoring, time of operations, times of ICU admission and discharge, time of hospital discharge or death, and other events were recorded in time elapsed after admission.

Calculation of Net Cumulative Deficit of Hemodynamic Variables

The area between the fluctuating monitored variables and either the normal values for blood pressure, $SapO_2$, and $PtcO_2/FiO_2$ or optimal value for cardiac index were calculated. This area was integrated over time to calculate the net cumulative amount of deficit or excess of each monitored variable for individual patient (21).

Stochastic Analysis and Control Program

The stochastic (probability) analysis and control program, system dynamics, and the calculation of the probability of survival were developed by Bayard et al (24) and are summarized in the Appendix. The program uses data from a homogeneous population, integrates a new patient's demographic characteristics, clinical diagnosis, and hemodynamic levels, and derives a survival probability for the patient in real time. The primary characteristics of this model include: a) the patient's course described by three-dimensional vectors, where baseline values are not required, b) the first derivative of the initial vector projects the patient's course if there are no inherent changes or external influences including "measurement noise", c) the second derivative tracks changes in the patient's course from either internal compensations, further deterioration, spontaneous improvement, or external influences such as changes in therapy as well as "process noise", and d) the integral sums up the total influences. The detailed formulation of the program and the derivation of the probability of survival are included in the Appendix.

Application to a Clinical Series

We tested this stochastic analysis and control program in a series of 177 severely injured patients whose hemodynamics were monitored noninvasively as early as admission to the ED. The patient population included the following diagnostic categories: truncal and nontruncal trauma, head injury, and brain death. Noninvasive monitoring was usually begun in the ED and the patient was followed to radiology department, operating room (OR), and ICU. Invasive pulmonary artery catheterization (PAC) was instituted when clinically indicated after the patient arrived in the ICU. The time and place of monitoring, time of operations, times of ICU admission and discharge or death, and other events were recorded relative to time elapsed after ED admission. In addition, the following data elements were included in the database: age, gender, presence of sepsis or activation of the systemic immune response system (SIRS), Glasgow coma score, injury severity score (ISS), primary injuries, hemodynamic patterns by invasive and noninvasive methods, organ failures, other complications, hospital days, ICU days, and hospital survival outcome. The protocol was approved by the Institution's Review Board.

Statistical Analyses

The survivors' and nonsurvivors' deficits of MAP, cardiac output, $SapO_2$, and transcutaneous O_2 were calculated for the periods of monitoring. For categorical variables, differences in proportions between survivors and nonsurvivors were tested using the Chi-square test or the two tailed Fischer's Exact test. For continous variables, the equality of the means between survivors and nonsurvivors was tested the two-sample t-test or the Wilkcoxon two-sample test. The effects of time (a repeated measure), outcome group, and their interaction on survival probability and on each hemodynamic parameter were analyzed by the mixed linear model using residual maximum likelihood with the unstructured covarience. The SAS statistical software (The SAS System, Release 8.2, SAS Institute Inc, Cary, NC) was used for all statistical computations.

RESULTS

Clinical Series

There were 151 males and 26 females. The mean age was 33.0 ± 15.5 years for all patients, 31.5 ± 13.7 years for survivors, and 36.7 ± 18.9 for nonsurvivors. Patients who were brain dead from severe neurological injury

were evaluated separately, because disruption of the autonomic centers usually produced hyperdynamic hemodynamic responses from unopposed peripheral vasodilatory mechanisms. There were 124 survivors, 53 nonsurvivors, of whom 10 were brain dead after severe head injury. The overall mortality was 30%. There were 151 males and 26 females. Sixty-four (52%) of the 124 survivors, and 22 (42%) of the 53 nonsurvivors were operated upon as part of their initial resuscitation. Another 25% were operated subsequent to their initia resuscitation. Table 1 lists the salient clinical features.

Temporal Patterns of Hemodynamic Values and Survival Probability from the Time of Admission

Figure 2 displays the hemodynamic data of surviving and nonsurviving emergency trauma patients during the first 24 hours after their ED admission. Mean values ± SEM are shown for survivors and nonsurvivor for cardiac index (CI), heart rate (HR), mean arterial pressure (MAP), arterial hemoglobin saturation ($SapO_2$) by pulse oximetry, transcutaneous oxygen tension indexed to the FiO_2 ($PtcO_2/FiO_2$) and the survival probability (SP) averaged over 2-hour intervals during the first 24 hour period. The mean SP of those who survived was >75% throughout the observation period. In nonsurvivors, it was <60% during this initial period.

Table 2 lists the mean values for both groups during the first 24 hours after admission. The CI, MAP, $SapO_2$, $PtcO_2/FiO_2$, and SP values of the survivors were significantly higher than the comparable values of those who died, while the HR was higher in the nonsurvivors. Initial studies showed that the differences between the measured SP values and the SP values predicted from the previous stage averaged 7 ± 6%, indicating satisfactory consistency and reproducibility.

Hemodynamic and Survival Probability Patterns Before and After the Survival Probability Nadir

Inspection of the data revealed 162 instances of abrupt hemodynamic deterioration that were identifiable by sudden reductions in the SP to 30.3% in nonsurvivors and 65.2% in survivors (Table 3). The CI, MAP, $SapO_2$, $PtcO_2/FiO_2$, and SP values patterns before and after the SP nadir of the survivors were significantly higher than the comparable nonsurvivors' values, while HR was higher in the nonsurvivors.

Comparison of Survival Probability with Actual Outcome

Of the 177 trauma patients, 124 survived, and 53 died; the mortality was 30%. Of the nonsurvivors, 2 died in the OR of uncontrollable blood loss, and 10 were brain dead, leaving 41 satisfactorily monitored nonsurvivors Of 41 patients predicted to die, 30/41 (73.2%) died. However, 11 patients predicted to survive, died of late medical complications in an average of 31 days (range 7-120 days). Complications included: ARDS (5 patients), cardiac failure (2), renal failure (1) and sepsis (3). Of those predicted to die, 5/124 (4%) survived after further resuscitation efforts. Of those who, in the initial resuscitation period, were predicted to survive 119/124 (96%) survived (Fig. 4 and Table 5). There were 16/165 (9.7%) misclassifications.

Comparison of APACHE scores with the Stochastic Survival Probabilities

The survivors' and nonsurvivors' APACHE scores of the present series were not significantly different during the first four days after admission, but were significantly different on the last day in the ICU (Fig. 5). However survival probabilities of survivors and nonsurvivors were significant different during the first 24 and 48 hours after admission (Table 2 and Fig. 5).

Hemodynamic Patterns in Severe Head Injury with Brain Death

Table 6 lists the mean CI, MAP, HR, $SapO_2$, $PtcCO_2$, and $PtcO_2/FiO_2$ values of nine patients with brain death from severe head injury. There were elevated values in each of these hemodynamic variables particularly CI MAP, HR, $SapO_2$, and $PtcO_2/FiO_2$ suggestive of augmented peripheral tissue perfusion from absent centra vasoconstriction.

Hemodynamic and Survival Probability Patterns Before, During, and After Emergency Surgery

Figure 3 and Table 4 show the temporal patterns of noninvasive hemodynamic variables and SP values of 64 survivors and 22 nonsurvivors before, during, and after emergency surgery as a part of their initial trauma resuscitation. The nonsurvivors' CI markedly decreased at the end of the operative procedure, while MAP values were approximately maintained intra-operatively. The survivors' CI, $SapO_2$, $PtcO_2/FiO_2$, and SP values were greater than the comparable values of those who died, while the HR was higher in the nonsurvivors.

Stochastic Control as a Decision Support System

The stochastic analysis of dynamic patterns also may be used for decision-making with a feedback control that, on the average, was shown to occur in similar patients, i.e., nearest neighbors, recorded in the database. Figures 6 and 7 are illustrative examples of nearest neighbors' responses to various therapeutic interventions in terms of their likely survival outcome.

DISCUSSION

A major assumption in the present approach is that circulatory deficiencies that ultimately lead to shock, organ failure, and death can be identified early by noninvasive monitoring and that the probability of survival may be predicted by stochastic analysis of dynamic patterns. The probability of survival is roughly equivalent to severity of illness. That is, the patient with a 90% likelihood of death is severely ill, while the patient with an estimated survival outcome of 90% may not be very ill. The accuracy and reliability of this approach depends on the size and comparability of the database needed to provide an adequate group of nearest neighbors.

The proposed mathematical representation of the circulation defines the patient's state by specific diagnostic categories, covariates, hemodynamic variables, their derivatives, and their integrals in a multi-dimension grid. Since the database contains over 9000 time-lines, each of which may represent a patient's state, there are many choices available for selection of the nearest neighbors. The average difference between a given patient's variables and the nearest neighbors' variable was <0.3 of the standard deviation. This suggests that we usually did not have to go far from the patient's state to find adequate numbers of nearest neighbors.

This approach was tested in the extenuating and sometimes chaotic circumstances of severely traumatized emergency patients in a large inner city public hospital. Under these circumstances, the SP was found to closely track changes at each point during the initial observation period and the SP correctly predicted outcome in over 90% of the patients during resuscitation monitoring within 24 hours of injury.

Early diagnosis and physiologic assessment is essential, because this allows therapy to be initiated sooner in the hope that earlier therapy may improve outcome in emergencies where time is crucial (8-15). Moreover, the stochastic analysis and control program provided an independent mathematical tool to evaluate therapeutic responses objectively.

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APPENDIX

The Stochastic Analysis Program based on a Trauma Database

Bayard et al (24) developed a stochastic analysis and control program to determine individual patients' survival probabilities (SP), from a database of patients with similar clinical-hemodynamic "states," which are defined in terms of the primary diagnosis, covariates, and hemodynamic variables. By "similar" is meant a group of patients, referred to as "nearest neighbors," with the same diagnosis, who share similar covariates, and have similar hemodynamic patterns to the patient under study. Mathematically, the stochastic analysis is defined as policy iteration with respect to conventional therapeutic policy used for each patient as the database was developed. It is motivated by methods of machine learning (33,34), and methods of dynamic programming for stochastic control (35,36).

The Stochastic Analysis and Therapeutic Decision Support Program

The therapeutic decision support program utilizes the database of therapeutic responses to evaluate the relative effectiveness of various therapies by the responses of each therapy in the patient's nearest neighbors (24). Figure 8 diagrams the stochastic analysis and control program synthesized from a database of therapeutic responses.

The state vector, *x(t)* at time *t* is described in terms of the various hemodynamic measurements, their derivatives, and their integrals. Assume that there are L different types of measurements taken on a given patient (e.g. cardiac index, blood pressure, pulse oximetry, and transcutaneous O_2 and CO_2 tensions) Specifically, for each measurement type, denoted as y_l, define the state vector as a concatenation of the value y_l itself, its first and second derivatives y_l', y_l'', and its first integral $\int y_l dt$, as follows:

$$x(t_k) = \left[y_1(t_k), y_1'(t_k), y_1''(t_k), \int_0^{t_1} y_1 dt, \ldots, \ldots, \ldots, y_L(t_k), y_L'(t_k), y_L''(t_k), \int_0^{t_1} y_L dt \right]^T$$

i.e., for L different measurement types there will be 4L states. In practice, the derivatives and integrals are approximated by finite differences and sums of the time-ordered data of the database. Specifically, we will calculate the approximations (24).

$$y_l^{'} \cong \frac{y_l(t_k) - y_l(t_{k-1})}{t_k - t_{k-1}}$$

$$y_l^{'}(t_{k-1}) \cong \frac{y_l(t_{k-1}) - y_l(t_{k-2})}{t_{k-1} - t_{k-2}}$$

$$y_l^{''}(t_k) \cong \frac{y_l^{'}(t_k) - y_l^{'}(t_{k-1})}{t_k - t_{k-1}}$$

$$\int_0^{t_k} y_l dt \cong y_l(t_k)(t_k - t_{k-1}) + \int_0^{t_{k-1}} y_l dt$$

Control Input Definition

The "Control Input" (mode of therapy) will be chosen from a finite set of control inputs that can be applied to the system.

System Dynamics

It is convenient to think of the propagation of the patient's state x_k at time t_k, to his state x_{k+1} at time t_{k+1} as obeying the following nonlinear dynamical system with process noise w_k and parameters p, i.e.:

$$x_{k+1}^j = f\left(x_k^j, u_i, p, w_k\right)$$

For simplicity, p is discrete, and is assumed to be drawn from a finite set formed by enumerating all useful combinations of clinical covariates,

$$p \in \{p_1, \ldots, p_M\}$$

Both the clinical covariates and process noise help to explain the variability of patient responses seen in the database. The covariates help to distinguish gross differences in responses due to patients with major differences in the nature of their disorders and complications. Process noise helps to explain small differences between patients with the same covariates but different responses to the same therapy. It is a measure of unmodeled dynamics, or intra-individual variability, due to other sources of variability in the system (24).

Probability of Survival

A patient's survival probability (SP) for a given state x is denoted by $S(x)$, which is calculated by first extracting the 40 (or 100) nearest neighbor states of patients having the same diagnosis and covariates as well as hemodynamic values that are closest to the given patients' values. The SP is then calculated as the fraction of these nearest neighbors that survived with this treatment. The SP may also serve as an outcome predictor as well as a measure of severity of illness (24).

LEGENDS

Figure 1. Effects of resuscitation therapy on sequential hemodynamic patterns and survival probability of a 26-years old male who sustained a gunshot wound to the right chest with multiple lacerations of the liver, stomach duodenum, small bowel, and kidney. Note the initial reductions in hemodynamic values and survival

probability in the first hour after admission and recovery with control of bleeding and surgical repair of injuries. Upper row: Cardiac index (CI); second row: Mean arterial pressure; Third row: Pulse oximetry ($SapO_2$); Fourth row: $PtcO_2/FiO_2$; Lowest row: Survival Probability. The dark areas represent the deficits of each variable. Time, in hours from ED admission, is noted below the bottom horizontal line. Therapies are outlined in boxes with vertical dotted lines marking onset an end of infusions. FFP, fresh frozen plasma; Hes, hespan (hydroxyethyl starch); rbc, packed red blood cell transfusion; alb, albumin; LR, lactated Ringer's solution. Time in OR and ICU indicated at lowest line.

Figure 2. Survivors' (solid line) and nonsurvivors' (dashed line) temporal patterns are shown for the first 24 hours after their ED admission. Mean values $\pm$ SEM are shown for cardiac index (CI), heart rate (HR), mean arterial pressure (MAP), pulse oximetry ($SapO_2$), transcutaneous oxygen tension indexed to the fractional inspired oxygen concentration ($PtcO_2/FiO_2$), and survival probability (SP). All values are keyed to the time of admission to the ED. Note the survivors' cardiac index, MAP, $SapO_2$, $PtcO_2/FiO_2$, and SP values were generally higher than those of the nonsurvivors. The mean survivors' SP values were significantly higher than the mean nonsurvivors' SP values in this initial resuscitation period.

Figure 3. Survivors' (solid line) and nonsurvivors' (dashed line) temporal patterns before, during, and for 24 hours after emergency surgical operations. Mean values $\pm$ SEM are shown for cardiac index (CI), heart rate (HR), mean arterial pressure (MAP), pulse oximetry ($SapO_2$), transcutaneous oxygen tension indexed to the fractional inspired oxygen concentration ($PtcO_2/FiO_2$), and survival probability (SP). Values are keyed to the time of the surgical operation. Note the survivors' cardiac index, MAP, $SapO_2$, $PtcO_2/FiO_2$, and SP values were higher than those of the nonsurvivors.

Figure 4. Comparison of the actual number of survivors and nonsurvivors (dark cross-hatched columns) with the number of predicted probabilities in each of these two groups (dotted columns).

Figure 5. APACHE scores of survivors and nonsurvivors over the first 4 days after admission compared with survival probabilities of the same series of patients. Vertical bars represent SD. Statistical significance was achieved on the last ICU day, but not during the first four days.

Figure 6. Window showing an illustrative patient's hemodynamic values at 0.43 hours after ED admission. The first column shows the number of nearest neighbors given each therapy. The second column shows the average PS of these nearest neighbors before therapy was given. Column 3 shows the number of nearest neighbors who were given each of the specified therapies in columns 5 through 12. Column 4 shows the PS of these nearest neighbors after administration of therapy specified in columns 5 through 12. WB/PRBC is whole blood or packed red cells, COLL is colloids, albumin or starch, XTALS is crystalloids, FFP fresh frozen plasma, DOB dobutamine, DOP dopamine, CRYO cryoprecipitate. Based on this information, FFP, COLL and WB/PRBC may be considered as likely to give an appropriate response. Figure 7. Left side: Values of a selected nearest neighbor (Pt. number 237), observed at 9.14 h after ED admission, with diagnostic data shown in the upper left (no blood loss, truncal injury, intra-operative monitoring, age 29, absence of head injury, male gender, and Survival outcome). Left: Hemodynamic values (CI, HR, MAP, SapO2, PtcCO2, PtcO2/FiO2, and hematocrit), integral, first derivative, and second derivative of this nearest neighbor are listed. Right: Hemodynamic values, integral, first derivative, and second derivative values of the monitored patient.

Figure 8. Flow chart showing procedures for the stochastic analysis and control program. First, the patient's state and covariates are defined and used to extract a given number of nearest neighbors. Second, the survival probability is calculated from the percentage of the nearest neighbors who survived. Third, the data is sorted according to the therapeutic interventions ("controls"). Fourth, the state response to the selected therapy is calculated. Fifth, the survival probabilities after each of the alternative therapeutic interventions are calculated. Sixth, the stochastic control is calculated.

TABLES

Table 1. Clinical Features of the Series:	Survivors (N=124)	Nonsurvivors (N=53)	P-Value
Age, years; Mean ± SD	31.5 ±13.7	36.7 ±18.9	0.041
Gender:			
Male, N; %	107/124 (60.5%)	44/53 (24.9%)	NS*
Female, N; %	17/124 (9.6%)	9/53 (5.1%)	NS
Mechanism of Injury:			
Fall, N (%)	5 (4%)	1 (2%)	NS
Gunshot wound, N (%)	61 (49%)	21 (40%)	NS
Blunt trauma, N (%)	35 (28%)	28 (54%)	0.003
Stab wound, N (%)	23 (19%)	2 (4%)	0.019
Bodily Injury**:			
Head, N (%)	16 (9.3%)	19 (25.3%)	NS
Spinal Cord, N (%)	4 (2.3%)	1 (1.3%)	NS
Chest, N (%)	56 (32.6%)	15 (20%)	NS
Abdomen, N (%)	66 (38.4%)	26 (38%)	NS
Back, flank, N (%)	13 (7.6%)	4 (5.3%)	NS
Extremity, N (%)	11 (6.4%)	4 (5.3%)	NS
Fractures, N (%)	6 (3.5%)	6 (8%)	NS
Injury Severity Score	20.2 ± 4.5	29.5 ± 5.1	<0.001

* NS, not statistically significant

** Not mutually exclusive

Table 2. Survival Probability and Hemodynamic Values for the First 24-hours After Admission

Variable, unit	Optimal Value	Survivors (N) Mean ± SEM	Nonsurvivors (N) Mean ± SEM	P value
SP, %	>80	(123) 81.5 ± 1.1	(52) 57.7 ± 2.3	<0.001
CI, L/min/m^2	4.0	(123) 4.34 ±0.07	(52) 3.96 ± 0.18	0.0005
MAP, mmHg	85	(122) 86 ± 1.2	(51) 76 ± 2.7	<0.0001*
HR, beat/min	<100	(123) 104 ± 1.6	(52) 117 ± 2.4	<0.0001
$SapO_2$, %	>98	(122) 99 ± 0.2	(51) 95 ± 1.1	<0.0001
$PtcCO_2$, torr	<50	(121) 47 ± 1.2	(51) 65 ± 9.2	0.0551
$PtcO_2/FiO_2$	>200	(120) 238 ± 12.4	(52) 109 ± 13.2	< 0.0001

*** by Wilcoxon two-sample test. SP Survival Probability, CI cardiac index, MAP mean arterial pressure, HR heart rate, $SapO_2$ arterial hemoglobin saturation by pulse oximetry, $PtcCO_2$ transcutaneous carbon dioxide tension , $PtcO_2/FiO_2$ transcutaneous oxygen tension indexed to FiO_2**

Table 3. Survival Probability and Hemodynamic Values at the time of the Probability Nadir*

Variable, unit	Optimal Value	Survivors (N=119) (N) Mean ± SEM	Nonsurvivors (N=43) (N) Mean ± SEM	P value P-Value*
SP, %	>80	(119) 65.2 ± 1.5	(43) 30.3 ± 2.1	<0.0001
CI, L/min/m^2	4.0	(119) 3.97 ± 0.11	(43) 3.16 ± 0.19	0.0002*
MAP, mmHg	85	(118) 82 ± 1.8	(42) 60 ± 3.7	<0.0001*
HR, beat/min	<100	(119) 109 ± 2.8	(43) 122 ± 4.0	0.0024
$SapO_2$, %	>98	(118) 98 ± 0.9	(42) 89 ± 2.0	<0.0001
$PtcCO_2$, torr	<50	(117) 49 ± 1.5	(42) 79 ± 12.1	0.0155
$PtcO_2/FiO_2$, torr	200	(116) 188 ± 13	(43) 49 ± 9.2	<0.0001

Only patients with a clearly defined nadir were included in this analysis

*** by Wilcoxon two-sample test SP Survival Probability, CI cardiac index, MAP mean arterial pressure, HR heart rate, $SapO_2$ arterial hemoglobin saturation by pulse oximetry, $PtcCO_2$ transcutaneous carbon dioxide tension ,**

Table 4. Survival Probability and Hemodynamic Values Before, During, and After Emergency Surgery

Variable, unit	Survivors			Nonsurvivors		
	Before (n) mean±	During (n) mean±	After (n) mean±	Before (n) mean±	During (n) mean±	After (n) mean±
Survival Percent, %	(34) 78.7±1.8	(76) 80.3±1.2	(53) 84.6±1.7	(11) 61.0±5.7	(27) 61.1±3.2	(13) 58.0±5.4
CI, L/min/m^2	(34) 4.59±0.18	(76) 4.34±0.10	(53) 4.18±0.11	(11) 4.27±0.49	(27) 3.81±0.18	(13) 3.75±0.26
MAP, mmHg	(34) 81±3.4	(76) 83±1.1	(52) 95±2.2	(11) 83±6.8	(26) 75±3.2	(13) 84±4.3
HR, beat/min	(34) 109±3.6	(76) 102±1.8	(53) 107±2.6	(11) 128±8.2	(27) 115±3.3	(13) 124±6.4
$SapO_2$, %	(34) 98±0.9	(76) 99±0.3	(52) 99±0.2	(11) 97±1.9	(27) 95±1.2	(13) 96±1.8
$PtcCO_2$,torr	(34) 45±2.9	(75) 46±1.5	(51) 41±1.1	(11) 59±6.5	(27) 62±8.6	(13) 48±6.0
$PtcO_2/FiO_2$	(34) 192±18.8	(74) 225±16.2	(51) 231±12.7	(11) 76±25.0	(27) 99±16.5	(13) 108±21.3

P-values for tests of equality of repeated measures were derived by the mixed linear models using the residual maximum likelihood with the unstructured covariance. CI: cardiac index; MAP: mean arterial pressure; HR: heart rate; $SapO_2$: arterial hemoglobin saturation by pulse oximetry; $PtcCO_2$: transcutaneous carbon dioxide tension; $PtcO2/FiO_2$: transcutaneous oxygen tension indexed to FiO_2.

Table 5. Classification Summary exclusive of brain death and death in the OR (N=165)

	Predicted to Die		Predicted to Live		Total	
	N	(Row%)	N	(Row%)	N	(Col%)
Actual Outcome						
Died	30	73.2%	11	26.8%	41	24.8%
Lived	5	4%	119	96%	124	75.2%
Total (%)	35	21.2%	130	78.8%	165	100.0%

Misclassification: 16/165=9.7%

Legend: Classification summary of those predicted to live and those predicted to die by survival probabilities, exclusive of those who were brain dead and those who died in the OR of massive uncontrollable hemorrhage.

Table 6. Hemodynamic Values of Head Injured Patients with Brain Death

Variable, unit	Normal Values	Brain Dead Patients (N=10)	P-values*
CI, L/min/m^2	3.2 ± 0.2	4.81 ± 0.17	0.01
MAP, mmHg	91 ± 3	89 ± 1	NS
HR, beat/min	<90	116 ± 2	0.01
$SapO_2$, %	>96	99 ± 0.2	NS
$PtcCO_2$, torr	<50	43 ± 1	NS
$PtcO_2/FiO_2$	185± 15	241 ± 16	0.05

***P-values calculated by unpaired Student's t-test**

CI cardiac index, MAP mean arterial pressure, HR heart rate, $SapO_2$ arterial hemoglobin saturation, $PtcCO_2$ transcutaneous carbon dioxide tension , $PtcO_2/FiO_2$ transcutaneous oxygen tension indexed to FiO_2

Figure 1A

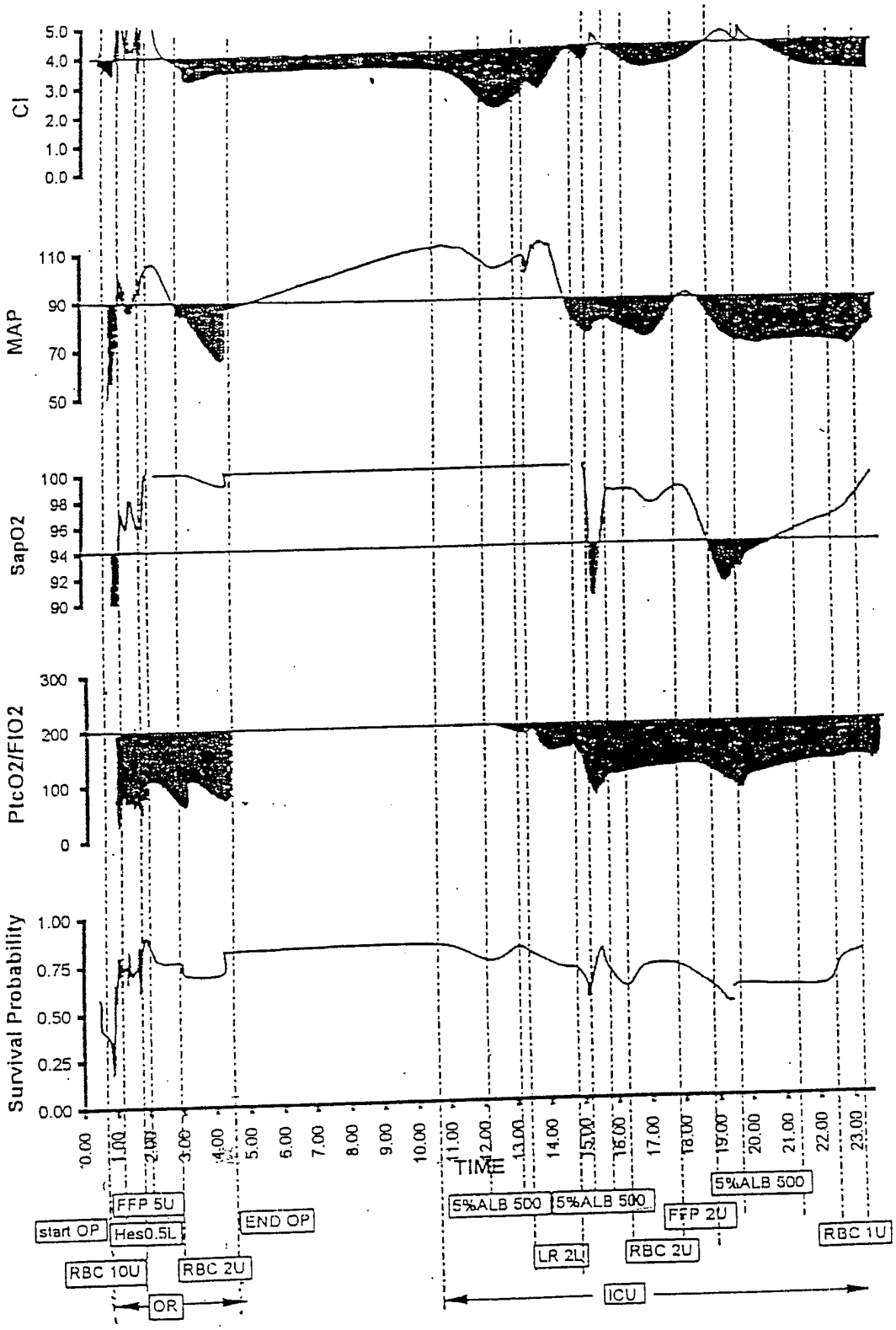

CI
5.0
4.0
3.0
2.0
1.0
0.0
MAP
110
90
70
50
SapO2
100
98
96
94
92
90
PtcO2/FIO2
300
200
100
0
Survival Probability
1.00
0.75
0.50
0.25
0.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
13.00
14.00
15.00
16.00
17.00
18.00
19.00
20.00
21.00
22.00
23.00
TIME
start OP
FFP 5U
Hes0.5L
END OP
RBC 10U
RBC 2U
OR
5%ALB 500
5%ALB 500
LR 2L
RBC 2U
FFP 2U
5%ALB 500
RBC 1U
ICU

Figure 1B

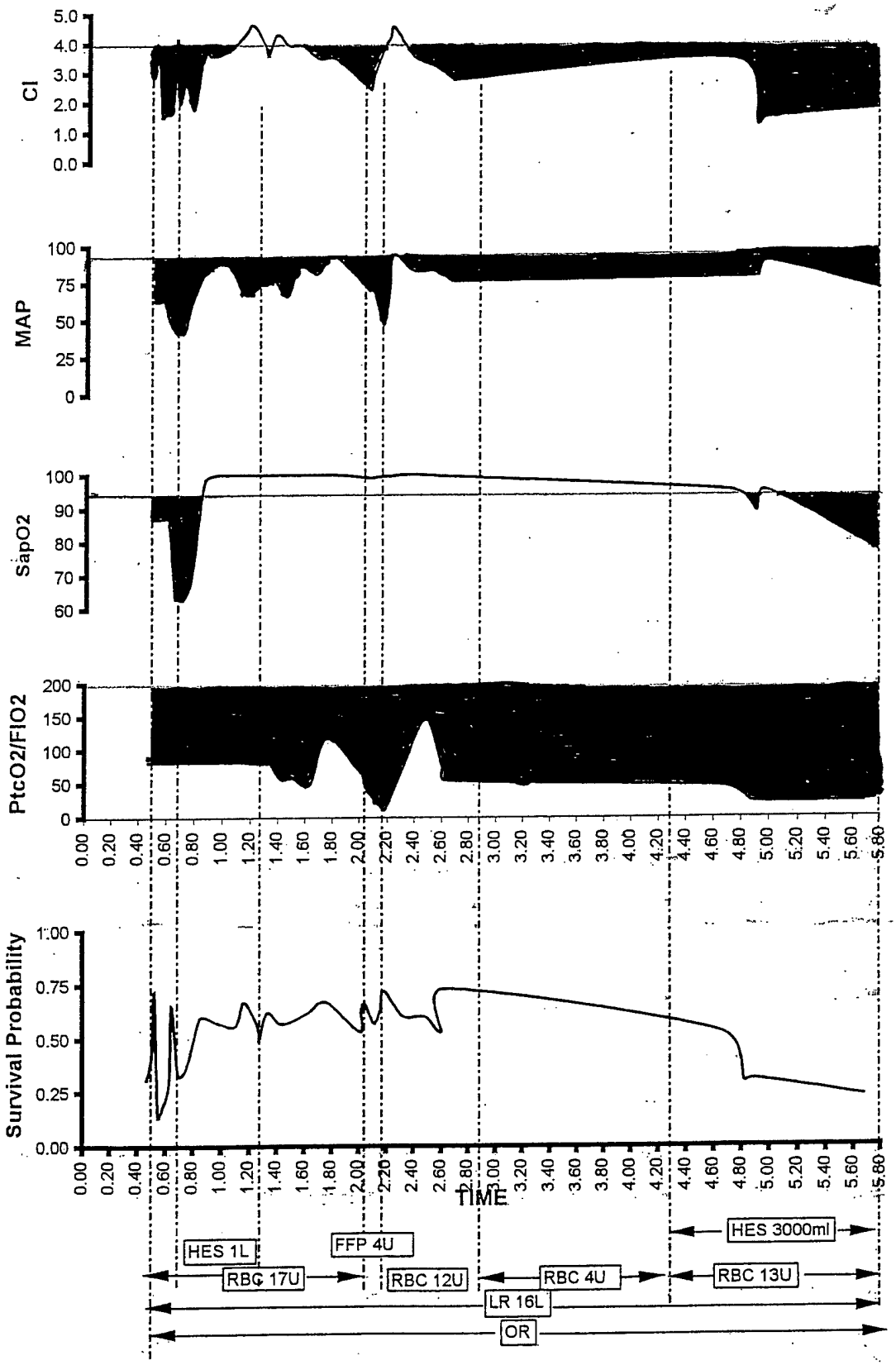

CI
5.0
4.0
3.0
2.0
1.0
0.0
MAP
100
75
50
25
0
SapO2
100
90
80
70
60
PtcO2/FIO2
200
150
100
50
0
0.00 0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80 5.00 5.20 5.40 5.60 5.80
Survival Probability
1.00
0.75
0.50
0.25
0.00
TIME
HES 1L
FFP 4U
HES 3000ml
RBC 17U
RBC 12U
RBC 4U
RBC 13U
LR 16L
OR

Figure 2

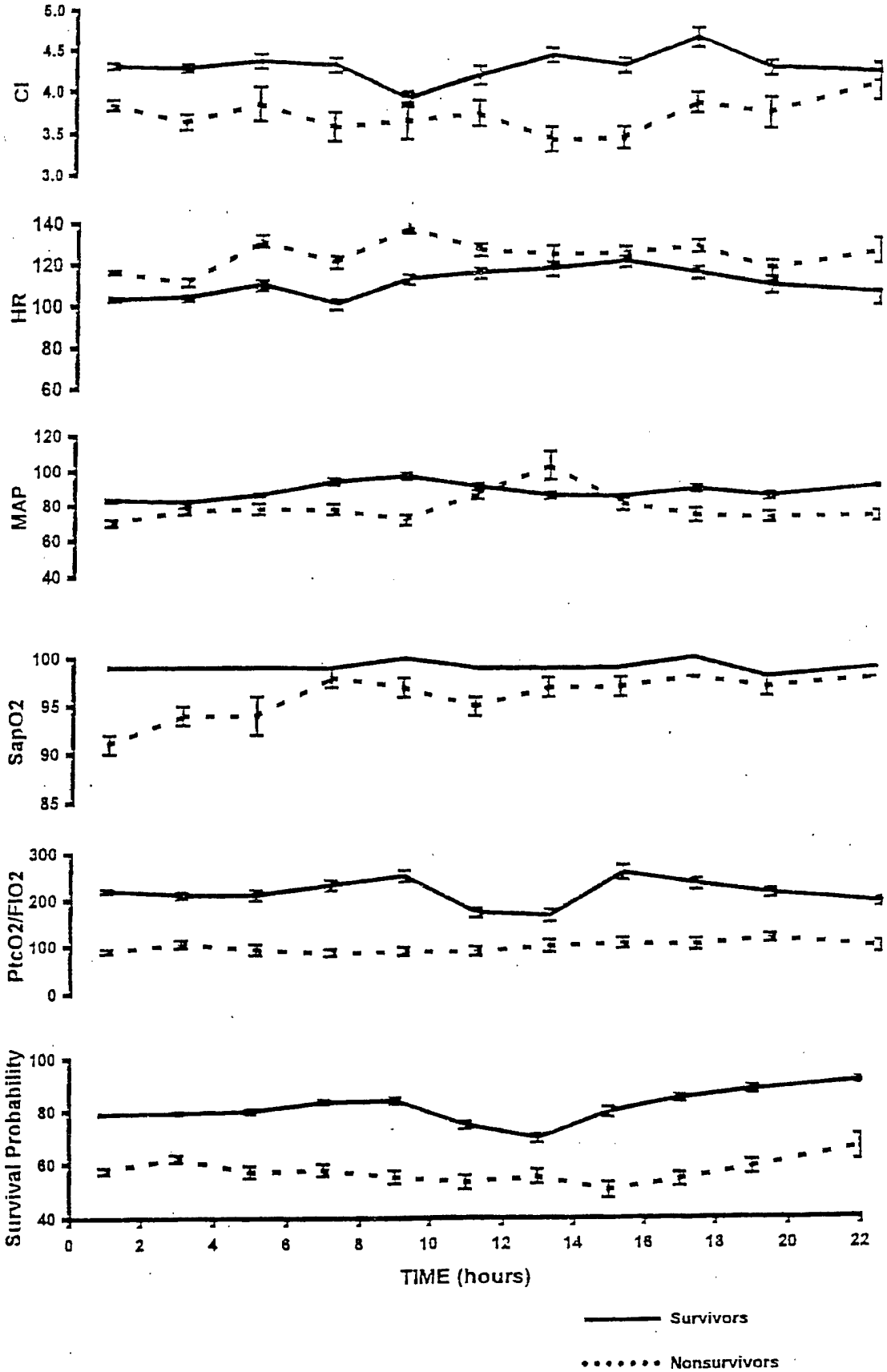

CI
5.0
4.5
4.0
3.5
3.0
HR
140
120
100
80
60
MAP
120
100
80
60
40
SapO2
100
95
90
85
PtcO2/FIO2
300
200
100
0
Survival Probability
100
80
60
40
0
2
4
6
8
10
12
14
16
18
20
22
TIME (hours)
Survivors
Nonsurvivors

Figure 3

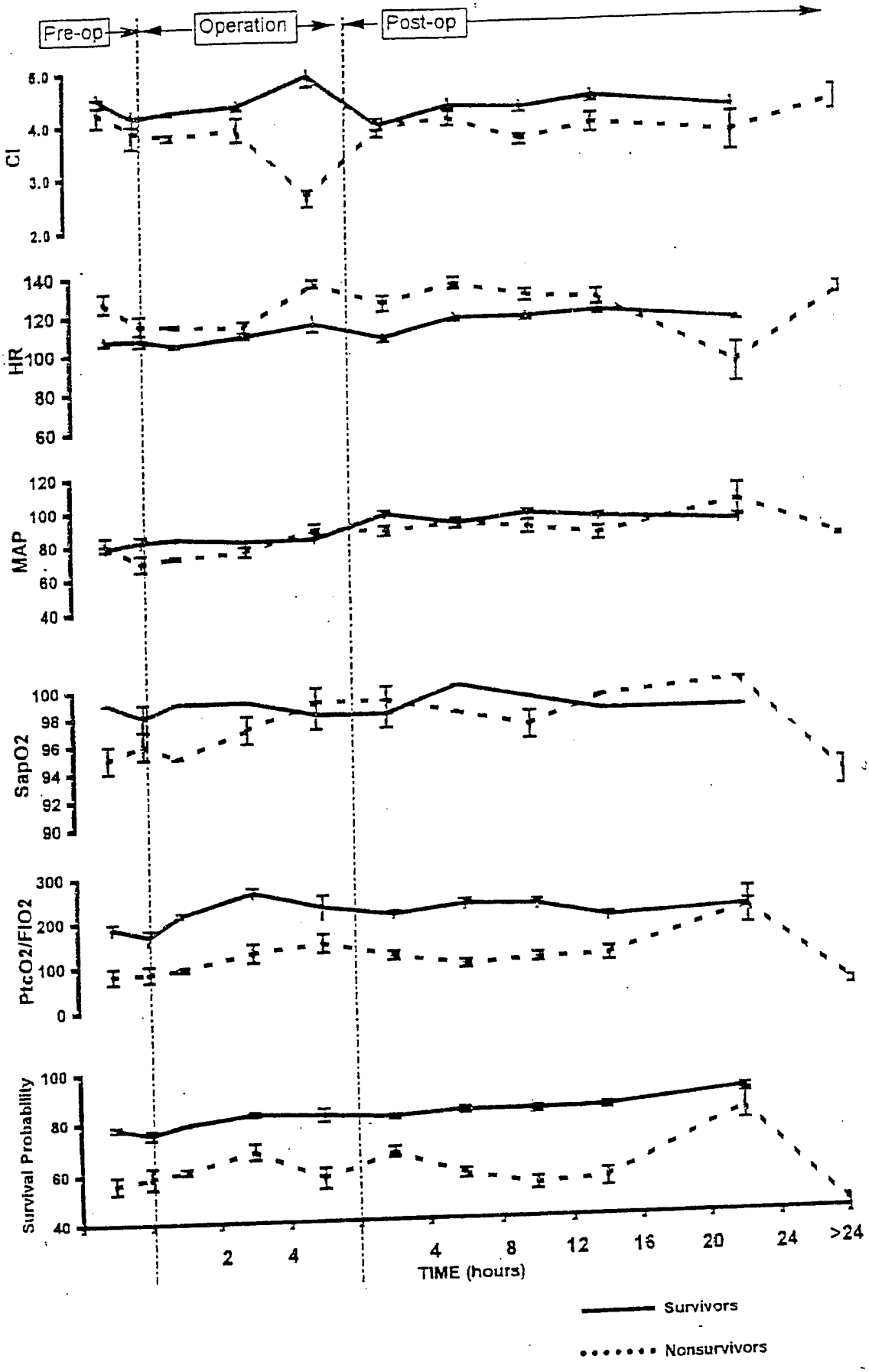

Pre-op
Operation
Post-op
CI
5.0
4.0
3.0
2.0
HR
140
120
100
80
60
MAP
120
100
80
60
40
SapO2
100
98
96
94
92
90
PtcO2/FIO2
300
200
100
0
Survival Probability
100
80
60
40
2
4
4
8
12
16
20
24
>24
TIME (hours)
Survivors
Nonsurvivors

Figure 4

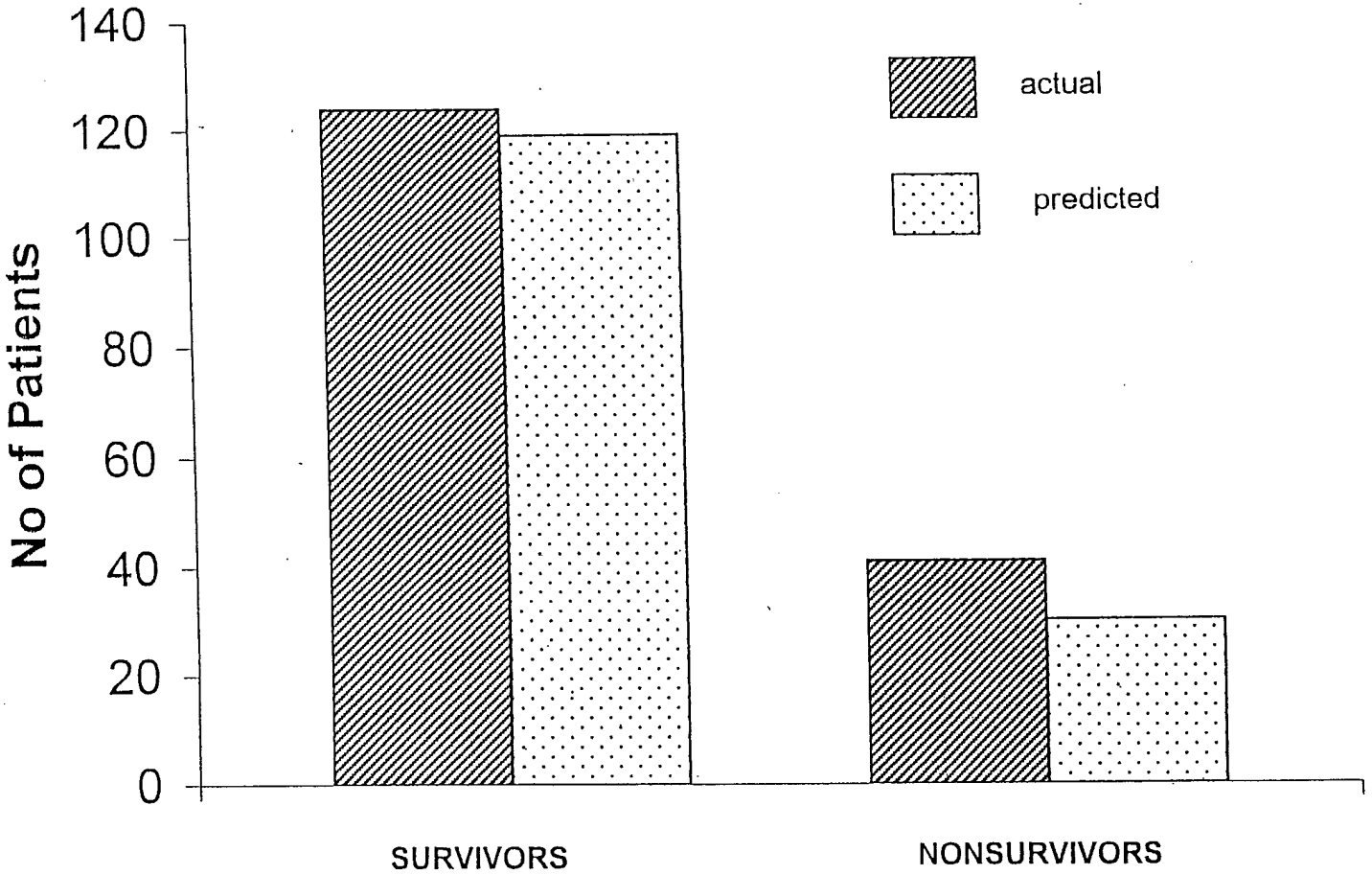

140
120
100
80
60
40
20
0
No of Patients
actual
predicted
SURVIVORS
NONSURVIVORS

Figure 5

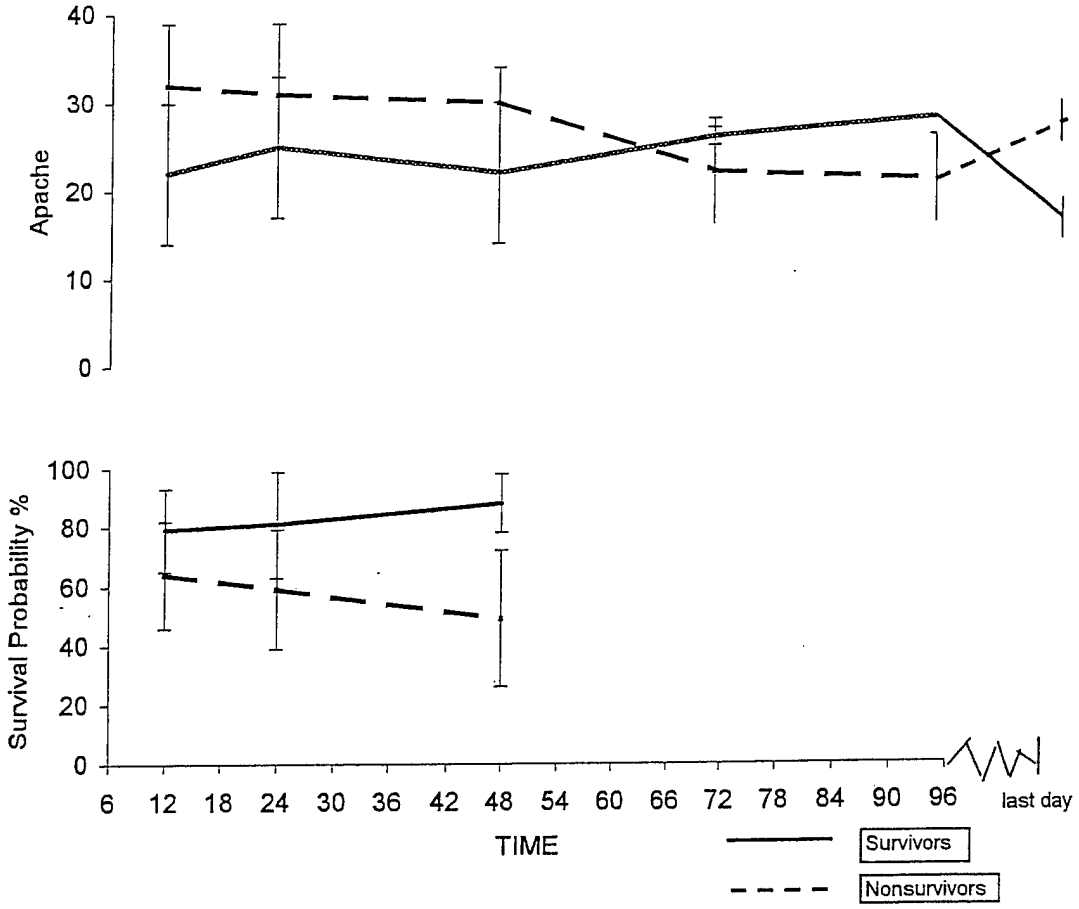

Apache
40
30
20
10
0
Survival Probability %
100
80
60
40
20
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
last day
TIME
Survivors
Nonsurvivors

Figure 6

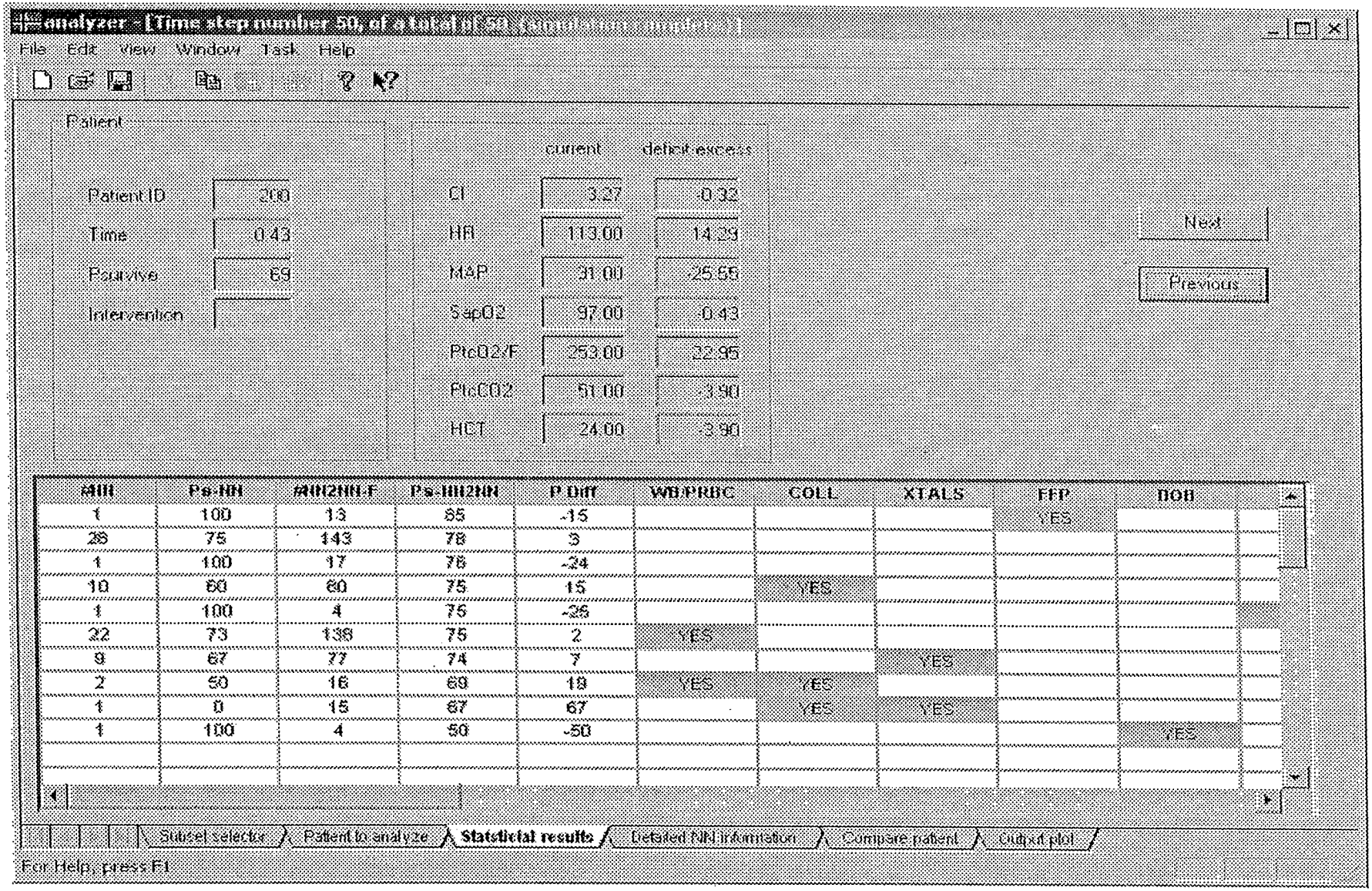

File Edit View Window Task Help
Patient
current
defect excess
Patient ID
200
Time
0.43
Post vive
69
Intervention
CI
3.27
0.32
HR
113.00
14.29
MAP
31.00
25.55
SapO2
97.00
0.43
PtcO2/F
253.00
22.95
PtcCO2
51.00
3.90
HCT
24.00
3.90
Next
Previous
#HR
Ps-HR
#HR2HR F
Ps-HR2HR
P Diff
WB/PRBC
COLL
XTALS
FFP
DOB
Subset selector
Patient to analyze
Statistical results
Detailed NN information
Compare patient
Output plot
For Help, press F1

Figure 7

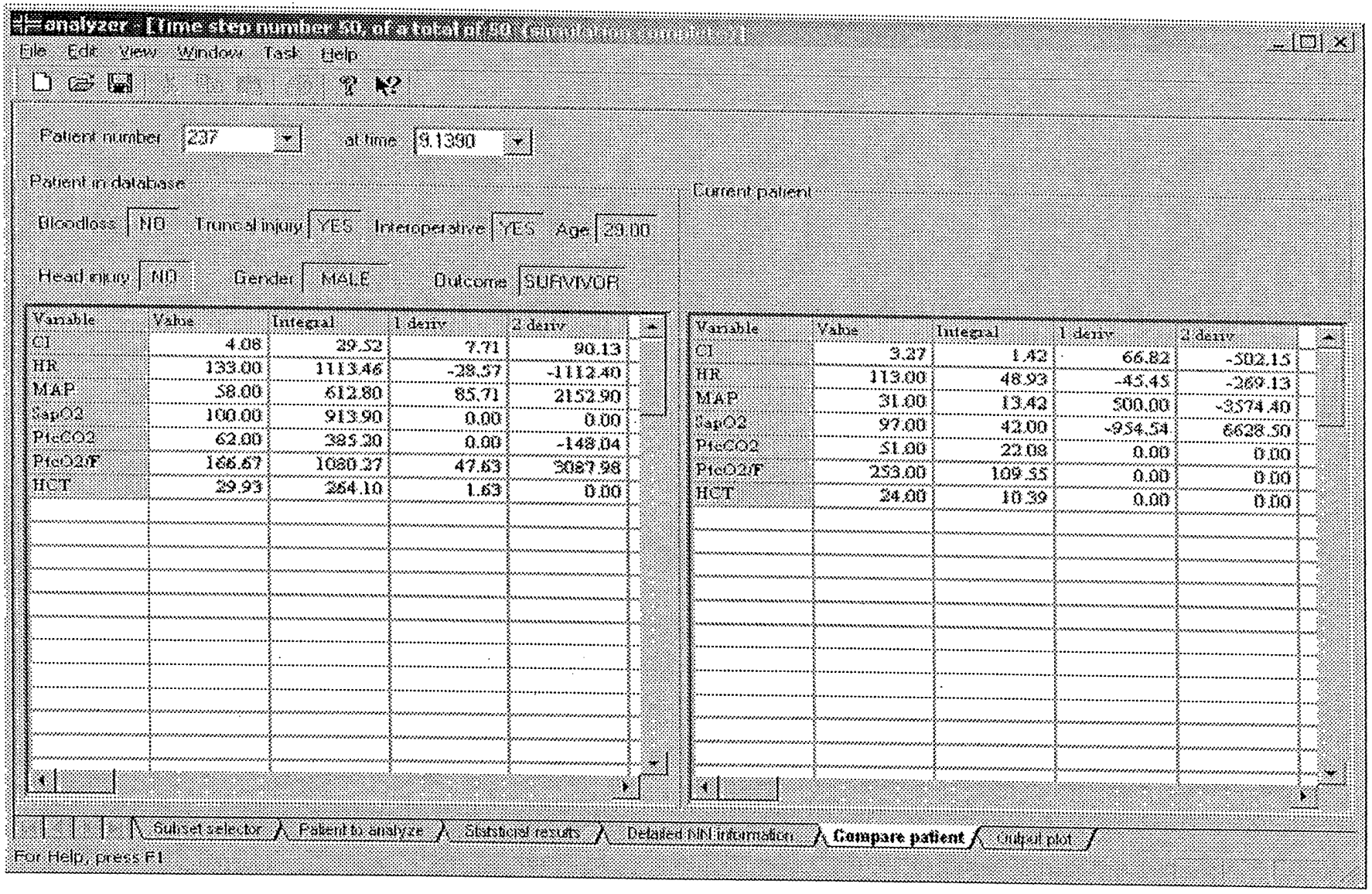
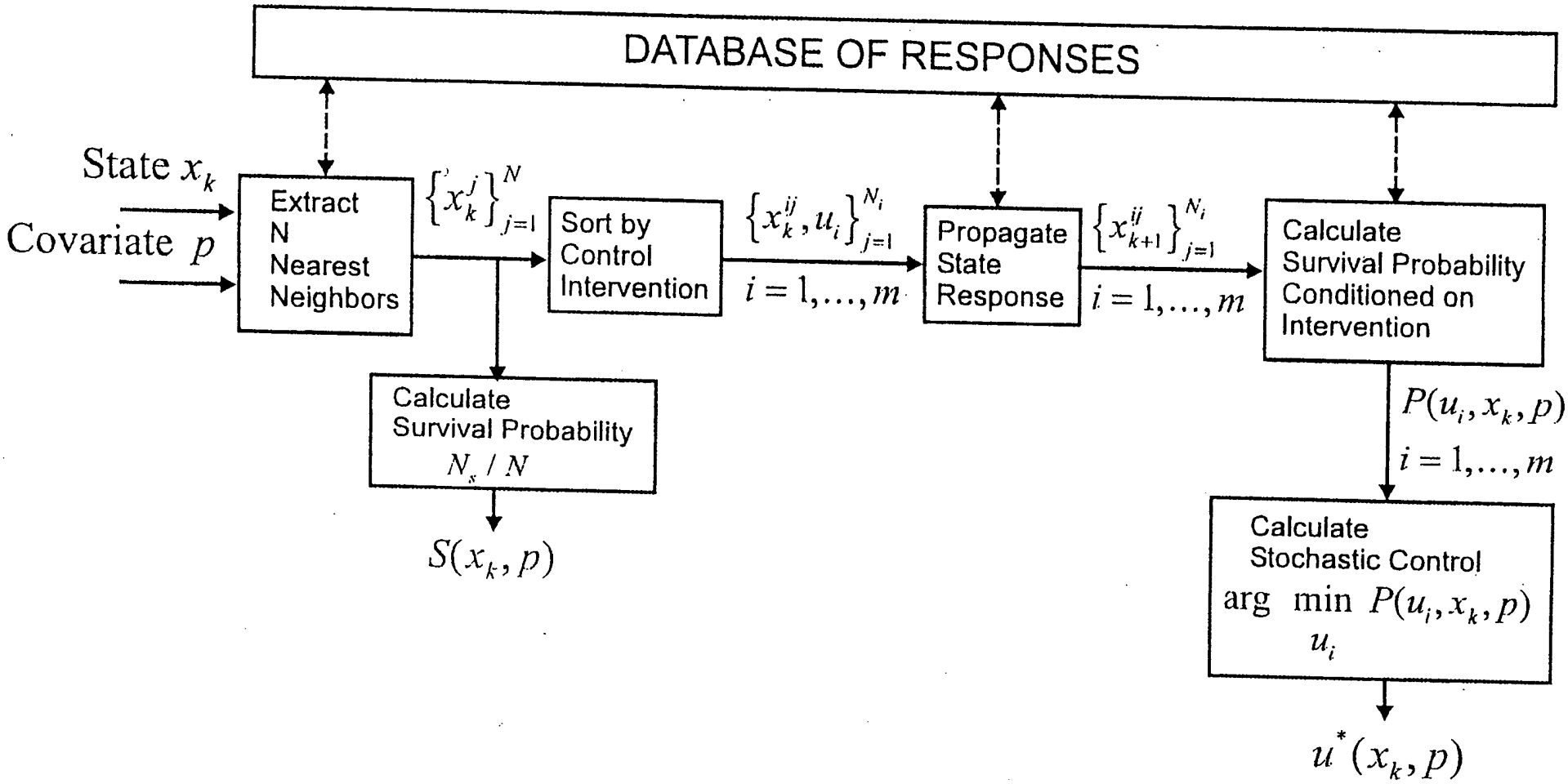

analyzer - [Time step number 30 of a total of 90
File Edit View Window Task Help
Patient number 237
at time 9.1380
Patient in database
Bloodloss NO
Truncal injury YES
Interoperative YES
Age 29.00
Head injury NO
Gender MALE
Outcome SURVIVOR
Variable Value Integral 1 deriv 2 deriv
CI 4.08 29.52 7.71 90.13
HR 133.00 1113.46 -28.57 -1112.40
MAP 58.00 612.80 85.71 2152.90
SaO2 100.00 913.90 0.00 0.00
PaCO2 62.00 385.30 0.00 -148.04
PaO2F 166.67 1020.37 47.63 3087.98
HCT 39.93 264.10 1.63 0.00
Current patient
Variable Value Integral 1 deriv 2 deriv
CI 3.27 1.42 66.82 -502.15
HR 113.00 48.93 -45.45 -269.13
MAP 31.00 13.42 500.00 -3574.40
SaO2 97.00 42.00 -954.54 6638.50
PaCO2 51.00 22.08 0.00 0.00
PaO2F 253.00 109.55 0.00 0.00
HCT 24.00 10.39 0.00 0.00
Subset selector
Patient to analyze
Statistical results
Detailed NN information
Compare patient
Output plot
For Help, press F1

Figure 8


DATABASE OF RESPONSES
State x_k
Covariate p
Extract N Nearest Neighbors
$\{x_k^j\}_{j=1}^{N}$
Sort by Control Intervention
$\{x_k^{ij}, u_i\}_{j=1}^{N_i}$
$i = 1,\ldots,m$
Propagate State Response
$\{x_{k+1}^{ij}\}_{j=1}^{N_i}$
$i = 1,\ldots,m$
Calculate Survival Probability Conditioned on Intervention
$P(u_i, x_k, p)$
$i = 1,\ldots,m$
Calculate Survival Probability
N_s / N
$S(x_k, p)$
Calculate Stochastic Control
$\arg \min_{u_i} P(u_i, x_k, p)$
$u^*(x_k, p)$

Autonomic Activity in Trauma Patients Based on Variability of Heart Rate and Respiratory Rate

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Running title: Autonomic Activity in Trauma Patients

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ABSTRACT

Objective: To evaluate the effects of sympathetic (SNS) and parasympathetic nervous system (PSNS) activity on the heart rate and other hemodynamic variables in acute emergency patients with mild to moderately severe trauma.

Setting: Fourteen trauma patients studied immediately after admission to the emergency department (ED) in a level 1 university-run trauma service.

Methods: We measured heart rate (HR) and respiratory rate (RR) variability by spectral analysis in mild to moderately injured patients and compared the patterns of the low (Lfa), and high frequency (Hfa) areas of variability. The Lfa is the area under the spectral analysis curve within the frequency range of 0.04 to 0.10 Hz. This area reflects primarily the tone of the sympathetic nervous system as mediated by the cardiac nerve. The respiratory, or high frequency area (Hfa) is a 0.12 Hz-wide frequency range centered around the fundamental respiratory frequency (FRF) defined by the peak mode of the respiratory power spectrum. It is indicative of vagal outflow reflecting parasympathetic nervous system activity. The Lfa/Hfa, or "(L/R) ratio," reflects the balance between the sympathetic and parasympathetic nervous systems. To explore the hemodynamic effects of bursts of autonomic activity in response to injury, HR and RR variability were studied along with noninvasive hemodynamic monitoring. Bioimpedance cardiac output, HR, and mean arterial pressure (MAP) were used to measure cardiac function, and transcutaneous oxygen ($PtcO_2$) to reflect tissue perfusion.

Results: During sudden surges of autonomic activity, we observed a consistently positive relationship of HR variability to Lfa, and to a lesser degree, HR variability to Hfa. However, the L/R ratio did not show a statistically significant correlation with HR variability in this series. These HR variabilities that reflect autonomic activity were associated with increased mean arterial pressure (MAP), cardiac index (CI), as well as HR, but decreased tissue perfusion indicated by the decreased $PtcO_2/FiO_2$ ratio.

Conclusions: Surges in autonomic activity in the period immediately after ED admission of trauma patients were associated with immediate and pronounced increases in hemodynamic parameters, especially heart rates, and reduced tissue oxygenation.

Key Words: Heart rate variability; Respiratory rate variability; Estimation of autonomic nervous system activity, Parasympathetic nervous system activity; Sympathetic nervous system activity; Blunt trauma; Acute emergencies.

INTRODUCTION

The observation that heart rate and blood pressure vary from beat to beat was first made by Stephen Hales, who, in the 18th century, performed the first quantitative measurement of arterial blood pressure. He also observed correlation among respiratory cycle, beat-to-beat systolic pressure, and the intervals between beats.

Understanding of the role of autonomically mediated neural input to the heart is needed for analyses of hemodynamic responses to acute emergency conditions. A quantitative, noninvasive means of autonomic monitoring based on heart rate (HR) and respiratory rate (RR) variability techniques provides a methodology for clinically evaluating autonomic nervous activity as a whole as well as the sympathetic and parasympathetic components (1-6). Sympathetic modifying drugs and clinical tests produce repeatable and sensitive changes in the low-frequency areas (Lfa) and the (L/R ratio), suggesting that these parameters reflect sympathetic nervous system (SNS) activity. Further, parasympathetic modifying drugs and clinical tests produce repeatable and sensitive changes in the high-frequency areas (Hfa), suggesting that these Hfa values reflect parasympathetic nervous system (PSNS) activity (7-11). Cardiac autonomic monitoring leads to earlier recognition of patients' physiologic state and may suggest timely, proactive therapy.

The present study uses noninvasive autonomic nervous system monitoring based on real-time HR variability technology in the initial period of patients recently admitted for blunt trauma. Early mild to moderate cases were selected because severe trauma cases particularly in the late stages had many associated extraneous and confounding influences that obscured underlying hemodynamic patterns. The interacting components of the ANS were evaluated by spectral analysis of HR variability and RR variability to reflect the tone of the autonomic nervous system (ANS) (1-5). The clinical usefulness of these values is based on the concept that the normal SNS and PSNS work harmoniously to maintain homeostasis which results in a stable power spectral content that maintains a balance of power contained in the LFa and the HFa.

In response to the long-standing recognition of beat-to-beat HR variations and their clinical relevance, Akselrod and colleagues (2) have explored the physiologic mechanisms that generate these fluctuations. Spectral analysis characterizes mathematically the physiologic mechanisms that generate variations in RR intervals. Spectral analysis calculates the frequency content of time-varying signals and offers a breakdown of the successive RR intervals into their frequency components (1-5).

The present study was designed to evaluate sympathetic and parasympathetic activity in the very early stage of acute emergencies of mild to moderate severity. Responses of patients with severe life-threatening injuries have too many confounding and conflicting influences to be easily evaluated.

METHODS

Clinical Series

We studied 14 mild to moderately severely injured patients by noninvasive monitoring of autonomic nervous system activity within the first 1 to 4 hours after admission to the emergency department (ED) before and during the initial fluid resuscitation, but before radiological diagnostic studies, and before the use of sedation, anesthesia, or vasoactive agents. There were 2 females and 12 males, mean age 33.86 years, ranging between 15 to 66 years of age. The mean injury severity score (ISS) was 10.1 ± 4.8. The patients were not under the effect of anesthesia; in three patients mild sedation or one dose of pain medication was given. All these study patients were discharged alive and well after two to 23 days of hospitalization. The demographic, salient clinical features and medications are given in table 1.

Patients were selected based on the following criteria: hypotension (systolic blood pressure <100 mmHg or mean arterial pressure < 70 mmHg), tachycardia (heart rate >100 beats/min), multiple long bone fractures, head injuries or blunt abdominal and thoracic injuries.

HR and RR Variability as Markers of Sympathetic and Parasympathetic Activities

HR variability is defined as recurrent changes in beat-to-beat, measured by RR intervals. Two skin electrodes placed on each side of the chest in the standard Lead II ECG configuration measure the instantaneous heart rate. The heart beat intervals are recorded and the HRV is plotted in the frequency domain to separate the high frequency components from the low frequency components by spectral analysis. When HR variability is plotted in the time-domain, it is difficult to distinguish the high frequency components from the low, because the curve reflects the sum total of all frequencies in that signal.

Variability in the instantaneous beat-to-beat heart rate intervals is a function of sympathetic and parasympathetic activity that regulates the cardiac functional response to the body's level of metabolic activity. The SNS primarily generates the low frequency components of HR variability associated with more gradually increasing HR variability, because the sympathetic branch usually responds in four or five seconds, which is slower than parasympathetic responses (12,13). The PSNS primarily generates the high frequency components and has sharper increases, since it typically responds in one to two seconds (12,13).

In addition to analyzing the ECG signals, a respiratory signal is obtained by the same electrodes through impedance plethysmography estimated by chest expansion. Incorporating respiratory signal analysis enables us to independently measure each branch of the ANS. This

provides the essential dimension missing from classical heart rate variability monitoring as it pertains to independent assessment of the ANS branches.

When the low frequency and high frequency components were isolated within the HR variability spectrum, their respective areas under the curve were calculated as Lfa and Hfa. The Lfa and Hfa values were demonstrated to reflect sympathetic and parasympathetic tone, respectively, by independent digital measurements (2-5). The Lfa is computed as the area under the heart rate spectrum from 0.04 Hz to 0.10 Hz. The Hfa, sometimes referred to as the respiratory frequency area, is computed as the area within a 0.12 Hz-wide portion of the heart rate spectrum centered around the fundamental respiratory frequency (FRF), which is defined by the peak mode of the respiratory power spectrum. The Hfa is indicative of the vagal outflow and reflects the parasympathetic nervous system (PSNS) influence on heart rate control. These measurements have been demonstrated to be reliable, repeatable, and specific for sympathetic and parasympathetic function (1-14).

Hemodynamic Monitoring

Cardiac Output

An improved thoracic bioelectric impedance device (Yantagh Inc., Bristol, PA) was applied shortly after arrival in the ED. The noninvasive disposable prewired hydrogen electrodes were positioned on the skin and three ECG leads were placed across the precordium and left shoulder (15,16). A 100 kHz, 4 mA alternating current was passed through the patient's thorax by the outer pairs of electrodes and the voltage was sensed by the inner pairs of electrodes; the voltage sensed by the inner electrodes captured the baseline impedance (Zo), the first derivative of the impedance waveform (dZ/dt), and the ECG. The signal processing algorithm used a time-frequency distribution (modified Wigner Distribution) analysis that increased signal-to-noise ratios (15,16). Previous studies have documented satisfactory correlations between thermodilution and bioimpedance cardiac output values for trauma patients in the ED, OR, and ICU conditions (17-19).

Transcutaneous Oxygen Tension

Standard transcutaneous oxygen tension measurements were continuously monitored throughout the observation period. This technology uses the same Clark polarographic oxygen electrode routinely employed in standard blood gas measurements (19-26). The oxygen tensions were measured in a representative area of the skin surface heated to 44^0C to increase diffusion of oxygen across the stratum corneum and to avoid vasoconstriction in the local area of the skin being measured (24-25). Previous studies demonstrated the capacity of transcutaneous oxygen tensions to estimate skin oxygen tension as a reflection of tissue perfusion (19-26). Transcutaneous oxygen tension ($PtcO_2$) has been shown to reflect the delivery of oxygen to the local area of skin; it also paralleled the mixed

venous oxygen tension (SvO_2) values (20). While oxygen tension of a segment of the skin does not reflect the state of oxygenation of all tissues and organs, it has the advantage of being the most sensitive early warning tissue for the adrenomedullary stress response; vasoconstriction of the skin is an early stress responses of hypovolemia and other shock syndromes (27-29). Transcutaneous oxygen tension was indexed to the FiO_2 to give a $PtcO_2/FiO_2$ ratio because of marked $PtcO_2$ changes produced by increased inspired oxygen. Limitations of the transcutaneous methods are the thermal environment must be reasonably constant, marked changes in room temperature from drafts or open windows must be avoided; the electrode must be changed to a nearby thoracic or shoulder site and be re-calibrated to avoid first degree skin burns.

Experimental Design

Continuous monitoring of HR variability with ANS-R1000 (Ansar Inc., Philadelphia, PA) was started shortly after admission and before use of anesthesia or ionotropic agents, and when possible, before pain medication. HR variability was monitored continuously for two to four hours in each patient to identify, record, and compare patterns of Lfa, Hfa and L/R ratio with the HR changes. We made effort to exclude times when extraneous confounding events may have played a role by noting, recording, and eliminating from consideration the time periods of agitation, pain, cough, needle insertion, withdrawal of the needle from skin, local anesthesia, suturing of minor skin injuries, changes in position, dressing changes, talking, presence of friends and family members, the patient's reaction to environmental sounds, need to urinate, and other disturbing events. Periods when the patient was quiet and stable were considered in the present analysis.

Sequential Changes in Lfa, Hfa, L/R ratio in Relation to Heart Rate

Spectral analysis of HRV and RRV were automatically determined and displayed as Lfa, Hfa, L/R ratio, and mean HR. The monitoring was continuous in a format of consecutive 32-second segments. Since observed changes occurred over varying lengths of time, we evaluated the patterns of changes occurring during varying time periods. Sequences of consecutive segments were used to differentiate relatively longer term patterns than the 32-second segments routinely analyzed and displayed by the monitoring device.

Changes in Lfa, Hfa, L/R ratio and their Correlation with Hemodynamic Values

We compared changing patterns of Lfa, Hfa and L/R with simultaneous hemodynamic changes in MAP, HR, CI, SI, $PtcO_2/FiO_2$ values during the initial post-admission period in the emergency department.

RESULTS

Sudden Abrupt Changes in Lfa, Hfa, L/R ratio

We studied 31.5 hours of continuous second-by-second monitoring of Lfa, Hfa and L/R ratio and compared them with simultaneous changes in CI, SI, HR, MAP, and $PtcO_2/FiO_2$. Figures 1-3 illustrate continuous simultaneous patterns of HR with Lfa, Hfa, and L/R ratios. Inspection of the continuous measurements revealed the presence of sudden bursts or surges of autonomic activity of widely varying magnitude that occurred along with increases of heart rate. The HR changes were better correlated with simultaneous Lfa values (Fig. 1) and to a lesser degree with simultaneous Hfa (Fig. 2), but not well correlated with L/R ratio (Fig. 3).

Lfa changes including relatively small changes were associated with HR changes 86% of the monitored time; range 81% to 92% (Table 4). Similarly, 65% of Hfa changes were associated with HR changes; this ranged between 55% and 86% in different patients (Table 4). L/R ratio had the least relationship (57%) with the changes in mean HR; in different patients this ranged between 45% and 75% (Table 3).

The dynamic range of HR changes did not affect the sensitivity of HR pattern to Lfa and Hfa changes. That is, there were pronounced changes in HR in the range of 70 beat/min. as well as in the tachycardia range of over 100 beat/min. The range of changes in HR was roughly proportional to the simultaneous Lfa changes (Fig. 1).

Correlation of Sudden Changes in Lfa with Hemodynamic Values

The increase of Lfa and Hfa was associated with significant increases in HR, CI, and MAP (Table 2). There was a trend toward increased SI that did not achieve statistical significance., indicating that the increased CI and HR did not occur at the expense of reduced SI . Similarly, the decreases in Lfa and Hfa was associated with significant reductions in CI, HR, and MAP, and with trends toward reduced Hfa, and SI values (Table 4).

Tissue perfusion reflected by $PtcO_2/FiO_2$ values decreased significantly with surges of increased Lfa and Hfa values, and tended to increase with the reduction in Lfa and Hfa values.

DISCUSSION

Spectral analysis of heart rate and respiratory rate variability converts the heart and respiratory time domain signals to the frequency domain signals for analysis and calculation of the LFa and the HFa. The LFa is a measurement that includes information from both sympathetic and parasympathetic components of the ANS carried by the cardiac nerve, a branch of the vagus nerve

that receives sympathetic input (3-6). However, the LFa and the HFa, can provide insight into the cardiac sympathovagal balance (2) as well as the health and functioning of the ANS, both centrally and, to a lesser extent, peripherally (1,12,13).

The frequency of the peak mode of the respiratory spectrum in defined as the fundamental respiratory frequency, which is equivalent to the inverse of the respiratory rate at rest during normal breathing. The HR variability is affected by both SNS and PSNS activity. When the fundamental respiratory frequency is superimposed on the HR variability spectral frequency axis, the high frequency PSNS component can then be isolated. The low frequency SNS component is also isolated within the heart rate variability signal based on classical spectral analysis theory.

The clinical usefulness of spectral analysis of HR variability and RR variability is based on the hypothesis that ANS monitoring provides evidence that a particular level of treatment is adequate or insufficient. Noninvasive ANS monitoring provides this information as numerical trends in real time. The goal is to titrate intervention to the level that is most effective at that given time. Noninvasive monitoring of ANS by spectral analysis of HR and RR variability has been used in different studies in the different health conditions such as sepsis (6), trauma and shock (14), depth of anesthesia (30-32), cardiac dysfunction (32-35), cardiopulmonary diseases (35), diabetic neuropathy (36-38), pain management, neonatal development as infant monitoring (39), pharmaceutical interactions (40) and brain death (14). In these studies, patients have been monitored for short periods of time to compare the average values of Lfa, Hfa, L/R ratio and mean HR with values of normal populations.

Invasive monitoring remains the most definitive means of evaluating circulatory function in high risk patients, but it is costly, personnel intensive, has complications and is often started late in the course of illness after ICU admission and the onset of organ failure. Delays in management of trauma patients have led to circulatory deficiencies, organ failures, and death. However, noninvasive monitoring techniques that are recently coming of age may reduce the risk and cost of monitoring by invasive techniques (14,17-19). New noninvasive methodologies, such as ANS monitoring based on real-time HR variability, may also reduce delays in instituting therapy and thereby improve outcomes.

As ANS monitoring becomes more widely used and as more is learned about the underlying mechanisms driving the ANS monitored parameters, more specific and appropriate descriptive hemodynamic correlations will develop (1-14).