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13. ABSTRACT ***(Maximum 200 Words)***

Traumatic brain injury (TBI) contributes to combat morbidity/mortality. Studies in models of TBI have focused on novel mediators and mechanisms. We used controlled cortical impact (CCI), a contemporary model of TBI in rats to study *field-oriented treatments*. The following technical objectives were addresses: 1) What is the optimal ventilation strategy? 2) Is hypothermia beneficial? and 3) what is the optimal sedative/analgesic? A fourth objective, combining hypothermia plus other therapies was abandoned due to the limited efficacy of hypothermia. The most important findings/publications include: Objective #1), in a report published in the *Journal of Neurosurgery*, we demonstrated that early aggressive hyperventilation worsened neuronal death, Objective #2), we published the first report showing that hypothermia was ineffective in the combat-relevant scenario of CCI followed by secondary hypoxemia. That work is *in press* in *Critical Care Medicine*, Objective #3), we reported remarkably poor outcome in rats treated with narcotics (fentanyl) versus general anesthesia (isoflurane) after CCI. That study is extremely relevant since narcotics are the current field treatment. That work was presented at the 1999 meeting of the National Neurotrauma Society by research trainee Dr. Kimberly Statler, who received the Women in Neurotrauma Award. The paper was submitted to *Journal of Neurosurgery*.

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(5) INTRODUCTION

Please note that although we have performed, presented and published a considerable number of studies, as outlined in this *final report*, we are still completing a few aspects of work on the third technical objective. There are also a number of manuscripts and abstracts that are either *in press*, *in submission* or *in preparation.* After discussion with our contracting officer, it was recommended that we submit a supplement to this report and its appendix. **A supplement will be forwarded to your office on July 14, 1999.** Additional supplements will follow for subsequent publications beyond that date.

Traumatic brain injury (TBI) is an important contributor to combat casualty morbidity and mortality. Although progress has been made in the development of rodent models of TBI (such as the controlled cortical impact (CCI) model), most of the studies with these models have focused on molecular mechanisms of damage. Because there has been a paucity of application of **practical emergency interventions** in TBI models, we felt that it was important to address this deficiency since this could have important implications for field and emergency management of both soldiers and civilians. Our overall **hypothesis** is that optimal manipulation of practical interventions applicable in the emergency treatment of severe TBI (respiratory management, temperature control, and sedation) can reduce secondary brain injury in a rat model of brain contusion, and thereby improve functional and/or neuropathological outcome.

Funding year 1

In yr. 1 of funding, we addressed the **first Technical Objective**, namely, to investigate the effects of mechanical ventilation strategies (as applied by the first responder in the field) on functional and neuropathological outcome in our model. **We found that aggressive, prophylactic hyperventilation (HV) applied for 4 h immediately after injury is detrimental (vs normal $PaCO_2$), and leads to increased neuronal death in selectively vulnerable brain regions.** This study was published as a full manuscript in the ***Journal of Neurosurgery*** (1). The reviewers indicated that this was an important study that would be cited often. Dr. M. Forbes, a Critical Care Medicine fellow training in research with Dr. Kochanek authored the study.

To set the stage for the evaluation of therapies after injury (as proposed studies in technical objectives 2-4), it appeared that it would be optimal to have TBI models both with and without a secondary insult-- since such insults are common in the field. This was accomplished by two studies assessing our model (2,3), including, adding a 30-min of moderate hypoxemia to the CCI. Characterization of that model was described in the 1997 report and was presented in 1998 at the National Neurotrauma Society Meeting (3). During yrs. 2 and 3, we used both the CCI model and the CCI plus secondary hypoxemia model to test therapies.

Funding year 2

In yr. 2 we performed three studies addressing Technical Objective 2 and part of Objective 3. These studies included: 1) assessment of the effect of transient (4 h),

moderate hypothermia on outcome after TBI with a secondary insult, 2) assessment of the effect of prolonged (12-h) moderate hypothermia on outcome after TBI, and 3) assessment of the effect of the anti-excitotoxic therapy (the NMDA-receptor antagonist MK-801) early after TBI. **The results of these studies showed that hypothermia (32 °C, for either 4 h or 12 h) reduced DNA damage early after injury but beneficial effects on long-term outcome could not be demonstrated in the model. It was particularly ineffective after the combined CCI plus hypoxemia. In contrast, we were surprised to find that MK-801 improved functional outcome. However, neither treatment improved brain histopathology after injury.** Three research fellows (Drs. C. Robertson, M. Whalen, and R. Ruppel) worked on these projects with the PI (Dr. Kochanek) during yr-2. Dr. Robertson presented an abstract at the 1999 annual meeting of the Society of Critical Care Medicine (4). That work on hypothermia is *in press* as a full manuscript in the journal *Critical Care Medicine* (5). We also reported that 4 h of moderate hypothermia attenuates DNA damage after injury (6,7). That work was presented last year at the Society of Critical Care Medicine Meeting and the manuscript is in preparation. Our work on hypothermia in TBI was summarized in an invited review article published by the International Trauma, Anesthesia and Critical Care Medicine Society (ITACCS)(8). Dr. Randall Ruppel presented the work on MK-801 at the 1999 Meeting of the National Neurotrauma Society (9). It was one of 12 papers selected for oral presentation out of over 200 papers submitted. The manuscript is in preparation for submission to the *Journal of Neurotrauma.*

Funding year 3

During yr. 3, we carried out a comprehensive study of sedation/analgesia comparing a narcotic (fentanyl) to a conventional general anesthetic (isoflurane). Fentanyl or morphine are the most commonly used narcotics after human head injury while isoflurane is the most commonly used anesthetic in rat models. **We reported remarkably poor outcome in rats treated with narcotics (fentanyl) versus general anesthesia (isoflurane) after CCI. That study is extremely relevant since narcotics are the current field treatment for combat casualties.** That work was presented by Critical Care Medicine fellow research trainee Dr. Kimberly Statler at the 1999 meetings of the National Neurotrauma Society, the Society for Neuroscience, and the Society of Critical Care Medicine (10-12). **Dr. Statler received the 1999 Women in Neurotrauma Award at the National Neurotrauma Meeting and an Educational Scholarship from the Society of Critical Care Medicine.** The full paper was recently submitted to *Journal of Neurosurgery* (13). **The lack of beneficial effect of hypothermia in our model coupled with the remarkably powerful effect of isoflurane suggested the elimination of technical objective 4 in favor of a more comprehensive study of sedatives/analgesics early after CCI (i.e., expansion of proposed technical objective 3).** As this progress report is being prepared, we are completing work on the final study in this proposal, namely a comparison of 7 sedative/analgesic treatments applied in a field relevant paradigm.

(6) BODY

(a) Technical Objective 1: Mechanical ventilation strategies after severe TBI in rats (see summary for 1996-1997 [yr-1] and reference 1, both in appendix).

We showed that aggressive, early HV after TBI augments neuronal death in CA3 hippocampus in a rat model of cerebral contusion. The further reduction of CBF with HV during the low cerebral blood flow state immediately after injury coupled with alkalosis may increase the vulnerability of selected neurons to damage. The results of this study reinforce the meticulous attention necessary to prevent secondary injury after TBI. A risk in the use of HV is demonstrated.

Recommendation: Unless signs of impending herniation (unilateral pupillary dilation, hypertension, bradycardia) are present, this study supports the targeting of normocarbia for mechanical ventilation in the emergency stabilization of the brain trauma victim.

(b) Technical Objectives 2: Testing of field-relevant therapies (notably hypothermia) in experimental models of severe TBI (with and without a secondary hypoxemic insult) in rats.

(b1) *Effect of transient (4 h), moderate (32°C) hypothermia on functional and histological outcome after experimental TBI with a superimposed secondary hypoxemic insult in rats.* **(see summary for 1998-1999 [yr-2] and reference 5, both in appendix).**

We tested the effect of 4 h of hypothermia in our model of TBI with a 30-min secondary hypoxemic insult. Hypothermia is effective in a variety of experimental models with transient application (1-4 h) and in a single-center study in humans (32°C applied for 24 h). However, in neither experimental nor clinical TBI has hypothermia been tested when applied after the combination of TBI with a secondary insult. We found no benefit from hypothermia after this field-relevant combined insult. This suggests three possibilities. First, the combination of TBI plus a secondary hypoxemic insult may be so severe that no single treatment will be effective. Second, the insult is so severe that no therapies will be effective. Third, based on our work in technical objective 3, it is possible that a beneficial effect of hypothermia is being masked by using isoflurane anesthesia (vida infra). This work is in press as a full paper in the journal *Critical Care Medicine* (5).

Recommendation: Even in centers where hypothermia was shown to be effective after TBI, this has not been the case for severely injured patients GCS 3-4. It is likely that severe injuries, such as that modeled by CCI with a 30-min hypoxemic secondary insult, will require combination therapies or may be refractory to all therapy. Also, based on the work by our group on technical objective #3, it will be important in future studies to test hypothermia using a sedative/analgesic approach that is similar to that used in the field or clinic—i.e., with narcotics. To our knowledge, such a study has never been carried out in a rodent model of TBI.

(b2) *Effect of prolonged (12 h), moderate (32°C) hypothermia on functional and histological outcome after experimental TBI in rats.* **(see summary for 1998-1999 [yr-2] in appendix for detailed methods).**

Based on the aforementioned study, in the CCI model, we sought to test, to our knowledge for the first time in any laboratory, the prolonged application of hypothermia in a rodent model of TBI. This included over 13-h of controlled mechanical ventilation and physiological monitoring. To date, only brief 1-4 h applications have been tested. In this study, we examined TBI without a secondary insult. **As described in last year's progress report, we failed to observe important beneficial effects of 12-h of hypothermia on functional or histopathological outcome after CCI.** This is a surprising finding which is discussed below.

Recommendation: Studies of 12 h of hypothermia in any experimental animal model are very labor intensive. The negative result of this study suggests one of two possibilities. First, there may be both beneficial and deleterious aspects to the use of hypothermia. Despite promising data from single clinical sites, recently, a randomized, controlled multi-center trial of hypothermia in human head injury failed to yield a positive result. **Second, once again based on the work by our group on technical objective #3, it will be important in future studies to test hypothermia using a sedative/analgesic approach that is similar to that used in the field or clinic—i.e., with narcotics.** It is our recommendation that this be tried first in a rodent model using either morphine or fentanyl anesthesia followed by either 1 or 4 h of hypothermia vs normothermia.

(b3) *Effect of the anti-excitotoxic NMDA-receptor antagonist MK-801 on functional and histological outcome after experimental TBI in rats.* **(see summary for 1998-1999 [yr-2], in appendix, for detailed methods).**

In the third treatment trial in year two, we sought to test the effect of the traditional NMDA-receptor antagonist MK-801 in our TBI model (without secondary insult). **The NMDA antagonist MK-801 was effective in improving both motor function and some aspects of cognitive function after CCI.** The motor effects were more dramatic than those seen with hypothermia. In a separate pilot study, MK-801 was not effective when tested in our TBI plus secondary hypoxemia model, again suggesting this insult may be too severe for any single therapy. Although this specific agent is not available for clinical use, it suggests that this category of agents –targeting excitotoxicity—represents a viable strategy.

Recommendation: This finding is particularly relevant since our studies addressing technical objective #3 suggested that an anti-excitotoxic general anesthesia strategy such as isoflurane produced a markedly better outcome than treatment with the narcotic analgesic fentanyl. Although there have been several negative clinical trials of anti-excitotoxic therapy, there is frequently a delay in administration of treatment for as much as 6 h in these trials. Several reports have suggested that important components of the excitotoxic response may occur in the initial 1-2 h after injury. **Based on our findings,**

anti-excitotoxic strategies should not be abandoned, rather consideration should be given to the field application of these strategies. In addition, sedative/analgesics with anti-excitotoxic properties must be extensively studied in experimental TBI, in both small animal and large animal models.

(c) Technical Objective 3: Testing of the optimal field-relevant sedative/analgesic therapy in an experimental model of severe TBI in rats (see reference 13 in appendix).

(c1) *Comparison of the effects of TBI on functional and histological outcome after experimental TBI in rats anesthetized with fentanyl or isoflurane* **(described below).**

Currently, in clinical practice, fentanyl is the most commonly used emergency sedative for the intubated patients with severe TBI. Fentanyl, has little direct anti-excitotoxic properties. Thus, to begin investigating this area, we tested how fentanyl treatment compared to standard isoflurane anesthesia in our model.

Outcome protocol

Rats were initially anesthetized with N_2O:O_2 (2:1) and 4% isoflurane and then endotracheally intubated and mechanically ventilated. Anesthesia was maintained for the for surgery with 2 - 2.5% isoflurane and N_2O:O_2 (2:1). Pancuronium bromide (0.1 mg/kg/h) was given iv for muscle relaxation. Femoral venous and arterial vessels were cannulated for continuous blood pressure measurement, blood sampling, and administration of medications. A rectal probe was inserted to monitor core temperature. The rat was then placed in a stereotaxic frame and a left parietal craniotomy was performed. The dura and bone flap were left in place until immediately before CCI. A burr hole was drilled into the left frontal bone for temperature probe placement into the frontal lobe. Continuously monitored physiologic parameters included arterial blood pressure and rectal and brain temperatures. Blood glucose, hematocrit, and arterial blood gas samples were assessed every 15 min for the initial hour and every 30 min thereafter. $PaCO_2$ was controlled at 35 - 45 mm Hg. This protocol produced a PaO_2 of greater than 70 mm Hg in all preparations. Both brain and rectal temperatures were maintained at $37.0 \pm 0.5\,^{\circ}C$.

Rats were allowed to stabilize for 5 min after completion of surgical preparation and then randomized to receive either fentanyl or isoflurane anesthesia. In the fentanyl group (n=9), isoflurane was discontinued and 10 μg/kg of fentanyl was administered iv, followed by a continuous iv infusion at 50 μg/kg/h. In the isoflurane group (n=9), inspired isoflurane concentration was reduced to 1%, and normal saline, the vehicle for fentanyl-treated rats, was administered to match the volume received by fentanyl infusion. Both anesthetic groups continue to receive N_2O:O_2 (2:1). After 30 min equilibration, TBI was induced by CCI. In pilot studies comparing isoflurane and fentanyl using our standard CCI model (6-mm tip, 4 m/s velocity, 50 msec duration of deformation and 2.5-mm deformation depth), all of the fentanyl-treated rats developed pulmonary edema and died early after injury. Thus, to compare the effect of isoflurane

vs fentanyl on long-term outcome in our model, our standard injury was reduced (2.0-mm deformation depth). After CCI, the bone flap was replaced and sealed with dental cement, and the scalp incision was closed. Anesthesia was continued for 4 h in the isoflurane group and 3.5 h in the fentanyl group to facilitate similar extubation times. At the end of the anesthetic period, rats received 100% oxygen, were allowed to awaken and resume spontaneous breathing, and were then extubated and returned to their cages. Sham rats underwent identical preparation/anesthesia, but no CCI (n=6 per group).

Motor function, including beam balance and beam walking tasks, was tested by an observer blinded to group assignment on days 1-5 after injury. Morris Water Maze (MWM) testing was performed using an acquisition paradigm on days 14-20 after injury. Lesion volume and hippocampal neuron survival were assessed on day 21.

ICP Protocol

Based both on results of the above protocol showing that fentanyl-treated rats had higher MAP throughout the experiment and on a recent report that increased MAP may exacerbate injury after TBI, ICP and percent brain water were monitored in a separate cohort of rats (n=9 per anesthetic group) subjected to either fentanyl or isoflurane anesthesia and CCI in an identical paradigm to that used to assess functional outcome.

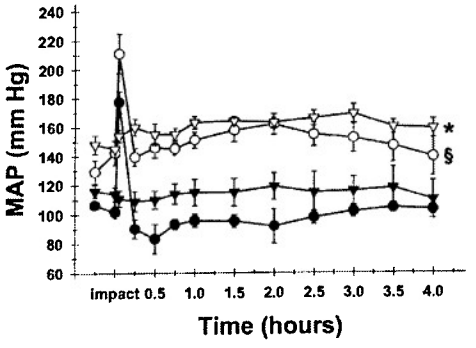

Fig 1: MAP vs time after injury. MAP in fentanyl-treated injured (open circles) and sham (open triangles) rats was ~50 mm Hg higher than in isoflurane-treated rats at all time points (injured shown by closed circles and shams by closed triangles). * $p < 0.05$, isoflurane vs fentanyl at each time after injury, § $p < 0.05$, isoflurane vs fentanyl at all time points, including baseline, in shams.

Surgical preparation, randomization, anesthetic administration, and CCI were identical to the outcome protocol, with minor exceptions. Specifically, an intraparenchymal ICP monitor (Codman microtransducer) was inserted through a burr hole in the frontal bone into the contralateral (right) frontal cortex at the time of craniotomy. After CCI, anesthesia was continued and ICP was monitored for 4 h in both anesthetic groups. Cerebral perfusion pressure (CPP) was calculated as the difference between MAP and ICP. Rats were killed by decapitation at the end of the anesthetic period. Brains were immediately removed and a 3-mm coronal slice was made through the center of the contusion. Per cent brain water was determined in the coronal slice using the wet-dry weight method. Brain water content was determined in both the injured and the homologous region of the uninjured hemispheres.

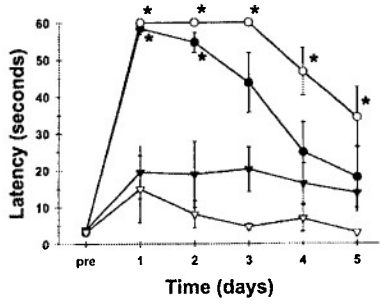

Fig 2: Beam walking latency vs d after injury. Isoflurane-treated rats recovered by post-injury d 3, fentanyl-treated rats failed to regain normal function by the end of the 5-d period. *$p < 0.05$, injured vs sham. Beam balance latency showed similar benefit of isoflurane vs fentanyl..

As an added control, a separate cohort of rats (n=3) was subjected to CCI and allowed to

recover without anesthesia. These rats were prepared for CCI under isoflurane anesthesia as above, allowed to recover a tail-pinch response and then subjected to CCI. Arterial MAP was monitored via a femoral arterial catheter for 4 h during recovery without anesthesia.

Results

Time to extubation did not differ after injury between isoflurane and fentanyl treatment groups (269 ± 7 min vs 275 ± 15 min, p = 0.29). Physiologic values, including $PaCO_2$, PaO_2, blood glucose and Hct did not differ between anesthetic groups. In contrast, MAP was higher in injured rats treated with fentanyl compared to their isoflurane counterparts ($p < 0.05$) during the entire posttrauma period (Fig 1). Similarly, MAP was higher in shams treated with fentanyl vs isoflurane ($p < 0.05$) during the entire duration of anesthesia (Fig 1). Fentanyl-treated rats had a MAP of ~150 mm Hg compared to ~105 mm Hg in the isoflurane groups.

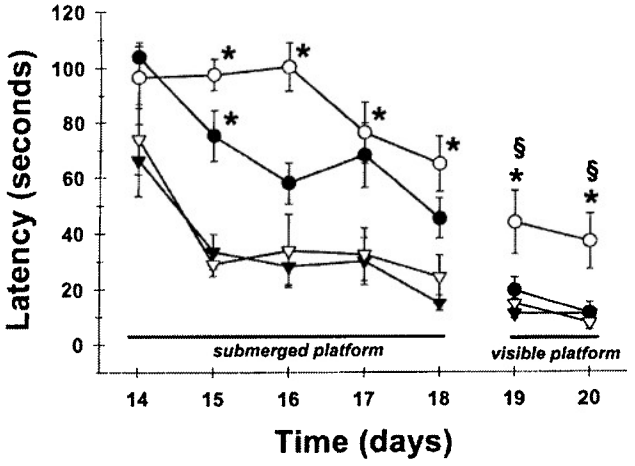

Fig 3: Latency to find platform vs time after injury in an acquisition paradigm of the MWM. Shams anesthetized with isoflurane (closed triangles) or fentanyl (open triangles) had similar performances. During the first few d of hidden platform testing, injured rats in fentanyl (open circles) and isoflurane (closed circles) groups had impaired performance vs sham. By d 3, latencies to find the hidden platform were similar in injured isoflurane-treated rats and shams. In contrast, longer latencies persisted in injured fentanyl-treated rats throughout the 5-d hidden platform testing. Latencies in all groups improved during visible platform testing; but, shams and injured isoflurane-treated rats performed better than injured fentanyl-treated rats during visible platform testing. $*p < 0.05$, injured vs sham; § $p < 0.05$, isoflurane vs fentanyl.

Rats anesthetized with isoflurane performed better on beam balance and beam walking tasks after TBI compared to their fentanyl counterparts ($p < 0.05$, Fig 2). Following injury, isoflurane-anesthetized rats also performed better than their fentanyl-treated counterparts during MWM testing with a hidden platform ($p < 0.05$, Fig 3). Motor and MWM performances did not differ between sham groups.

Lesion volume, expressed as mm^3 or as percent of uninjured hemisphere, at 21 d did not differ between treatment groups (see reference 13 in appendix for details). In contrast, neuron counts in the injured CA1 hippocampus were markedly greater in isoflurane-treated rats ($p < 0.05$, Fig 4). Neuron counts in the injured CA3 hippocampus, however, did not differ significantly between treatment groups

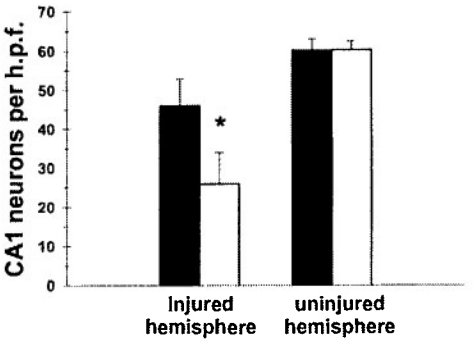

Fig 4: Neuron counts in injured CA1 hippocampus were greater in isoflurane- vs fentanyl-treated rats. Neuron counts in uninjured CA1 hippocampus were similar in both treatment groups. $*p < 0.05$, injured vs uninjured.

(see reference 13 in appendix for details).

In the ICP protocol, again physiologic values, including $PaCO_2$, PaO_2, glucose and Hct, did not differ between anesthetic groups. As in the outcome protocol, MAP was higher in rats treated with fentanyl compared to their isoflurane counterparts ($p < 0.05$). ICP was similar in both anesthetic groups; however, there was a trend toward higher ICP in rats anesthetized with isoflurane by 3-4 h after TBI (Fig 5). This strongly suggests that the higher MAP in fentanyl vs isoflurane treated rats did not exacerbate intracranial hypertension. As expected from the difference in MAP, CPP was higher in the fentanyl treatment group (Fig 6).

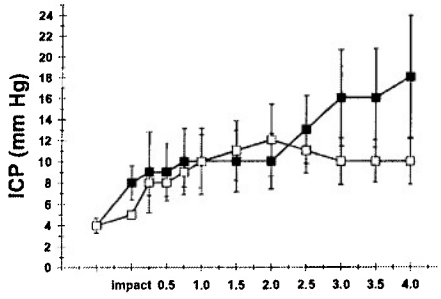

Fig 5: ICP vs time after injury. Initial ICP was ~4 mm Hg in both isoflurane (open squares) and fentanyl (closed squares) groups. ICP gradually increased, reaching 10-18 mm Hg by 4 h. ICP was similar between groups; but, isoflurane-treated rats showed a trend toward higher ICP after injury (vs fentanyl) that did not reach significance.

Brain water content, assessed at 4 h after injury, was higher in the injured vs uninjured hemisphere ($p < 0.05$) for both anesthetic groups (Fig 7). However, brain water in either the injured or uninjured hemisphere did not differ between isoflurane- and fentanyl-treated rats, indicating that edema was not exacerbated in the fentanyl group.

Average MAP during the 4 h post-injury observation period did not differ significantly between fentanyl-treated rats and those recovering from CCI without anesthesia (157 ± 6.2 mm Hg vs 147 ± 7.1 mm Hg, NS). In contrast, isoflurane-anesthetized rats had lower MAP (105 ± 5.5 mm Hg) vs both fentanyl-treated rats and rats recovering without anesthesia ($p < 0.05$ vs both groups).

Recommendation: The theoretical advantages of isoflurane vs fentanyl are compelling; however, explanations for the observed improvement in neurologic outcome after TBI in isoflurane-anesthetized rats remain to be determined. What has become clearer is that anesthetic agents may have considerable impact upon outcome following TBI. **The results of this study suggest two important potential ramifications. First, isoflurane may not represent the optimal anesthetic in experimental TBI since it may mask potential benefits of novel therapies. Second, despite common clinical and field use, narcotics such as morphine or fentanyl may not be the optimal sedative/analgesic agent to administer to patients in the acute phase after severe head injury. We feel this has considerable relevance since narcotics (either fentanyl or morphine) are first line agents in field or emergency**

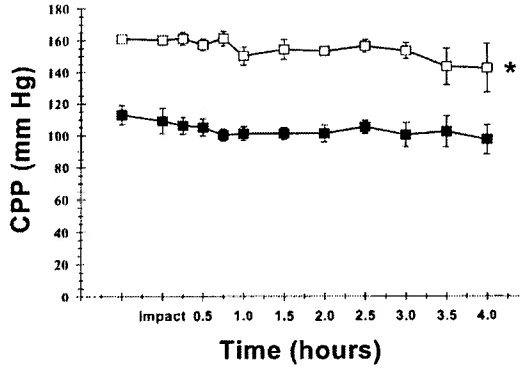

Fig 6: CPP vs time after injury. CPP was increased in rats treated with fentanyl (open squares) vs isoflurane (closed squares), * $p < 0.05$, isoflurane vs fentanyl at all time points except 3.5 and 4h.

department. Consideration should also be given to the possibility that like isoflurane anesthesia could be provided in the field. However, additional studies in rodent and large animal models of TBI are indicated. Specifically, further study of the mechanistic differences between isoflurane and fentanyl anesthesia is warranted. Our suspicion is that narcotics are not deleterious, rather general anesthetics such as isoflurane are powerfully beneficial. Defining the factors responsible for improved outcome with isoflurane may help to direct the clinical application of more optimal sedative or analgesic agents and possibly to identify novel therapies. **Finally, since the immediate post-trauma sedative/analgesic regimen has such a powerful effect on both functional and histopathological outcome, a comprehensive comparison of field-relevant sedative/analgesic agents is suggested by this study and is underway (as described below).**

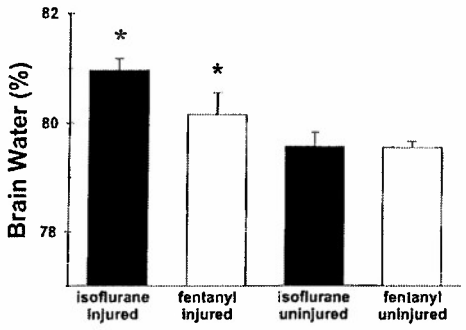

Fig 7: Percent brain Water 4 h after TBI. Percent brain water in the injured hemisphere was increased vs respective non-injured hemisphere in both isoflurane- and fentanyl-treated rats; however, brain water did not differ between anesthetic groups. * $p < 0.05$, isoflurane vs fentanyl.

(C2) Randomized, blinded study in the rat model of CCI of seven different sedative/analgesic strategies for field use in TBI.

We are currently in the midst of completing a nine group (seven anesthetic) study in our model. Rats are prepared for TBI exactly as described in our protocol comparing isoflurane and fentanyl above. Anesthesia for surgical preparations is 2% isoflurane in nitrous oxide/oxygen. After surgical preparation, anesthesia is discontinued until tail-pinch response is obtained and then CCI is delivered. Rats are then randomized to one of the 8 groups below (n = 8 per group, Table 1). There is also a sham group (thus, a total of 9 groups). The sedation or anesthesia is maintained for a period of 60 min and the rats are then weaned and extubated when recovered. Outcome parameters are identical to the isoflurane vs fentanyl outcome study (motor and MWM function; lesion volume, hippocampal CA1 and CA3 cell counts. To date we have completed 30 of the studies.

Table 1. Sedation/analgesia study posttrauma

Anesthetic/Sedative	Dose
Isoflurane	1% by inhalation for 1 h
Pentobarbital	50 mg/kg iv
Morphine	
Fentanyl	
Diazepam	
Ketamine	
Propofol	
None[1]	NA
Sham	NA

[1]Isoflurane anesthesia discontinued and TBI immediately on return of tail pinch reflex.

Comment: This study will complete the expanded technical objective #3 (in lieu of elimination of objective #4) and is obvious, particularly since our isoflurane vs fentanyl study showed such a

powerful difference in outcome. **We anticipate completing this ambitious protocol by February 28, 2000.** Delay in beginning this protocol related to the need to perform pilot studies with each anesthetic in our CCI model.

(7) KEY RESEARCH ACCOMPLISHMENTS

In order of importance

Narcotics

- **Narcotics, the standard field-treatment of victims of severe head injury (after intubation) and a front line treatment in emergency departments in the civilian sector had not been compared head-to-head with general anesthesia in a contemporary rodent model of TBI. We found that after experimental TBI, rats anesthetized with isoflurane exhibited markedly better functional and histopathological outcomes versus those treated with a narcotic (fentanyl). Narcotics probably are not the optimal sedative/analgesic early after TBI. Consideration should also be given to the possibility that light isoflurane anesthesia could be provided in the field.**

Hyperventilation

- **Aggressive hyperventilation for 4-5 h early after TBI is associated with an exacerbation of hippocampal neuronal death in selectively vulnerable brain regions adjacent to the contusion site.**

Hypothermia

- **Transient, moderate hypothermia, effective after TBI alone in prior studies, was demonstrated to be ineffective after experimental TBI in rats subjected to TBI with a superimposed secondary insult (hypoxemia). This may be clinically important since hypoxemic patients have not been randomized in current clinical trials of hypothermia after TBI.**

Mechanisms

- **Moderate hypothermia reduces markers of injury (such as DNA damage) early after experimental TBI. However, sustained (12 h) of hypothermia was also surprisingly ineffective (on long-term outcome) after experimental TBI in rats. This suggests that although there are beneficial effects of hypothermia, there are potential side effects.**

(8) REPORTABLE OUTCOMES

Manuscripts[§]

Forbes ML, Clark RSB, Dixon CE, Graham SH, Marion DW, DeKosky ST, Schiding JK, Kochanek PM: Augmented neuronal death in CA3 hippocampus following hyperventilation early after controlled cortical impact. *Journal of Neurosurgery* 88:549-556, 1998.

Kochanek PM, Safar P, Marion DW, Tisherman SA, Clark RSB, DeKosky ST: Therapeutic Hypothermia After Traumatic Brain Injury or Hemorrhagic Shock: From Mild Cooling To Suspended Animation In: Hypothermia in Trauma: Deliberate or Accidental. ITACCS Monograph, CE Smith and CM Grande, Editors, Baltimore, Maryland, 1997, pp 17-20.

Robertson CL, Clark RSB, Dixon CE, Graham ST, Alexander HL, Wisniewski SR, Marion DW, Safar PJ, Kochanek PM: No Long Term Benefit from Hypothermia after Severe Traumatic Brain Injury with Secondary Insult in Rats. *Critical Care Medicine* (in press).

Statler KD, Kochanek PM, Dixon CE, Alexander HL, Warner DS, Clark RSB, Wisniewski SR, Graham SH, Jenkins LW, Marion DW, Safar PJ: Isoflurane improves long-term neurologic outcome vs. fentanyl after traumatic brain injury in rats. *Journal of Neurosurgery* (in submission).

[§]*Full manuscripts from abstracts (see below) Whalen et al., and Ruppel et al. are also in preparation.*

Abstracts and Presentations

Alexander HL, Robertson CL, Dixon CE, Clark RSB, Graham SH, Safar PJ, Kochanek PM: Vertical Versus Angled Controlled Cortical Impact in Rats. *Journal of Neurotrauma* 15:854, 1998.

Clark RSB, Robertson CL, Dixon CE, Alexander HL, Graham SH, Safar PJ, Kochanek PM: Effect of Hypothermia After Severe Traumatic Brain Injury with Secondary Hypoxemia in Rats. *Journal of Neurotrauma* 15:864, 1998.

Robertson CL, Clark R, Dixon CE, Graham S, Alexander H, Wisniewski S, Marion D, Safar P, Kochanek P: No Long-Term Benefit from Hypothermia After Severe Traumatic Brain Injury with Secondary Hypoxemia in Rats. *Critical Care Medicine* 27(1):A52, 1999.

Whalen M, Chen M, Clark R, Jin K, Kochanek P, Marion D, Graham S: DNA Damage is Temperature Dependent Early After Traumatic Brain Injury in Rats. *Society for Neuroscience Abstracts* 24:252, 1998.

Whalen M, Chen M, Clark R, Jin K, Kochanek P, Marion D, Graham S: DNA Damage is Temperature Dependent Early After Traumatic Brain Injury in Rats. *Critical Care Medicine* 27:A51, 1999.

Ruppel RA, Kochanek PM, Dixon CE, Alexander HL, Graham SH, Clark RSB, Wisniewski SR, Marion DW, Safar PJ: MK-801 improves functional outcome in rats after controlled cortical impact. *Journal of Neurotrauma* 16:986, 1999.

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Statler KD, Kochanek PM, Dixon CE, Alexander H, Warner DS, Clark RSB, Wisniewski S, Graham SH, Jenkins LW, Ma X, Marion DW, Safar P: Fentanyl versus isoflurane anesthesia: Effect on outcome after traumatic brain injury in rats. *Journal of Neurotrauma* 16:965, 1999.

Statler KD, Kochanek PM, Dixon CE, Alexander HL, Warner DS, Clark RSB, Wisniewski SR, Jenkins LW, Marion DW, Safar PJ: Isoflurane improves long-term neurologic outcome compared to fentanyl after traumatic brain injury in rats. *Critical Care Medicine* 27:A38, 1999.

AWARDS

(1999) Women in Neurotrauma Award to Dr. Kimberly Statler for her presentation to the 1999 Meeting of the National Neurotrauma Society entitled, "Fentanyl versus isoflurane anesthesia: Effect on outcome after traumatic brain injury in rats."

(2000) Educational Scholarship to Dr. Kimberly Statler from the Society of Critical Care Medicine for her abstract entitled, "Isoflurane improves long-term neurologic outcome compared to fentanyl after traumatic brain injury in rats."

(9) CONCLUSIONS

1. Based on the important finding in this study where the use of fentanyl in our CCI model produced deleterious effects on outcome after TBI, animal models should utilize clinically relevant sedative/analgesic treatments. The beneficial mechanisms of isoflurane (possibly promotion of cerebral blood flow or reduction of excitotoxicity) should be investigated for the development of novel treatments. **Narcotics may not be the optimal sedative/analgesic early after TBI. Better field therapy than narcotics must be developed.** Finally, more powerful sedative agents already in clinical use may represent better alternatives (and are currently under investigation in studies completing technical objective 3).

2. Based on studies in rats using the CCI model, we have demonstrated tangible risk to aggressive, indiscriminate hyperventilation early after injury—specifically—augmentation of neuronal death in selectively vulnerable brain regions. This suggests that aggressive hyperventilation should not be indiscriminately used in the field treatment of TBI, rather it should be applied if there are signs and/or symptoms of herniation. Mild hyperventilation (used in our control group) or normocapnia may be preferable.

3. Based on our studies in rats, hypothermia, although showing some beneficial effects, particularly early after TBI (such as a reduction in DNA damage, etc), may have some deleterious effects which result in only modest overall beneficial effects on long-term outcome. This is particularly true in the setting of severe injury (such as TBI plus secondary hypoxemic insults) where it is possible that there is little to gain except side effects. Also based on our narcotic (fentanyl) vs isoflurane study, hypothermia should be re-examined in future studies with narcotic anesthesia, since beneficial effects of isoflurane may be masking any benefit from hypothermia.

(10) REFERENCES

1. Forbes ML, Clark RSB, Dixon CE, Graham SH, Marion DW, DeKosky ST, Schiding JK, Kochanek PM: Augmented neuronal death in CA3 hippocampus following hyperventilation early after controlled cortical impact. *Journal of Neurosurgery* 88:549-556, 1998.

2. Alexander HL, Robertson CL, Dixon CE, Clark RSB, Graham SH, Safar PJ, Kochanek PM: Vertical Versus Angled Controlled Cortical Impact in Rats. *Journal of Neurotrauma* 15:854, 1998.

3. Clark RSB, Robertson CL, Dixon CE, Alexander HL, Graham SH, Safar PJ, Kochanek PM: Effect of Hypothermia After Severe Traumatic Brain Injury with Secondary Hypoxemia in Rats. *Journal of Neurotrauma* 15:864, 1998.

4. Robertson CL, Clark R, Dixon CE, Graham S, Alexander H, Wisniewski S, Marion D, Safar P, Kochanek P: No Long-Term Benefit from Hypothermia After Severe Traumatic Brain Injury with Secondary Hypoxemia in Rats. *Critical Care Medicine* 27(1):A52, 1999.

5. Robertson CL, Clark RSB, Dixon CE, Graham ST, Alexander HL, Wisniewski SR, Marion DW, Safar PJ, Kochanek PM: No Long Term Benefit from Hypothermia after Severe Traumatic Brain Injury with Secondary Insult in Rats. *Critical Care Medicine* (in press).

6. Whalen M, Chen M, Clark R, Jin K, Kochanek P, Marion D, Graham S: DNA Damage is Temperature Dependent Early After Traumatic Brain Injury in Rats. *Society for Neuroscience Abstracts* 24:252, 1998.

7. Whalen M, Chen M, Clark R, Jin K, Kochanek P, Marion D, Graham S: DNA Damage is Temperature Dependent Early After Traumatic Brain Injury in Rats. *Critical Care Medicine* 27:A51, 1999.

8. Kochanek PM, Safar P, Marion DW, Tisherman SA, Clark RSB, DeKosky ST: Therapeutic Hypothermia After Traumatic Brain Injury or Hemorrhagic Shock: From Mild Cooling To Suspended Animation In: Hypothermia in Trauma:

Deliberate or Accidental. ITACCS Monograph, CE Smith and CM Grande, Editors, Baltimore, Maryland, 1997, pp 17-20.

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11. Statler KD, Kochanek PM, Dixon CE, Alexander H, Warner DS, Clark RSB, Wisniewski S, Graham SH, Jenkins LW, Ma X, Marion DW, Safar P: Fentanyl versus isoflurane anesthesia: Effect on outcome after traumatic brain injury in rats. *Journal of Neurotrauma* 16:965, 1999.

12. Statler KD, Kochanek PM, Dixon CE, Alexander HL, Warner DS, Clark RSB, Wisniewski SR, Jenkins LW, Marion DW, Safar PJ: Isoflurane improves long-term neurologic outcome compared to fentanyl after traumatic brain injury in rats. *Critical Care Medicine* 27:A38, 1999.

13. Statler KD, Kochanek PM, Dixon CE, Alexander HL, Warner DS, Clark RSB, Wisniewski SR, Graham SH, Jenkins LW, Marion DW, Safar PJ: Isoflurane improves long-term neurologic outcome vs. fentanyl after traumatic brain injury in rats. *Journal of Neurosurgery* (in submission).

(11) APPENDIX

1. **Figure 1**
   Figure 2
   Figure 3
   Figure 4
   Figure 5
   Figure 6
   Figure 7

2. **1997 Report**

3. **1998 Report**

4. **Curriculum Vitae**

5. **Manuscripts:**

Forbes ML, Clark RSB, Dixon CE, Graham SH, Marion DW, DeKosky ST, Schiding JK, Kochanek PM: Augmented neuronal death in CA3 hippocampus following

hyperventilation early after controlled cortical impact. *Journal of Neurosurgery* 88:549-556, 1998.

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6. Abstracts:

Alexander HL, Robertson CL, Dixon CE, Clark RSB, Graham SH, Safar PJ, Kochanek PM: Vertical Versus Angled Controlled Cortical Impact in Rats. *Journal of Neurotrauma* 15:854, 1998.

Clark RSB, Robertson CL, Dixon CE, Alexander HL, Graham SH, Safar PJ, Kochanek PM: Effect of Hypothermia After Severe Traumatic Brain Injury with Secondary Hypoxemia in Rats. *Journal of Neurotrauma* 15:864, 1998.

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(12) BINDING (N/A)

(13) FINAL REPORTS

a) **Bibliography of all publications and abstracts (see appendix)**

b) **List of personnel receiving pay from the research effort**

Patrick M. Kochanek, M.D.
Peter Safar, M.D.
Henry Alexander
Scott Heineman
Marci Provins
Linda Amick

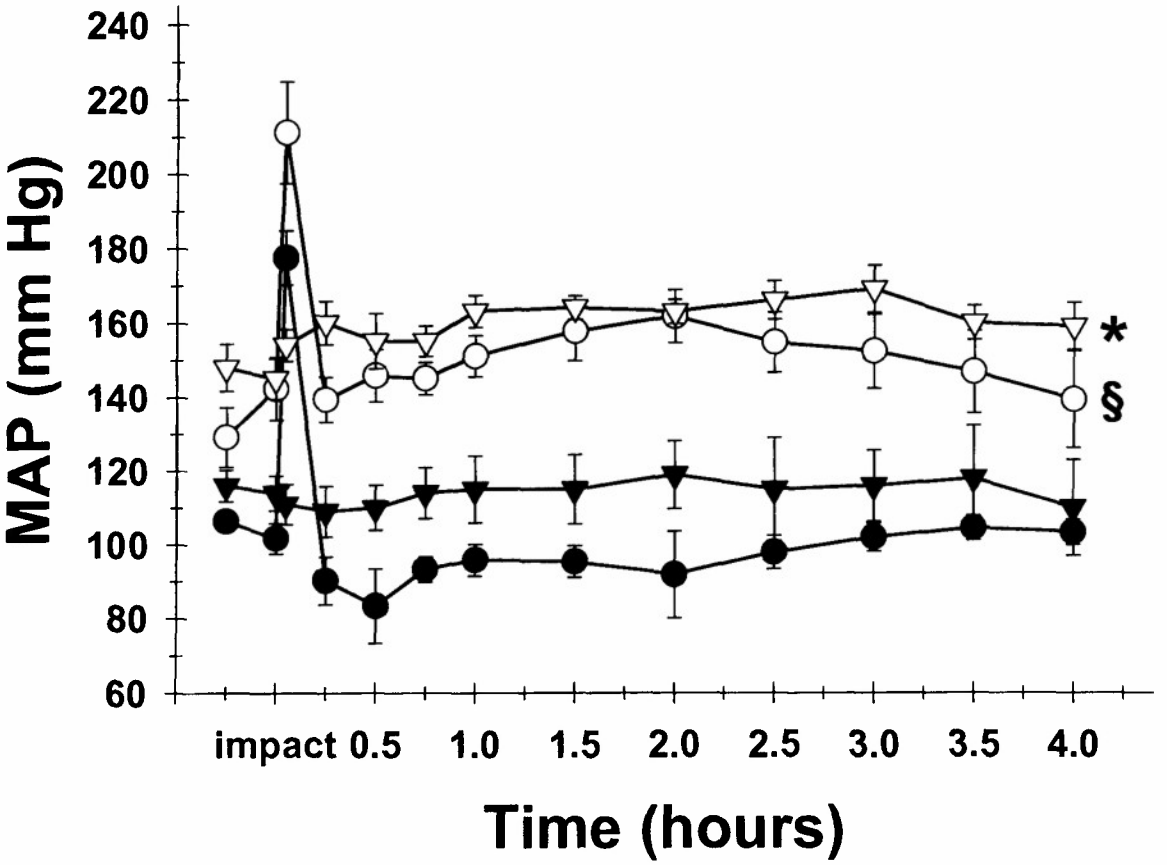
240
220
200
180
160
140
120
100
80
60
MAP (mm Hg)
impact
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Time (hours)
*
§

FIGURE 1

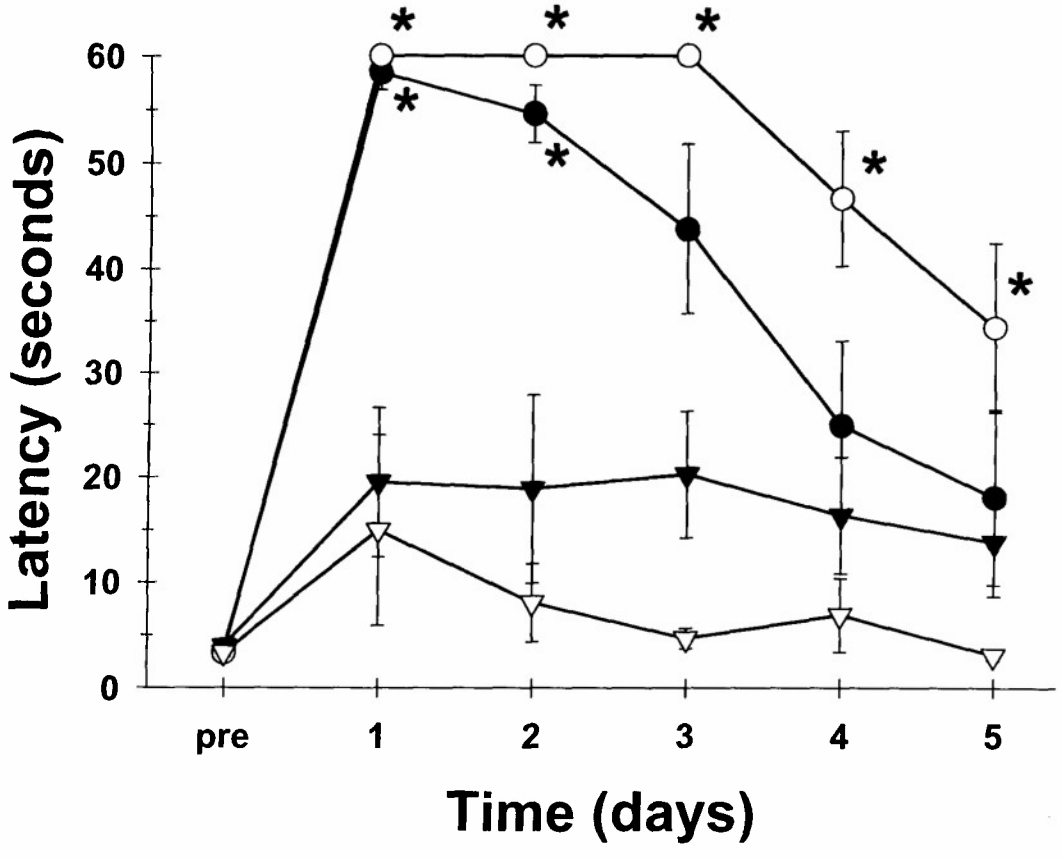

FIGURE 2

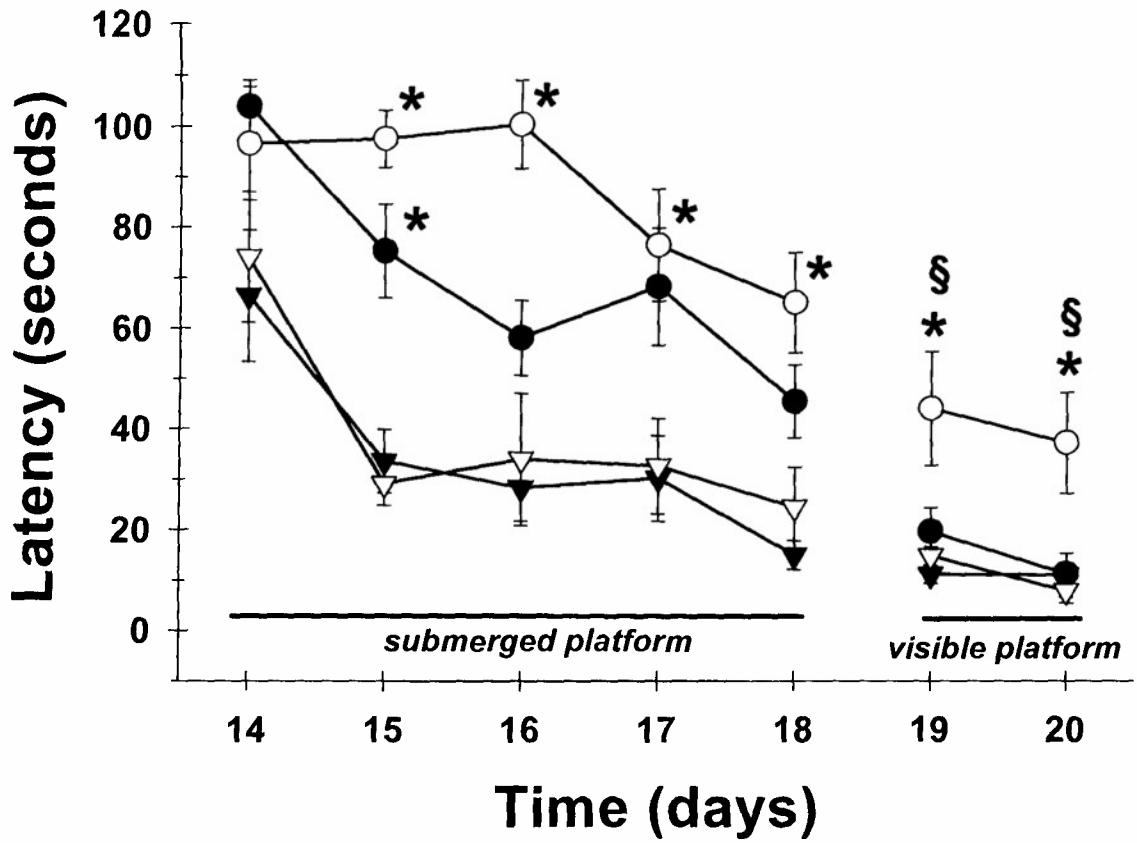
Latency (seconds)
120
100
80
60
40
20
0
*
*
*
*
*
§
*
§
*
submerged platform
visible platform
14
15
16
17
18
19
20
Time (days)

FIGURE 3

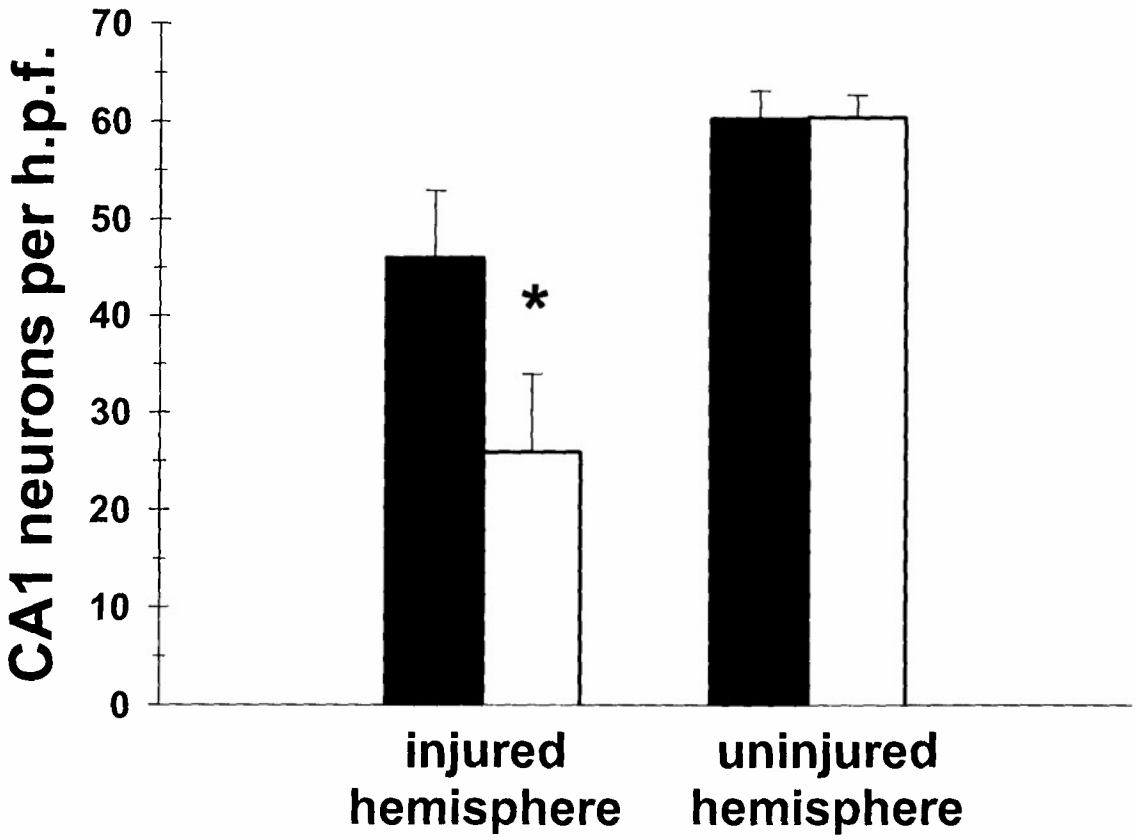
CA1 neurons per h.p.f.
70
60
50
40
30
20
10
0
*
injured hemisphere
uninjured hemisphere

FIGURE 4

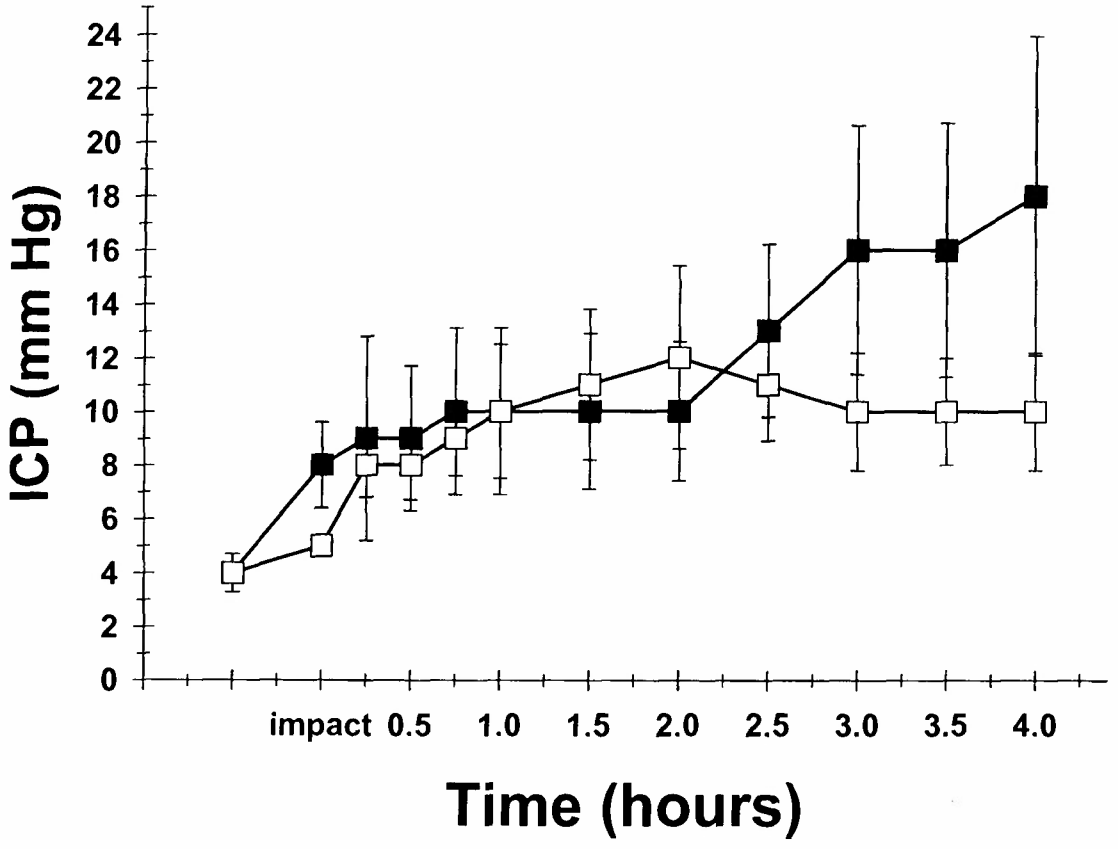
ICP (mm Hg)
24
22
20
18
16
14
12
10
8
6
4
2
0
impact
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Time (hours)

FIGURE 5

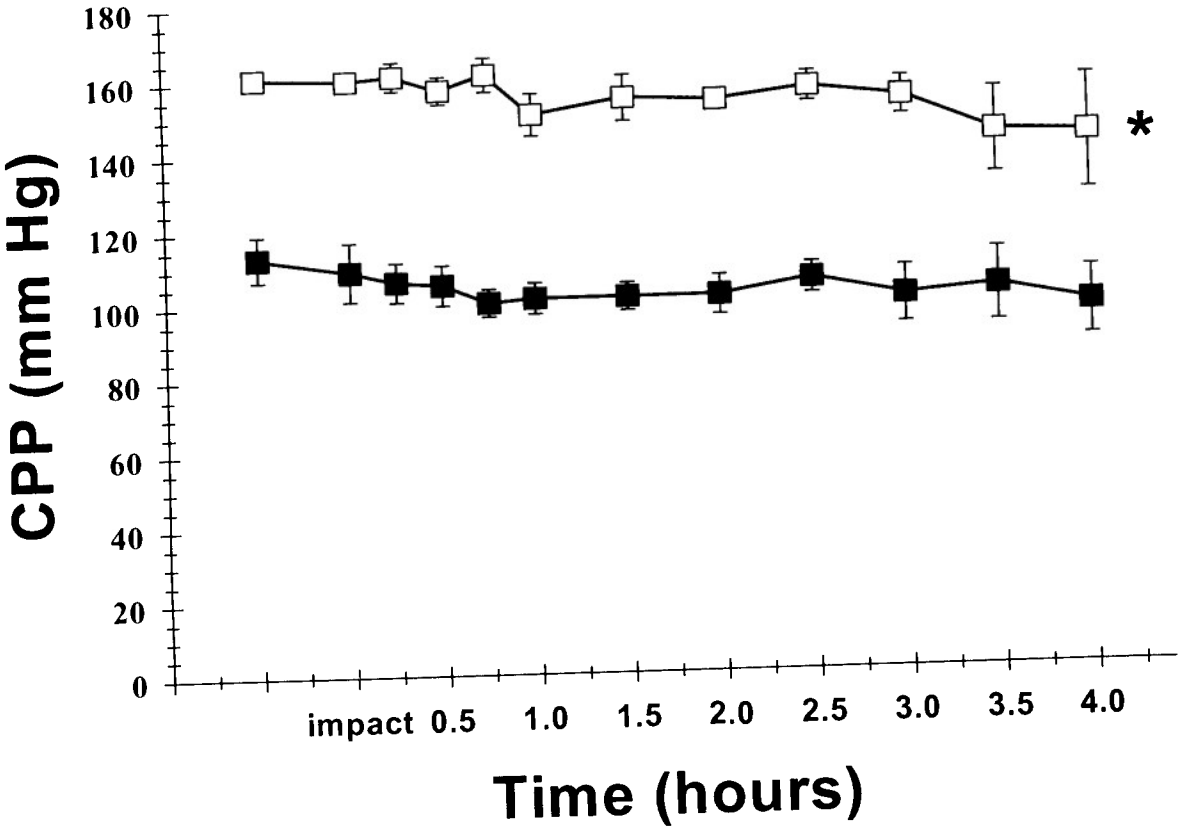
180
160
140
120
100
80
60
40
20
0
CPP (mm Hg)
*
impact 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0
Time (hours)

FIGURE 6

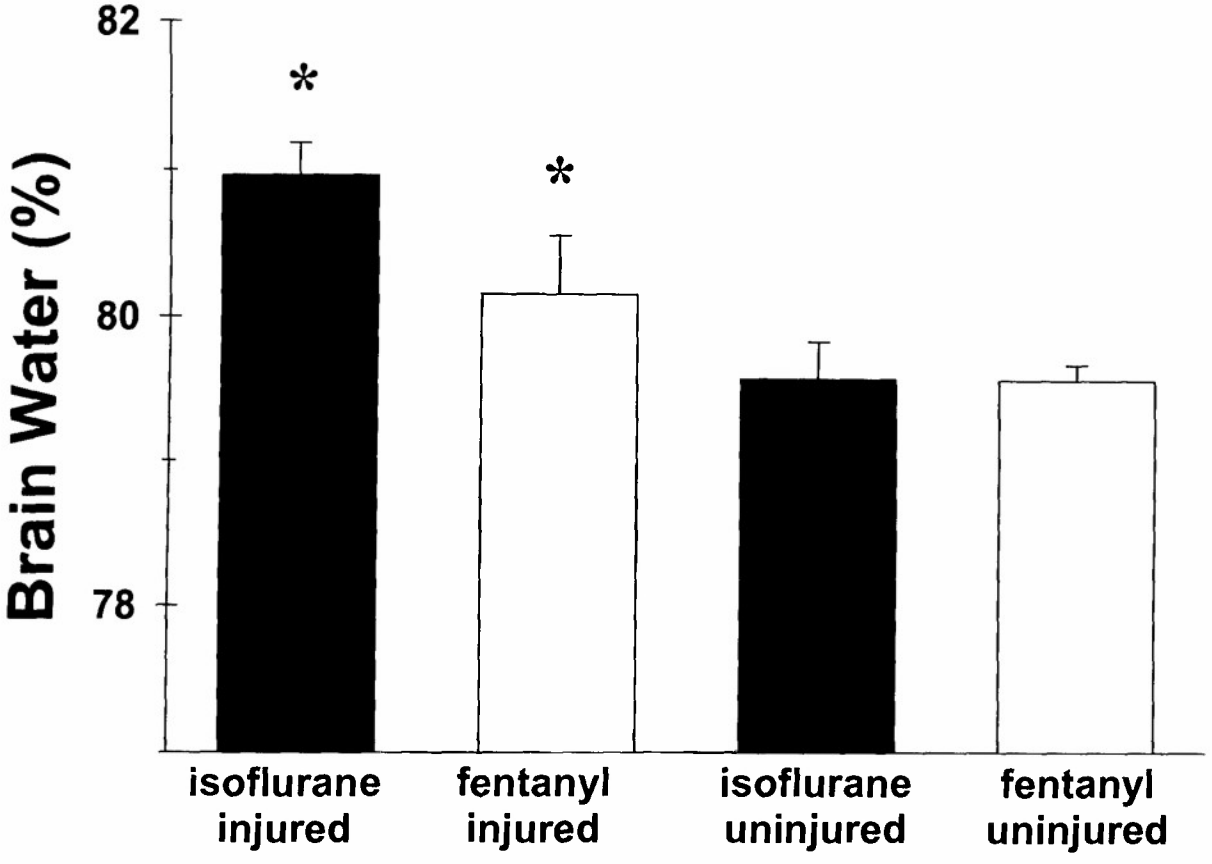
82
80
78
Brain Water (%)
*
*
isoflurane injured
fentanyl injured
isoflurane uninjured
fentanyl uninjured

FIGURE 7

AD_____

Grant Number DAMD17-97-1-7009

TITLE: Emergency Interventions After Severe Traumatic Brain Injury in Rats: Effect on Neuropatholgy and Functional Outcome

PRINCIPAL INVESTIGATOR: Patrick M. Kochanek, M.D.

CONTRACTING ORGANIZATION: University of Pittsburgh
Pittsburgh, Pennsylvania 15260

REPORT DATE: January 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5400

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6. AUTHOR(S)
Patrick M. Kochanek, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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Pittsburgh, Pennsylvania 15260

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13. ABSTRACT *(Maximum 200*

Traumatic brain injury (TBI) contributes to combat morbidity/mortality. We hypothesized that optimal emergency treatment can reduce brain injury in a rat model. Our ultimate goal is translation to the human condition. In yr-1, we studied mechanical ventilation strategies. We found that aggressive hyperventilation early after TBI is detrimental. Also, we developed a model of TBI plus secondary hypoxemia to study therapies, since secondary insults are common. In yr-2, we performed 3 studies, and began a 4th --addressing objectives 2-3. We found that TBI plus secondary hypoxemia was refractory to 4 h of hypothermia--suggesting the need for combination therapies. We also tested prolonged hypothermia (12 h) in our model. Hypothermia improved motor function early after injury. However, by 2 wks, rats treated with hypothermia deteriorated and were ultimately worse (vs normothermia). This suggests the need for studies of hypothermia plus other therapies. We found that the NMDA antagonist MK-801 improved outcome after TBI-- suggesting excitotoxicity as a promising therapeutic target. Fentanyl is used in patients with TBI; but lacks anti-excitotoxic properties. We are evaluating fentanyl in our model. Two fellows worked with the PI, and presented 3 abstracts (2-4). We also published an invited review (6).

14. SUBJECT TERMS
Head Injury, Hyperventilation, Hypothermia, Trauma, Apoptosis Excitotoxicity, Sedation, Oxygen

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✓ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

NA For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

NA In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

NA In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

I/C 13-Jan-99

PI - Signature Date

U.S. Army Medical Research Acquisition Activity

1998 Annual Technical Report

Table Of Contents

INTRODUCTION

In our application, we highlighted the fact that traumatic brain injury (TBI) is an important contributor to combat casualty morbidity and mortality. We also stated that although progress has been made in the development of rodent models of TBI (such as the controlled cortical impact (CCI) model), most of the studies with these models have focused on molecular mechanisms of damage. Because there has been a paucity of application of **practical emergency interventions** in TBI models, we felt that it was important to address this deficiency and that this approach could have important implications for field and emergency management of both soldiers and civilians with severe TBI.

Our overall **hypothesis** is that optimal manipulation of practical interventions applicable in the emergency treatment of severe TBI (respiratory management, temperature control, and sedation) can reduce secondary brain injury in a rat model of brain contusion, and thereby improve functional and/or neuropathological outcome.

In the yr-1 of funding, we addressed the most important aspect of the **first Technical Objective** of our proposal -namely- to perform a comprehensive study of the effects of mechanical ventilation strategies (as applied by the first responder in the field) on both functional and neuropathological outcome in our model. **We found that aggressive, prophylactic hyperventilation (HV) applied for 4 hours immediately after injury is detrimental (vs ventilation to a normal $PaCO_2$), and leads to an increase in the amount of neuronal death in selectively vulnerable brain regions.** This study was published as a full manuscript in the ***Journal of Neurosurgery*** (1). We were pleased that the reviewers indicated that this was an important study that would be cited often.

Also, to set the stage for the evaluation of therapies targeting improvement in outcome after severe TBI (as proposed studies in technical objectives 2-4), it appeared that it would be optimal to have TBI models both with and without a secondary insult since such insults are common in the field. This was done by adding a 30 min period of moderate hypoxemia to the CCI insult. The characterization of that model was described in last year's report and presented this year at the National Neurotrauma Society Meeting (2). As evidenced below, during yr-2, we have used both the standard CCI model and the CCI plus secondary insult model to provide insight on important therapies.

This year we performed three comprehensive studies addressing Technical Objective III and part of Objective II. In addition, we have begun a fourth study. These studies included 1) assessment of the effect of transient (4 h), moderate hypothermia on outcome after TBI with a secondary insult, 2) assessment of the effect of prolonged (12 h) moderate hypothermia on outcome after TBI, 3) assessment of the effect of the application of anti-excitotoxic therapy (the NMDA-receptor antagonist MK-801) early after TBI in our model, and 4) comparison of injury using two different anesthetic regimens (isoflurane or fentanyl [the standard emergency department and ICU sedative]). The results of these studies are summarized below. Finally, two research fellows (Drs. C. Robertson and R. Ruppel) worked on these projects with the PI (Dr. Kochanek) during yr-2. Dr. Robertson presented two abstracts of this work-- at the 1999 annual meeting of the National Neurotrauma Society (2,3) --and will present another abstract at the

Annual Meeting of the Society of Critical Care Medicine (4). That work is currently being prepared in full manuscript form. Also, in related studies, we recently reported that 4 hours of moderate hypothermia attenuates DNA damage assessed at 4 hours after injury using the Klenow method (5). Finally, some of our work on hypothermia in TBI was summarized in an invited review article that we published in a monograph by the International Trauma, Anesthesia and Critical Care Medicine Society (ITACCS)(6).

(6) BODY

(a) Technical Objective 1: Mechanical ventilation strategies after severe TBI in rats (see summary for 1996-1997 [yr-1]). Also see reference 1.

Recommendation

We showed that aggressive, early HV after TBI augments neuronal death in CA3 hippocampus in a rat model of cerebral contusion. The further reduction of CBF with HV during the low cerebral blood flow state immediately after injury coupled with alkalosis may increase the vulnerability of selected neurons to damage. The results of this study reinforce the meticulous attention necessary to prevent secondary injury after TBI. A risk in the use of HV is demonstrated. Unless signs of impending herniation (unilateral pupillary dilation, hypertension, bradycardia) are present, this study supports the targeting of normocarbia for mechanical ventilation in the emergency stabilization of the brain trauma victim.

(b) Technical Objectives 2-4: Testing of field-relevant therapies in experimental models of severe TBI (with and without a secondary hypoxemic insult) in rats .

(b1) *Effect of transient (4 h), moderate (32℃) hypothermia on functional and histological outcome after experimental TBI with a superimposed secondary hypoxemic insult in rats.*

We tested the effect of 4 hours of hypothermia in our model of TBI with a 30-min secondary hypoxemic insult. Hypothermia has been shown to be effective in a variety of experimental models with transient application (1-4 h) and in humans (32°C applied for 24 h). **However, in neither experimental nor clinical TBI has hypothermia been tested when applied after the combination of TBI with a secondary insult.**

Method

All of the protocols for the studies outlined below were approved by the Animal Care and Use Committee of the University of Pittsburgh. Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats (n = 43) were subjected to CCI (4 m/s, 2.5 mm depth of deformation) followed by a 30 min controlled hypoxemic insult that reproducibly results in a PaO_2 of 40-45 mm Hg. Rats were treated with one of the following three regimens—1) Brain temperature maintained at 37°C applied throughout a 5 hours period (n = 19), 2) Brain temperature maintained at 32°C applied for 4 hours beginning after insult (beginning after both TBI and secondary hypoxemia) and then followed by re-warming over 1 hour (n = 14), and 3) Brain temperature maintained at 37°C applied immediately after TBI (before the secondary

hypoxemic insult) and continued for 4 hours and followed by re-warming over 1 hour (n = 10). After 5 h, rats were weaned from mechanical ventilation, extubated and returned to their cages. Beam balance/beam walking and Morris water maze (MWM) performance latencies were measured in eight rats from each group on days 1-5 and 14-20 post CCI, respectively. Rats were killed at 21 d. Serial coronal sections of brain were studied for contusion volume and hippocampal neuron counting [CA1, CA3] by a blinded observer.

Results

There were no significant differences in recovery of motor function (beam balance, beam walking, Figure 1) tested on days 1-5 after injury or cognitive function (spatial memory acquisition paradigm on the Morris water maze [MWM], Figure 2) tested between days 14-20 after injury. There were also no significant differences in lesion volume or hippocampal neuron counts between groups at 21 days after injury (Table 1). There was a trend toward reduced contusion volume in the immediate post injury group, however, it did not reach statistical significance.

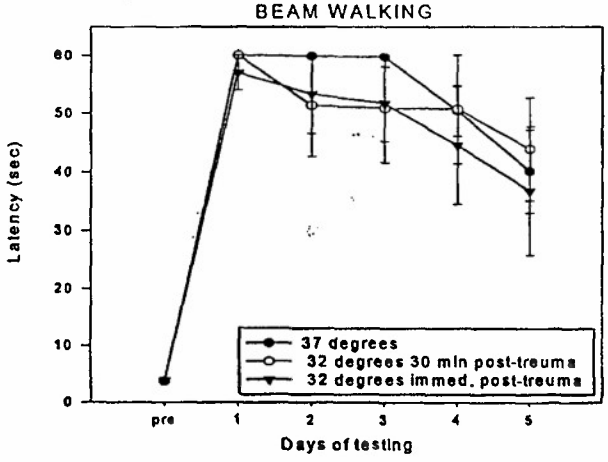

Figure 1. Effect of hypothermia on motor outcome after experimental TBI plus a secondary hypoxemic insult in rats. Mean beam walking performance latencies (mean ± SEM, in sec) in rats before and on days 1-5 after CCI (4 m/s, 2.5 mm cortical deformation depth). Analysis of variance with repeated measures revealed no difference between the three groups. Data are mean ± SEM.

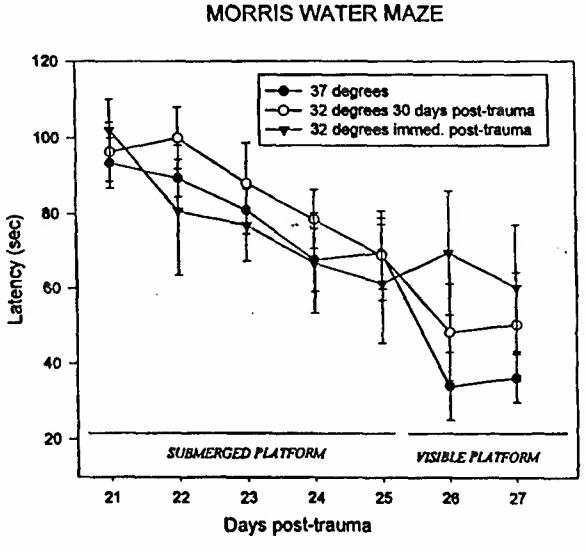

Figure 2. Effect of hypothermia on cognitive outcome after experimental TBI plus a secondary hypoxemic insult in rats. MWM performance latency to find a hidden platform (mean ± SEM, in sec) by rats on days 14-20 after CCI is depicted. There were no between group differences when performances were compared using ANOVA with repeated measures. Data are mean ± SEM.

Table 1. Effect of transient moderate hypothermia on histological outcome at 21 days after experimental TBI with secondary hypoxemic insult in rats.

GROUP	Rat survival rate	Contusion Volume	CA3 Survival, mean # neurons per hpf	CA1 Survival, mean # neurons per hpf
37°C	15/19 (78.95%)	mm^3 =65.34±6.94	19.8 ± 4.6	19.4 ± 4.2
32 °C, application delayed 30 min until after secondary hypoxemic insult	8/14 (57.14%)	mm^3=53.69±7.93	18.5 ± 7.3	13.7 ± 5.8
32 °C, application begun immediately after TBI, before secondary hypoxemic insult	8/10 (80.00%)	mm^3 =50.17±8.23	15.6 ± 7.3	13.2 ± 8.7

All data are mean ± SEM

Discussion

Surprisingly, we found that the combined insult of TBI plus secondary hypoxemia was refractory to 4 hours of moderate hypothermia. This is an important finding that was presented in November, 1998 at the annual Meeting of the National Neurotrauma Society, and will be presented in January, 1999 Meeting of the Society of Critical Care Medicine. It suggests the need for combination therapies in this setting. Alternatively, it was possible that the combined TBI plus hypoxemia insult was too severe to favorably effect outcome with any therapy. To address that possibility, we proceeded to perform two studies. These are outlined below.

(b2) *Effect of prolonged (12 h), moderate (32°C) hypothermia on functional and histological outcome after experimental TBI in rats.*

The need for combined therapies was suggested, again by the second trial of hypothermia we performed this year. In the second experimental paradigm, we sought to test, to our knowledge for the first time in any laboratory, the prolonged application of hypothermia in a rodent model of TBI. This included over 13 hours of controlled mechanical ventilation and physiological monitoring. To date, only brief 1-4 hours applications have been tested. In this study, we examined TBI without a secondary insult.

Method

Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats (n = 20) were subjected to CCI (4 m/s, 2.5 mm depth of deformation) and treated with one of the following two regimens—1) Brain temperature maintained at 37°C applied throughout a 13 hours period (n = 10),

2) Brain temperature maintained at 32°C applied for 12 hours beginning after insult (beginning after TBI and followed by re-warming over 1 hour [n = 10]). Rats were then weaned from mechanical ventilation, extubated and returned to their cages. They tolerated the procedure remarkably well. Beam balance/walking and MWM performance latencies were measured in all

rats from each group on days 1-5 and 14-20 post CCI, respectively. Rats were killed at 21 days. Serial coronal sections of brain were studied for contusion volume and hippocampal neuron counting [CA1, CA3] by a blinded observer.

Results

Motor function, as quantified by beam walking task score recovered more rapidly in rats treated with hypothermia. (beam walking p=0.06 vs normothermia, Figure 3A). In contrast, rats deteriorated between 5 and 14 days after injury as reflected by the fact that cognitive function (spatial memory acquisition paradigm on the MWM, Figure 4) tested between days 14-20 after injury was worse in the hypothermia treated group. Histology, from these rats is currently being processed.

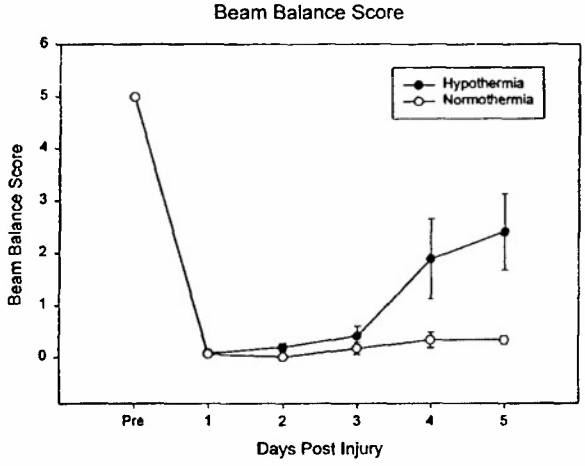

Figure 3. *Effect of prolonged (12 h) of hypothermia on motor outcome after experimental TBI in rats. Mean beam walking score (mean ± SEM, in sec) in rats before and on days 1-5 after CCI (4 m/s, 2.5 mm cortical deformation depth). Analysis of variance with repeated measures revealed a trend toward a significant difference in favor of hypothermia (p=0.06). Data are mean ± SEM.*

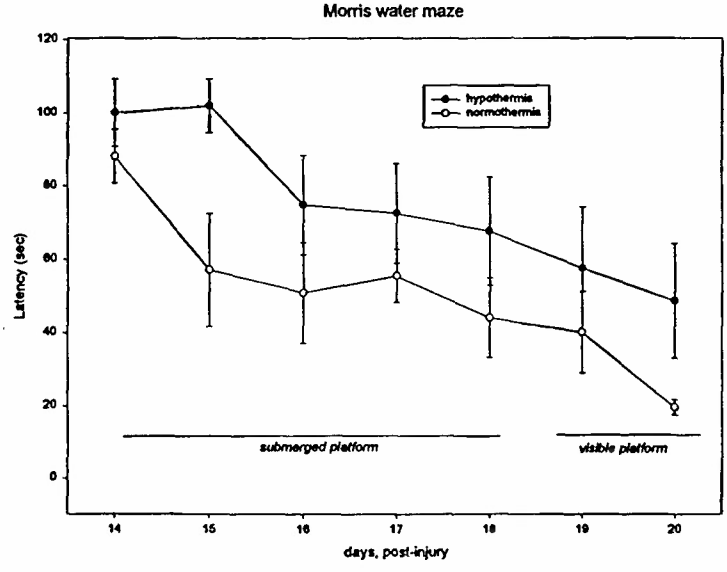

Figure 4. *Effect of prolonged (12 h) hypothermia on cognitive outcome after experimental TBI in rats. MWM performance latency to find a hidden platform (mean ± SEM, in sec) by rats on days 14-20 after CCI is depicted. There was a trend towards a worsening by hypothermia (p=0.082) when treatment groups were compared using ANOVA with repeated measures. Data are mean ± SEM*

Discussion

In this demanding experimental paradigm, testing 12 hours of hypothermia, we found that there were beneficial effects of hypothermia on motor function during the initial 5 days after TBI. However, by 2-3 wks after injury, rats treated with hypothermia had deteriorated and their performance on cognitive outcome tasks (MWM) was worse than the group treated with normothermia. One possible explanation for this is the inhibition of nerve growth factor synthesis by hypothermia (previously shown by our co-investigator, S. DeKosky). Thus, acute benefits of hypothermia on mechanisms such as cerebral swelling may be counterbalanced by detrimental effects on "regeneration" or other mechanisms yet to be defined. It is our opinion that this may be an extremely important finding. These data also again strongly suggest the need for studies of hypothermia plus other therapies during and after re-warming. To further strengthen these data, in year 3 we will again compare 12 hours of hypothermia vs normothermia in a squadron of rats, examining its effect on brain edema, intracranial hypertension, and markers of neuronal death (DNA damage) early after in insult (at the completion of the 12 hours period of temperature control). If these markers are favorably affected (as anticipated), it would mirror the clinical condition, and strengthen the relevance of our model for the proposed studies in year 3 (combination treatments). Recently, we demonstrated that 4 hours of hypothermia reduces DNA damage in our CCI model (Whalen et al, Soc for Neurosci Abstract,

(b3) *Effect of the anti-excitotoxic NMDA-receptor antagonist MK-801 on functional and histological outcome after experimental TBI in rats.*

In experimental cerebral ischemia, Dietrich et al (*J Cereb Blood Flow Metab* 15:960, 1995) demonstrated efficacy of transient hypothermia plus sustained treatment (for several days after insult) with the anti-excitotoxic agent MK-801. The delayed deterioration after 1 wk in our model seen with the application of hypothermia suggests the possible need for combined therapies. In the third experimental paradigm, we sought to test the effect of the traditional NMDA-receptor antagonist MK-801 in our TBI model (without secondary insult), to set the stage for combination therapies.

Method

Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats (n = 30) were subjected to CCI (4 m/s, 2.5 mm depth of deformation) and treated with one of the following two regimens—1) MK-801 (a single 1 mg/kg IP dose immediately after injury) or vehicle. A separate sham group (all surgery including craniotomy, but no TBI was also studied. Brain temperature maintained at 37°C during TBI. Rats were then weaned from mechanical ventilation, extubated and returned to their cages. They tolerated the procedure remarkably well. Beam balance/walking and Morris water maze (MWM) performance latencies were measured in all rats from each group on days 1-5 and 14-20 post CCI, respectively. Rats were killed at 21 d. Serial coronal sections of brain were studied for contusion volume and hippocampal neuron counting [CA1, CA3] by a blinded observer.

Results

Motor function, as quantified by both beam balance and beam walking tasks recovered more rapidly in rats treated with MK-801 (Figure 5). MWM performance in MK-801-treated rats did not differ between treatment groups (Figure 6). However, a significantly improved performance in the probe trial (Figure 7) was seen in MK-801 vs vehicle groups. Lesion volume data did not differ between groups (Table 2). There was similar tissue loss in both MK-801 and vehicle treated groups in the injured hemisphere at 21 days after injury. Hippocampal cell counts are still being processed.

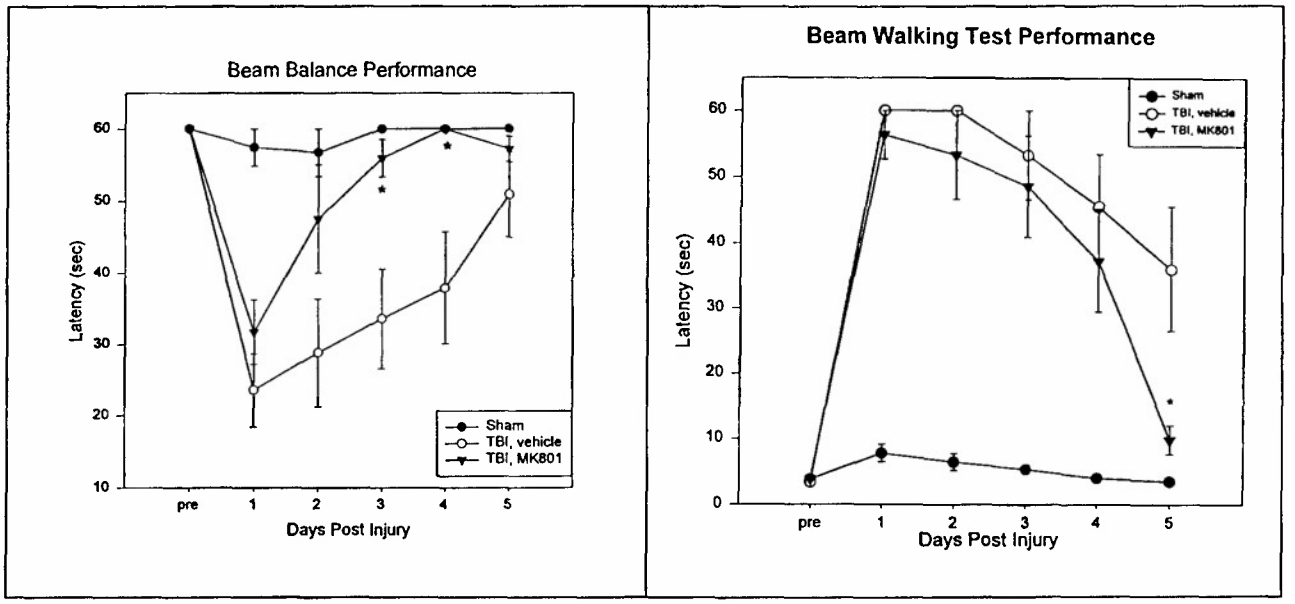

Figure 5A-B. *Effect of MK-801 treatment on motor outcome after experimental TBI in rats. Mean beam balance (A) and beam walking (B) performance latencies (mean ± SEM, in sec) in rats before and on days 1-5 after CCI (4 m/s, 2.5 mm cortical deformation depth). Analysis of variance with repeated measures revealed a significant group difference. For both tests, MK-801 treated groups recovered sooner than saline treated groups (*p<0.05 vs vehicle). Data are mean ± SEM.*

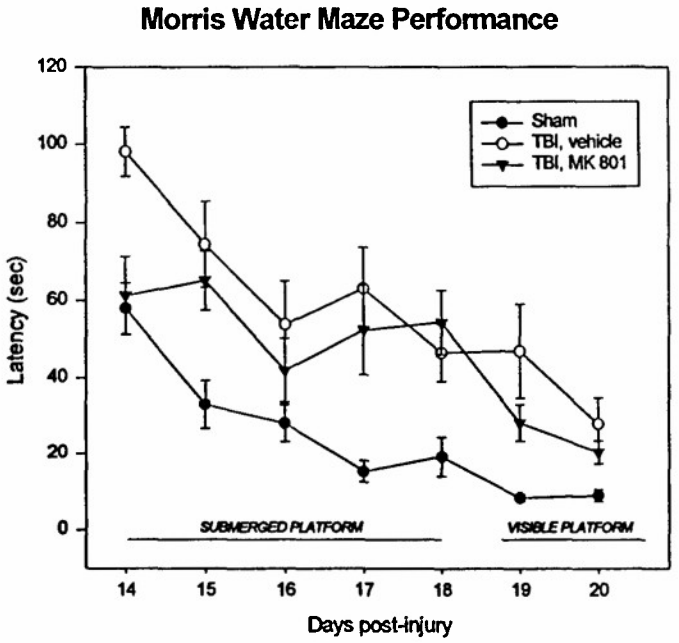

Figure 6. *Effect of MK-801 on cognitive outcome after experimental TBI in rats. MWM performance latency to find a hidden platform (mean ± SEM, in sec) by rats on days 14-20 after CCI is depicted. There was no significant effect of MK-801 treatment (vs vehicle). Data are mean ± SEM*

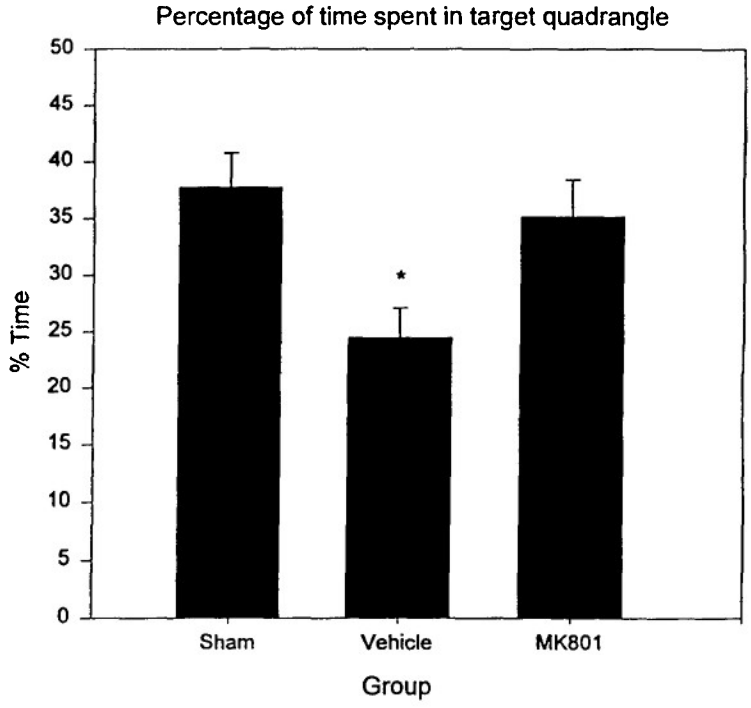

Figure 7. *Effect of MK-801 on cognitive outcome after experimental TBI in rats. MWM performance probe trial (percent of time spent in target quadrant, mean ± SEM) after CCI is depicted. There was a significant beneficial effect in favor of MK-801 treatment vs vehicle treatment. Data are mean ± SEM*

Table 2. Effect of MK-801 treatment on outcome after experimental TBI in rats.

Treatment	Lesion mm^3	Lesion % non-injured hemisphere	L hemisphere	R hemisphere
Vehicle	54.91 ± 8.35	12.68 ± 2.01	351.21 ± 17.93	435.92 ± 19.29
MK801	53.63 ± 10.00	13.07 ± 2.62	356.24 ± 23.29	424.61 ± 13.77
SHAM	---	---	451.29 ± 24.62	442/04 ± 21.62
p-value	.92	.90	**0.006 ***	.81

All data are mean ± SEM

Discussion

Remarkably, the NMDA antagonist MK-801 was effective in improving both motor function and some aspects of cognitive function after CCI. The motor effects were as dramatic or more dramatic than those seen with 12 hours of hypothermia. **In a separate pilot study, MK-801 was not effective when tested in our TBI plus secondary hypoxemia model, suggesting this insult may be too severe for any single therapy**. Although this specific agent is not available for clinical use, it suggests that this category of agents –targeting excitotoxicity—is a viable strategy for application with hypothermia.

(b4) *Comparison of the effects of TBI on functional and histological outcome after experimental TBI in rats anesthetized with isoflurane or fentanyl.*

Many, but not all, sedatives (such as barbiturates and Ketamine) target excitotoxicity. Currently, in clinical practice, fentanyl is the most commonly used emergency sedative for the

intubated patients with severe TBI. Fentanyl, has little direct anti-excitotoxic properties. Thus, we have begun to investigate how fentanyl anesthesia compared to standard isoflurane anesthesia in our model. In pilot studies, we noted that rats became markedly hypertensive and died early after TBI when anesthetized with fentanyl (but not isoflurane) in our standard TBI model. Thus, we are currently testing the use of fentanyl vs isoflurane anesthesia in our CCI model, using a slightly lesser degree of injury (2.0 mm depth of penetration rather than 2.5 mm depth—an insult with a low mortality rate in both groups). Since fentanyl is the standard of care in management of patients with TBI (in both the emergency department and the ICU), these results cold have important clinical implications if fentanyl is found to be deleterious in our model.

(7) CONCLUSION

In our work during the second year of funding addressing portions of Technical Objective #2 and 3, we demonstrated that hypothermia plus anti-excitotoxic therapies represent an excellent potential combination therapy to test in our model of experimental TBI. In addition, we demonstrated that the combination of TBI plus a secondary insult not only results in severe deficits and large lesions after injury, but is remarkably refractory to either hypothermia or anti-excitotoxic treatment. In addition, we have begun studies suggesting that the current agent used for sedation in emergency departments and ICUs (fentanyl) may not be an optimal sedative agent. In year three we are going to first define the optimal sedative approach for field use in our TBI model (Completing Objective 2 and 3). We will then combine that approach with hypothermia in an attempt to target Objective 4 and model the best possible clinically-relevant approach for field use, both in civilian and military settings.

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13. ABSTRACT *(Maximum 200* Traumatic brain injury (TBI) contributes to combat casualty morbidity and mortality. Our hypothesis is that optimal manipulation of practical interventions applicable in the emergency treatment of severe TBI(respiratory management, temperature control, and sedation) can reduce brain injury in a rat model of brain contusion, and thereby improve functional and neuropathological outcome. In the first year of funding, we addressed the first Technical Objective of our proposal - to perform a comprehensive study of the effects of mechanical ventilation strategies on outcome. We found that aggressive hyperventilation applied for 4 h immediately after injury is detrimental (vs normal ventilation), and increases neuronal death in vulnerable brain regions.

Also, to set the stage for the evaluation of therapies targeting improved outcome after TBI (proposed in Technical Objectives 2-4), the severity of the insult was increased in our model. This was done by more accurately simulating the field scenario (adding a secondary insult).

Finally, another injury station was established and a technician was trained to perform the studies proposed in y 2-3. Dr. Michael Forbes, a fellow completed his training during this year and was the first author of the manuscript described above

14. SUBJECT TERMS Head Injury, Hyperventilation, Hypothermia, Trauma, Apoptosis, Excitotoxcicity, Sedation, Oxygen

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FOREWORD

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1997 Annual Technical Report

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(5) INTRODUCTION

In our application, we highlighted the fact that traumatic brain injury (TBI) is an important contributor to combat casualty morbidity and mortality. We also stated that although progress has been made in the development of rodent models of TBI (such as the controlled cortical impact (CCI) model), most of the studies with these models have focused on molecular mechanisms of damage. Because there has been a paucity of application of **practical emergency interventions** in TBI models, we felt that it was essential to address this deficiency and that this strategy could have important implications for field and emergency management of both soldiers and civilians with severe TBI. Our overall **hypothesis** is that optimal manipulation of practical interventions applicable in the emergency treatment of severe TBI (respiratory management, temperature control, and sedation) can reduce secondary brain injury in a rat model of brain contusion, and thereby improve functional and/or neuropathological outcome.

In the first year of funding, we addressed the most important aspect of the **first Technical Objective** of our proposal -namely- to perform a comprehensive study of the effects of mechanical ventilation strategies (as applied by the first responder in the field) on both functional and neuropathological outcome in our model. **We found that aggressive, prophylactic hyperventilation (HV) applied for 4 h immediately after injury is detrimental (vs ventilation to a normal $PaCO_2$, normal ventilation [NV]), and leads to an increase in the amount of neuronal death in selectively vulnerable brain regions.** This study, is now ***in press*** as a full manuscript in the ***Journal of Neurosurgery*** (1), (also see Appendix #1). We were pleased that the reviewers indicated that this was an important study which would be cited often.

In addition, to set the stage for the evaluation of therapies targeting improvement in outcome after severe TBI (as proposed studies in technical objectives 2-4), it appeared that it would be optimal to increase the severity of the insult in our model. This was done by attempting to more accurately simulate the field scenario – i.e., adding a 30 min period of moderate hypoxemia to the insult. The characterization of that model for our future studies will also be described below.

During the first year of funding, Henry Alexander, an experienced technician assumed the technical duties of the injury model and has successfully learned the model to perform all of the subsequent injury studies in years 2 and 3. Also, a new injury device and station were purchased and is in operation for these studies. Finally, Dr. Michael Forbes, a fellow in Pediatric Critical Care Medicine completed his training during this first year of funding and was the team leader on our study assessing the effect of HV in our model. He was the first author of the manuscript describing that work. Dr. Forbes is now Associate Director of the Pediatric Intensive Care Unit at Allegheny General Hospital in Pittsburgh.

(6) BODY

(a) Technical Objective 1: Mechanical ventilation strategies after severe TBI in rats

For over two decades, HV has been one of the most utilized strategies in the management of TBI. Laboratory and clinical studies, however, have verified that early after TBI, there is usually a state of reduced cerebral perfusion that may increase vulnerability to secondary injury. HV reduces intracranial hypertension by reducing cerebral blood volume; however, this generally is accompanied by a reduction in cerebral blood flow. A recent clinical study

suggested that HV may worsen outcome after TBI. However, in the field or during the initial stabilization, HV is often used (either planned or iatrogenically) and the first blood gas of patients in the emergency room can reveal significant hypocarbia. Using the CCI model in rats, we tested the effect of 4 h of aggressive HV (vs NV), beginning immediately after injury, on functional and neuropathological outcome.

Methods:

All of the protocols for the studies outlined below were approved by the Animal Care and Use Committee of the University of Pittsburgh. Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats (n = 26) were subjected to CCI (4 m/s, 2.5 mm depth of deformation) and randomized after 10 min to either HV [n = 13, P_aCO_2 = 20.3 ± 0.7 mm Hg] or NV [n = 13, P_aCO_2 = 34.9 ± 0.3 mm Hg] for 5 h. Beam balance and Morris water maze (MWM) performance latencies were measured in eight rats from each group on d 1-5 and 7-11 post CCI, respectively. Rats were killed at 14 d. Serial coronal sections of brain were studied for contusion volume and hippocampal neuron counting [CA1, CA3] by a blinded observer.

Results:

HV was readily achieved and could be sustained for 4 h in our model, and produced the anticipated systemic alkalosis (Figure 1). In addition, other variables could be tightly controlled for 4 h in our model (Figure 1).

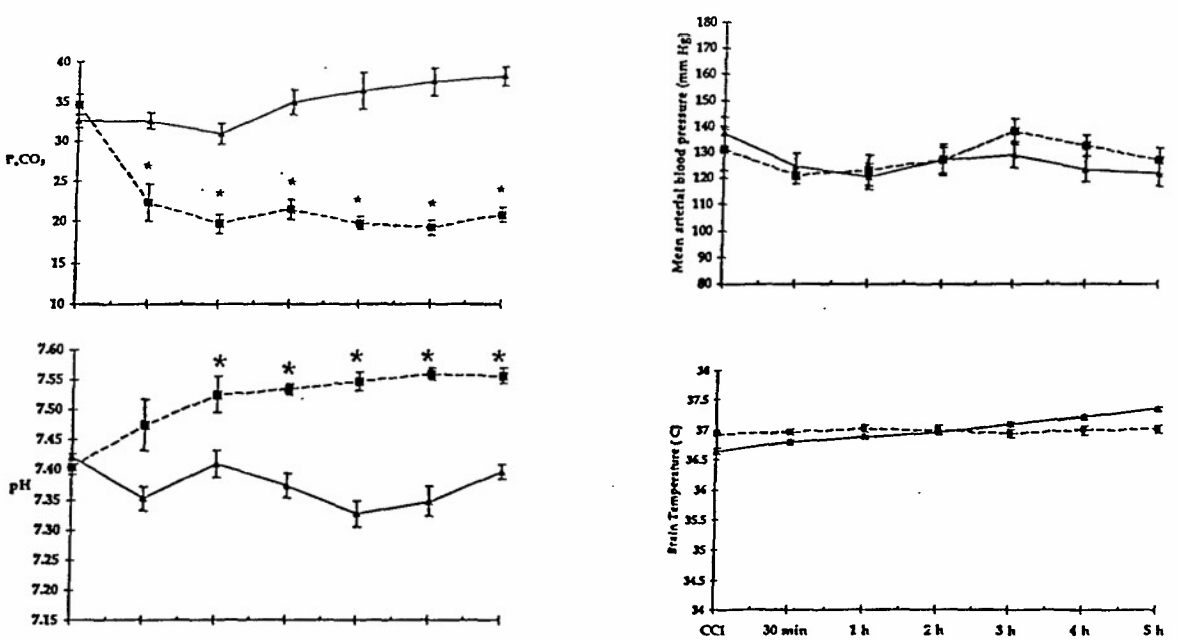

*Figure 1. Entire time course of (A) $PaCO_2$ (mm Hg), (B) arterial pH, (C) Mean arterial blood pressure (MABP, mm Hg), and (D) brain temperature (°C) in all rats treated with either NV (▲, n = 13) or HV (■, n = 13) after CCI. * $p < 0.05$ for NV vs HV. Data are mean ± SEM.*

Mortality rates were similar in both groups (2/13 vs 3/13, NV vs HV, respectively, *NS*). There were no differences between groups in mean arterial blood pressure, brain temperature, and serum glucose concentration. There were no differences between groups in either performance latencies for both beam balance (Figure 2) and MWM (Figure 3) or contusion volume (Figure 4*)*.

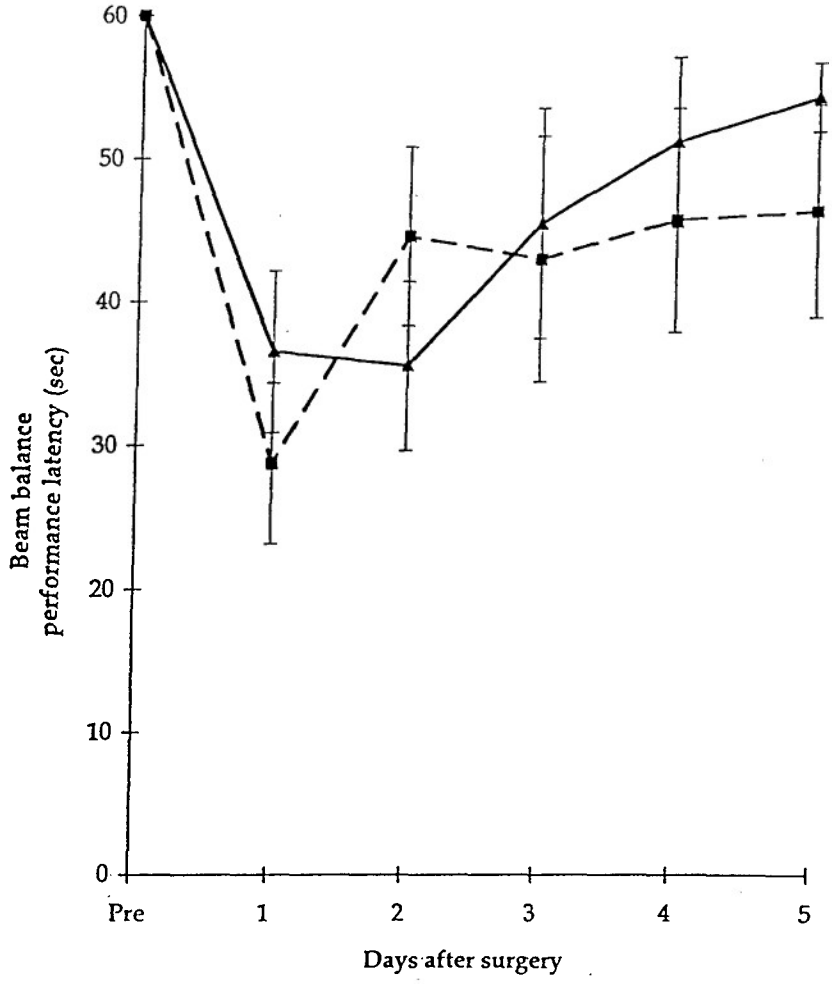

Figure 2. *Mean beam balance performance latencies (mean ± SEM, in sec) in rats before and on d 1-5 after CCI (4 m/s, 2.5 mm cortical deformation depth). Analysis of variance with repeated measures revealed no difference in duration of balance maintained between the two groups. (▲, NV, n = 8; ■, HV, n = 8). Data are mean ± SEM.*

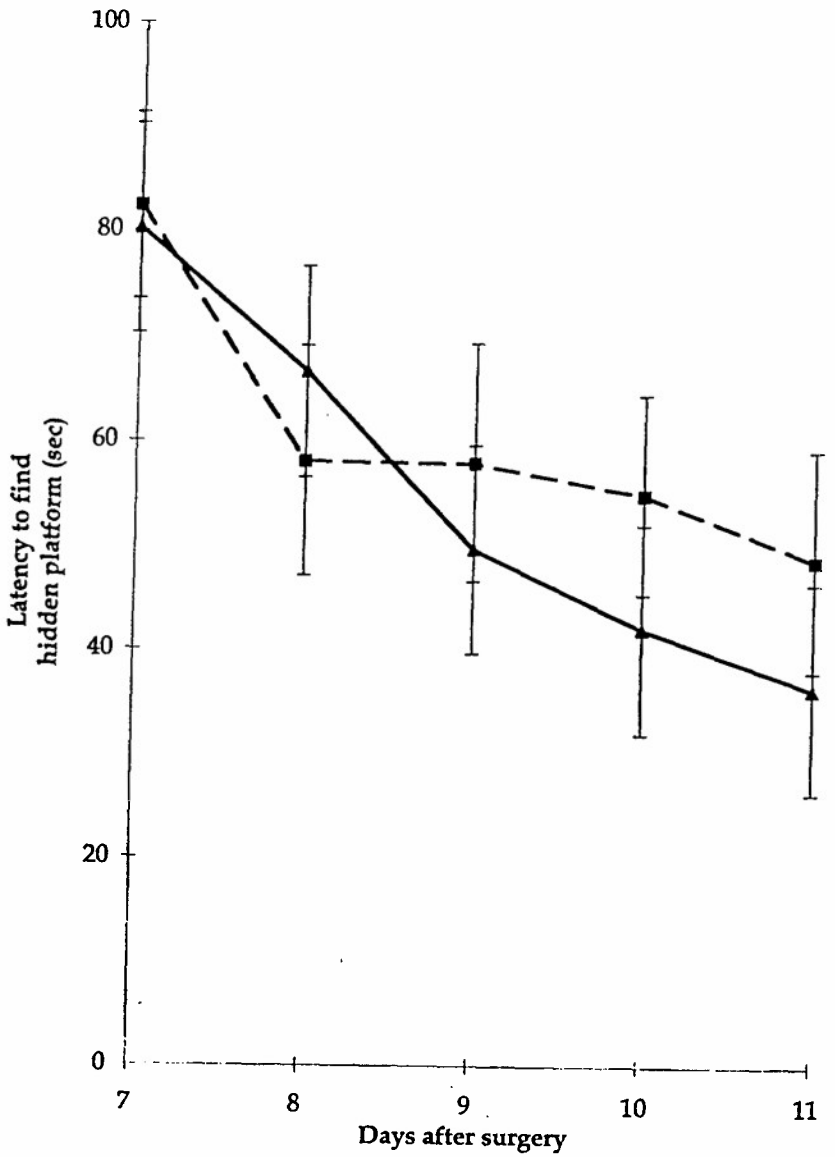

Figure 3. MWM performance latency to find a hidden platform (mean ± SEM, in sec) by rats on d 7-11 after CCI. There was no between group difference (▲, NV, n = 8; ■, HV, n = 8) when performances were compared using ANOVA with repeated measures. Data are mean ± SEM.

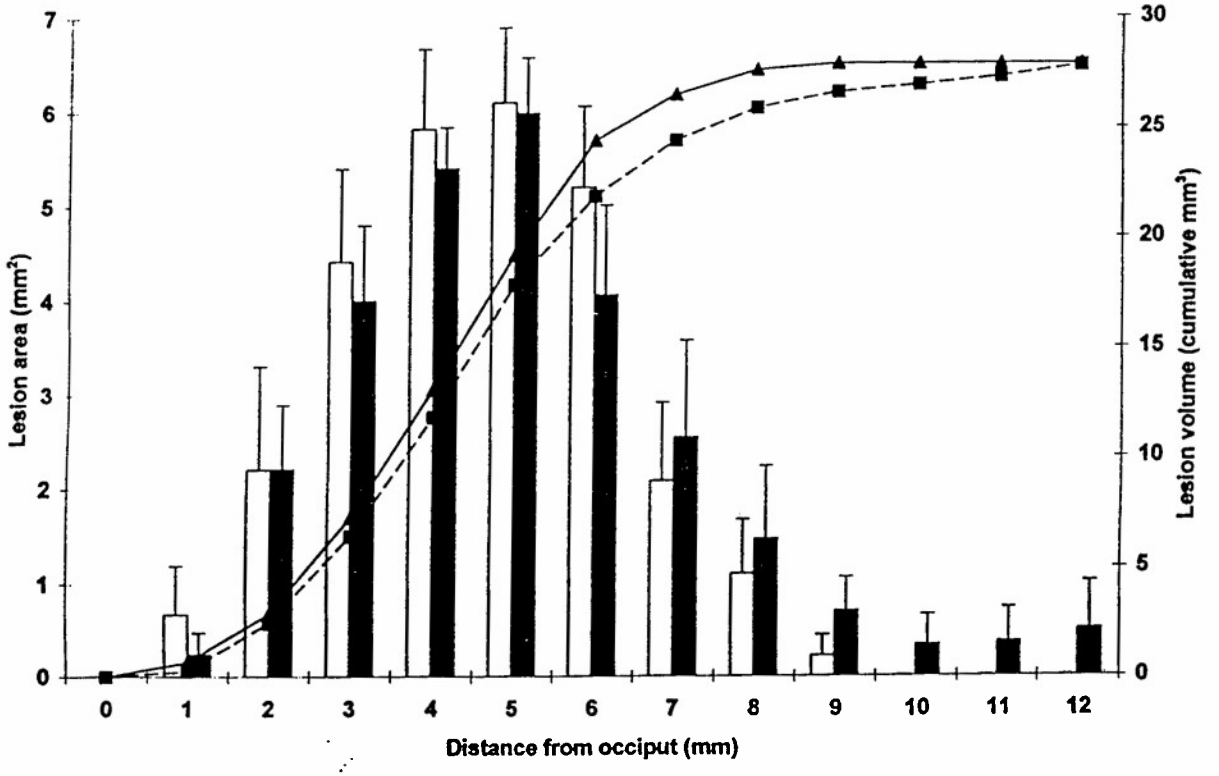

Figure 4. Graph depicting mean lesion area (left y-axis, mm^2) vs distance from occiput (mm) measured 14 d after CCI (NV, open bars, n = 11, HV, closed bars, n = 10). Contusion volume (mm^3) was calculated as the sum of these areas in each group and is depicted as cumulative volume (right y-axis) in the NV, ▲, and HV, ■, groups. There was no difference between groups in contusion volume (27.8 ± 5.1 vs 27.8 ± 3.1 mm^3 NV vs HV, mean ± SEM).

However, in brain sections through the center of the contusion, hippocampal neuronal survival in HV reduced the number of surviving hippocampal CA3 neurons (29.7 [24.2 - 31.7] vs 19.9 [17.0 - 23.7] cells/high power field (NV vs HV, median [25^{th}-75^{th} percentiles] $*p < 0.05$, Mann-Whitney Rank Sum Test, Figure 5). In contrast to the detrimental effect on CA3 neurons, CA1 neuronal death was not increased by aggressive HV.

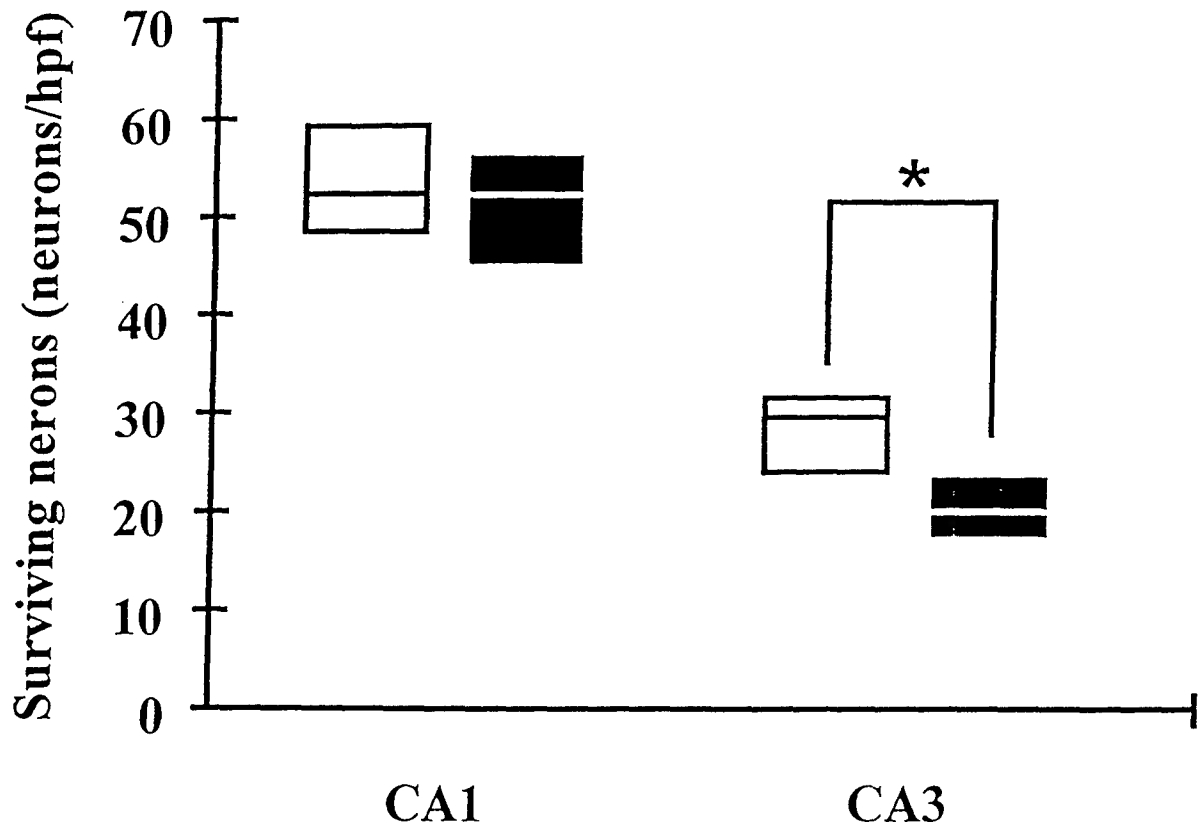

*Figure 5. Box plots representing the number of surviving CA1 and CA3 hippocampal neurons in coronal brain sections through the center of the lesion in the hemisphere ipsilateral to the contusion. Cells were counted 14 d after injury. The median line is placed within the shaded 25th - 75th range. There was a reduction in the number of surviving CA3 hippocampal neurons after injury comparing NV and HV groups (29.7 [24.2 - 31.7] vs 19.9 [17.0 - 23.7], cells/high power field (hpf), *$p < 0.05$, Mann-Whitney rank sum test).*

Conclusion:

Aggressive HV early after TBI augments CA3 hippocampal neuronal death; however, it did not impair functional outcome or expand the contusion. These data suggest that CA3 hippocampal neurons are selectively vulnerable to the effects of HV early after TBI. The injudicious application of HV early after TBI, such as in the field, may contribute to secondary neuronal injury.

Comment:

Hippocampal CA3 neurons are selectively vulnerable to delayed neuronal death after TBI. The mechanisms underlying this process remain speculative. Potential mechanisms include ischemia, TBI-induced excitotoxicity, apoptosis, and inflammation. We previously demonstrated that the hippocampus and cortex ipsilateral to the impact have marked flow reduction (at least 60%) at 2 h after TBI in the CCI model (2). CBF approaches ischemic levels in the core of the contusion at 2 h after injury. Although we have not evaluated the status of reactivity of the cerebral circulation to changes in $PaCO_2$ at 2 h after TBI in this model, we have reported that CO_2 reactivity is impaired, although still present (62-71% of baseline) in and around the contusion at 24 h after CCI in rats (3).

HV produces cerebral vasoconstriction and alkalosis. Alkalosis exacerbates N-methyl-D-aspartate (NMDA)-receptor mediated neurotoxicity. As a result of aggressive HV, the rats in our study were quite alkalotic as indicated by arterial pH measurements. Alkalosis appears to have deleterious effects on neurons. It could also be that the combined effect of alkalosis and further flow reduction by HV is deleterious in regions vulnerable to excitotoxicity such as CA3. Early, aggressive or prophylactic HV, therefore, in the context of reduced CBF, may exacerbate excitotoxic mechanisms and augment neuronal death.

Aggressive HV in the early low flow period did not worsen functional outcome or expand the contusion. The cognitive deficits in this model are modest. Indeed, to test therapies targeting an improvement in outcome, we may need a more severe injury (see below). Additional unilateral or bilateral hippocampal damage may be necessary to create more marked functional deficits. CA3 damage alone may not mediate post-TBI MWM deficits. However, hippocampal damage and memory deficits are common after TBI in humans, and exacerbation of neuronal death in any brain region would be highly undesirable.

This study does not completely address the uncommon situation where early after severe head injury marked intracranial hypertension is observed. HV may, in fact, be life saving in the setting of impeding herniation. Similarly, we did not measure ICP or titrate ventilation to control cerebral perfusion pressure, and we evaluated only one level of HV and injury severity. We did not attempt to model the clinical scenario of optimal titration of ventilation when ICP is increased. Rather, we chose to evaluate the field setting and apply the worst case scenario, aggressive HV during the early post-trauma period when flow is already low and excitotoxicity is peaking. Our study does, however, show that HV is associated with a tangible risk to vulnerable neurons. To our knowledge, this is the first *in vivo* study demonstrating that HV can augment neuronal injury after TBI. This suggests that there is indeed a trade-off associated with this intervention.

Recommendation

We have shown that aggressive, early HV after TBI augments neuronal death in CA3 hippocampus in a rat model of cerebral contusion. The further reduction of CBF with HV during the low cerebral blood flow state immediately after severe TBI coupled with alkalosis may increase the vulnerability of selected neurons to damage. The results of this study reinforce the meticulous attention necessary to prevent secondary injury after TBI. A risk in the use of HV is demonstrated. Unless signs of impending herniation (unilateral pupillary dilation, hypertension, bradycardia) are present, this study supports the targeting of normocarbia for mechanical ventilation in the emergency stabilization of the brain trauma victim.

(b) A field scenario of severe TBI for the evaluation of therapies proposed in Technical Objectives 2-4.

Using our standard CCI model, neuronal death in selectively vulnerable regions and MWM deficits are present but modest. In tat model, we able to nicely demonstrate exacerbation of damage with a deleterious strategy, namely HV. However, in technical objectives 2-4, our goal is to define strategies (hypothermia, anesthetics, anti-excitotoxic therapies) that will mitigate damage. Thus, the severity of damage must be increased, both from the standpoint of both hippocampal neuronal death and MWM deficit, to achieve this goal. Recently, in studies separate from this application, we published a variation of our CCI model that was designed to

increase the amount of hippocampal damage without totally destroying the hippocampus (and making in impossible to resuscitate)(4). This was achieved by adding a 30 min period of moderate hypoxemia ($FiO_2 = 0.11$) which also results in accompanying mild hypotension. This mimics the secondary insults in head injury victims so commonly seen in the field. In addition, in studies by our group separate from this application (5), we reported that both necrotic and apoptotic neuronal death is seen in this new variant of the CCI model. To be certain that this model would be suitable for technical objectives 2-4, it was essential to determine if the insult was accompanied by a significant MWM deficit.

Methods:

All of the protocols for the studies outlined below were approved by the Animal Care and Use Committee of the University of Pittsburgh. Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats (n = 20) were subjected to CCI (4 m/s, 2.5 mm depth of deformation) to the left parietal cortex using either a vertical or angled impact. Immediately after injury, the FiO_2 was reduced to 0.11 (inhalational anesthesia maintained constant by the addition of N2 to the ventilator circuit). At 5 min after reducing the FiO_2 and at the completion of the 30 min secondary hypoxemic insult, a blood gas is obtained to document the level of hypoxemia achieved, and then the FiO_2 is increased. Shams were subjected to all surgical procedures, but no insult (i.e., neither CCI nor hypoxemia). After the recovery periods, catheters were removed and anesthesia was discontinued. Rats were weaned from mechanical ventilation, extubated, and returned to their cages until further study. Motor and cognitive outcome were assessed as previously described.

Results:

Both vertical and angled impacts resulted in significant motor and cognitive deficits as assessed by beam balance, and MWM paradigms (Figures 6, and 7, respectively).

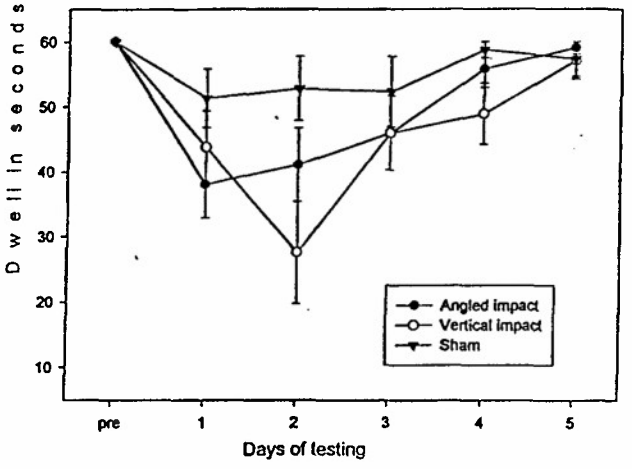

Figure 6. Mean beam balance performance latencies (mean ± SEM, in sec) in rats before and on d 1-5 after either vertical or angled CCI with secondary hypoxemic insult. Analysis of variance with repeated measures revealed no difference in duration of balance maintained between the two insults. Both insults were significantly different from sham.

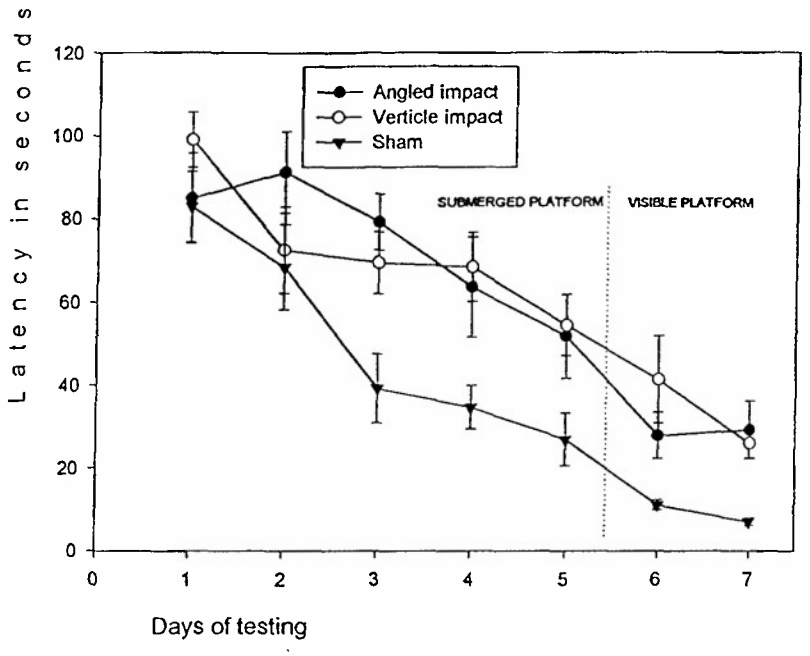

Figure 7. MWM performance latency to find a hidden platform (mean ± SEM, in sec) by rats on d 14-21 after either vertical or angled CCI with secondary hypoxemic insult. Analysis of variance with repeated measures revealed no difference between the two insults. Both insults were significantly different from sham.

Conclusion:

Combined with our prior publications showing both a well defined contusion and neuronal death by both apoptosis and necrosis in this model (4,5), the functional deficits produced with either a vertical or angled insult set the stage for studies proposed in Technical Objectives 2-4. We have chosen to use the vertical insult with hypoxemia, since all of our initial studies with HV used a vertical impact. These will be addressed in years 2-3 of the funding period. In accordance with this plan, we are currently evaluating the effect of hypothermia in this model of CCI with a secondary insult (as outlined in Technical Objective #2). We plan to address Technical Objective 2 and part of Technical Objective 3 in funding year 2.

(7) CONCLUSION

In our work during the first year of funding addressing Technical Objective #1, we demonstrated that aggressive HV early after TBI augments CA3 hippocampal neuronal death. These data suggest that CA3 hippocampal neurons are selectively vulnerable to the effects of HV early after TBI. The injudicious application of HV early after TBI, such as in the field, may contribute to secondary neuronal injury. As previously discussed, the results of this study reinforce the meticulous attention necessary to prevent secondary injury after TBI. A risk in the use of HV is demonstrated. Unless signs of impending herniation (unilateral pupillary dilation, hypertension, bradycardia) are present, this study supports the targeting of normocarbia for mechanical ventilation in the emergency stabilization of the brain trauma victim. Finally, by adding a secondary insult to our injury model, we have set the stage to address the optimal

application of treatments to improve outcome as outlined in Technical Objectives 2-4, and those studies are underway.

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APPENDIX

FACULTY DATA SHEET

NAME:	PATRICK MICHAEL KOCHANEK, M.D.	
CAMPUS ADDRESS:	Safar Center for Resuscitation Research	
CURRENT TITLE & EFFECTIVE DATE:	Associate Professor February 1, 1991	
DATE APPOINTED TO FACULTY:	July 1, 1986	TITLE: Assistant Professor
DATE APPOINTMENT EXPIRES:	June 1999	LENGTH OF TERM:
DATES OF PROMOTIONS:	February 1991	

CERTIFICATION:	ABA: American Board of Pediatrics
	Sub-Board of Pediatric Critical Care Medicine
TENURE:	Tenured - 1997

MEDICAL SCHOOL GRADUATE:	University of Chicago
HOSPITAL OF RESIDENCY:	University of California, San Diego
YEAR RESIDENCY COMPLETED:	1983

[redacted] [redacted]

[redacted] [redacted]

SOCIAL SECURITY NUMBER:	[redacted]
DATE OF BIRTH:	[redacted]
CITIZEN	USA
NAME OF SPOUSE:	Denise
UPDATED:	January 10, 2000

CURRICULUM VITAE

BIOGRAPHICAL

NAME:	Patrick M. Kochanek, M.D.	BIRTH DATE:	[redacted]
HOME ADDRESS:	[redacted]	BIRTH PLACE:	[redacted]
HOME PHONE:	[redacted]	CITIZENSHIP:	USA
BUSINESS ADDRESS:	Safar Center for Resuscitation Research 201 Hill Building 3434 Fifth Avenue Pittsburgh, PA 15260	SOCIAL SECURITY NO:	[redacted]
BUSINESS PHONE:	(412) 383-1900		

EDUCATION AND TRAINING

Undergraduate		
1972 - 1976	University of Michigan Ann Arbor, Michigan	B.S., 1976 Zoology
Graduate		
1976 - 1980	University of Chicago Chicago, Illinois	M.D., 1980
Post Graduate		
1980 - 1981	University of California San Diego, California	Pediatric Internship William Nyhan, M.D.
1981 - 1983	University of California San Diego, California	Pediatric Residency William Nyhan, M.D.
1983 - 1986	Children's Hospital National Medical Center Washington, DC	Pediatric Critical Care Fellowship Peter Holbrook, M.D.

APPOINTMENTS AND POSITIONS

ACADEMIC		
1986-1991	University of Pittsburgh School of Medicine Department of Anesthesiology/CCM Pittsburgh, PA	Assistant Professor
1991 - present	University of Pittsburgh School of Medicine Department of Anesthesiology/CCM Pittsburgh, PA	Associate Professor

1986 - 1991	University of Pittsburgh School of Medicine Department of Pediatrics Pittsburgh, PA	Assistant Professor
1991 - present	University of Pittsburgh School of Medicine Department of Pediatrics Pittsburgh, PA	Associate Professor

NON ACADEMIC

1983 - 1986	Hyperbaric Medicine Program Center/Naval Medical Research Institute Washington, DC	Guest Scientist
1986 - present	Children's Hospital of Pittsburgh One Children's Place Pittsburgh, PA	Associate Director Pediatric Intensive Care Unit
1992 - present	Children's Hospital of Pittsburgh One Children's Place Pittsburgh, PA	Director Pediatric Critical Care Research
1994 - present	Safar Center for Resuscitation Research 3434 Fifth Avenue Pittsburgh, PA 15260	Director

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CERTIFICATION AND LICENSURE

SPECIALTY CERTIFICATION

1985	Diplomate, American Board of Pediatrics, #32806
1987	American Board of Pediatrics Sub-board of Pediatric Critical Care Medicine, #0081
1995	American Board of Pediatrics Sub-board of Pediatric Critical Care Medicine, Recertification

MEDICAL LICENSURE

1981	California, #G46392
1985	Maryland, #D32599
1986	District of Columbia, #15785
1986 - present	Pennsylvania, MD# 035634-E

MEMBERSHIPS IN PROFESSIONAL AND SCIENTIFIC SOCIETIES

1985	American Association for the Advancement of Science
1987	Society of Critical Care Medicine
1988	Pennsylvania Society of Critical Care Medicine
1988	International Society of Cerebral Blood Flow and Metabolism
1988	New York Academy of Sciences
1989	Stroke Council, American Heart Association
1989	Neurotrauma Society
1990	Society for Neuroscience
1990	Society for Pediatric Research
1994	Council on Critical Care, American Heart Association
1996	International Neurotrauma Society
1997	The American Association of Neurological Surgeons, Associate Member

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HONORS

1976	Phi Beta Kappa
1980	Alpha Omega Alpha
1977 - 1980	Joseph Collins Foundation Scholar
1984 - 1985	Fellow of the Year Department of Critical Care Medicine Children's Hospital National Medical Center
1985 - 1986	Fellow of the Year Department of Critical Care Medicine Children's Hospital National Medical Center
1991	Fellow, American College of Critical Care Medicine
1992	Cited in "Best Doctors in America", Woodward/White, Inc.
1992	Named one of "Pittsburgh's Best Doctors", Pittsburgh Magazine
1993	Society of Critical Care Medicine Pediatric Award
1993 - 1995	Society of Critical Care Medicine Established Investigator Award
1993	Cited in "Best Doctors in America", 2nd Edition
1993	Neurotrauma Society Poster Award (Research Mentor to Dr. Susan Kaczorowski)

1993	Society of Critical Care Medicine Educational Scholarship (Research Mentor to Dr. Susan Kaczorowski)
1994	Outstanding Faculty University of Pittsburgh Honors Convocation
1995	Society of Critical Care Medicine (Scientific Award) (Research Mentor to Dr. Robert Clark)
1996	Presidential Citation Society of Critical Care Medicine
1996	Poster Award Finalists Neurotrauma Society of Medicine (Research mentor to Dr. Michael Bell and Dr. Michael Forbes)
1996 - 1997	Who's Who in America
1997	Educational Scholarship Society of Critical Care Medicine (Research mentor to Dr. Michael Bell)
1997	Young Investigator's Award Society for Neurosurgical Anesthesiology and Critical Care (Research mentor to Dr. Elizabeth Sinz)
1997	Poster Award Finalists Neurotrauma Society of Medicine (Research mentor to Dr. Michael Bell)
1998	The American Board of Pediatrics Sub-Board in Pediatric Critical Care Medicine
1998	SCCM In-Training Fellow Award Society of Critical Care Medicine (Research mentor to Dr. Michael Bell)
1999	Women in Neurotrauma Award National Neurotrauma Society (Research Mentor to Dr. Kimberly Statler)

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Refereed Articles.

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194. Satchell M, Clark RSB, Chen M, Melick J, Szabo C, Kochanek PM: Poly (ADP-ribose) synthetase activation and NAD depletion after traumatic brain injury in rats. Crit Care Med 27:A34, 1999.

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PROFESSIONAL ACTIVITIES

TEACHING:

March 1987	Grand Rounds Controversy Surrounding Corticosteriods Administration in Septic Shock Children's Hospital of Pittsburgh Pittsburgh, PA
October 1987	Instructor - Pediatric Advanced Life Support Children's Hospital of Pittsburgh Pittsburgh, PA
March 1988	Pediatric Grand Rounds The Pediatric Arrest Children's Hospital of Pittsburgh Pittsburgh, PA
April 1988	University of Pittsburgh Anesthesiology/CCM Grand Rounds Reperfusion Injury after Cerebral Ischemia Pittsburgh, PA
October 1988	University of Pittsburgh Anesthesiology/CCM Research Conference How Important is Inflammation to the Evolution of Brain Injury? Pittsburgh, PA
April 1989	Research Conference Granulocytes and Cerebral Trauma International Resuscitation Research Center University of Pittsburgh, Pgh, PA
June 1989	Instructor for Board Review Course - Pediatric Critical Care Medicine Section on Cerebral Resuscitation New Orleans, LA
November 1989	Pediatric Grand Rounds Pathobiology of the Pediatric Arrest Children's Hospital of Pittsburgh Pittsburgh, PA
November 1989	Research Conference Inflammation and Brain Injury: An Update International Resuscitation Research Center University of Pittsburgh Pittsburgh, PA
February 1990	Pediatric Grand Rounds Pathobiology of the Pediatric Arrest Mercy Hospital Pittsburgh, PA

March 1990	Neonatology Grand Rounds Pathobiology of the Pediatric Arrest Magee-Womens Hospital Pittsburgh, PA
April 1990	Scientific Affairs Research Conference University of Pittsburgh, Anesthesia Department "Activation of Endogenous Neutrophils in the Cerebral Circulation" Pittsburgh, PA
April 1991	Children's Hospital of Pittsburgh Trauma Conference "Age-Related Differences in the Cerebrovascular Response to Neurotrauma" Pittsburgh, PA
April 1991	IRRC Hornbein Research Symposium "Adult Brain Distress Syndrome" International Resuscitation Research Center Pittsburgh, PA
May 1991	University of Pittsburgh Neurosurgery Department Basic and Clinical Science Conference "Biochemistry of Cellular Injury and Repair" Mentor to Dr. Peter Miller Children's Hospital of Pittsburgh Pittsburgh, PA
May 1991	Neuroanesthesia and CCM Lecture Series "Inflammation and Brain Injury" Eye and Ear Hospital Pittsburgh, PA
April 1992	IRRC Research Conference "Trauma Studies at CHP" International Resuscitation Research Center Pittsburgh, PA
November 1992	IRRC Research Conference Blood Flow after Brain Injury International Resuscitation Research Conference Pittsburgh, PA
November 1992	University of Pittsburgh Interdisciplinary Seminars in Cerebral Blood Flow and Metabolism "Cerebral Blood Flow After Traumatic Brain Injury" (with Dr. Walter Obrist) Pittsburgh, PA
November 1993	Novel Therapeutic Approach to Traumatic Brain Injury Pediatric Grand Rounds Children's Hospital of Pittsburgh Pittsburgh, PA

November 1993	Head Injury in Children Pulmonary Medicine Conference Children's Hospital of Pittsburgh Pittsburgh, PA
November 1993	Inflammatory Response to Traumatic Brain Injury Pediatric Clinical Pharmacology Conference Children's Hospital of Pittsburgh Pittsburgh, PA
January 1994	Update on Traumatic Brain Injury Studies International Resuscitation Research Center Pittsburgh, PA
June 1994	Plans for the Future International Resuscitation Research Center Pittsburgh, PA
November 1994	Acute Inflammatory Response to Traumatic Brain Injury Department of Neurology University of Pittsburgh Medical Center Pittsburgh, PA
February 1995	Inflammatory Response to Traumatic Brain Injury Neuroscience Seminar Program University of Pittsburgh Medical Center Pittsburgh, PA
April 1995	Mini Symposium Department of Anesthesiology and Critical Care Medicine Safar Center for Resuscitation Research Overview and Traumatic Brain Injury Program University of Pittsburgh Medical Center Pittsburgh, PA
April 1995	Traumatic Brain Injury Safar Center Bendixon Symposium University of Pittsburgh Medical Center Pittsburgh, PA
May 1995	Pathophysiological Mechanisms in Head Injury Center for Clinical Pharmacology University of Pittsburgh Pittsburgh, PA
March 1996	Inflammatory Response to Traumatic Brain Injury Research Minisymposium for Dr. Paul Knight Department of Anesthesiology/CCM Pittsburgh, PA
February 1997	MRI-assessment of Cerebrovascular Failure after Traumatic Brain Injury in Rats Pittsburgh NMR Center for Biomedical Research Carniege Mellon University, February 12, 1997 Pittsburgh, PA

February 1997	MRI-Applications to TBI in Rats Safar Center Monthly Lecture Series Department of Anesthesiology/CCM University of Pittsburgh, February 12, 1997 Pittsburgh, PA
February 1997	Adhesion Molecules and Quinolinic Acid in CSF after Head Injury in Humans University of Pittsburgh Brain Trauma Research Center University of Pittsburgh, February 25, 1997 Pittsburgh, PA
March 1997	MRI-assessment of Head Injury in Rats Pittsburgh NMR Center for Biomedical Research Carniege Mellon University, March 11, 1997 Pittsburgh, PA
August 1997	Traumatic Brain Injury in Children: From Bench to Bedside Pediatric Grand Rounds Children's Hospital of Pittsburgh, August 14, 1997 Pittsburgh, PA
December 1997	MRI-Facilitated Assessment of Outcome After Traumatic Brain Injury in Rats Pittsburgh NMR Center NIH Site Visit Carnegie Mellon University, December 3, 1997 Pittsburgh, PA
December 1997	Mechanisms and Pharmacology in Suspended Animation Suspended Animation Investigators Meeting University of Pittsburgh, December 6, 1997 Pittsburgh, PA
March 1998	New Concepts in Traumatic Brain Injury Safar Center Monthly Lecture Series Department of Anesthesiology/CCM University of Pittsburgh, March 11, 1998 Pittsburgh, PA
April 1998	Traumatic Brain Injury in Children: From Bench to Bedside Trauma Conference Children's Hospital of Pittsburgh, April 9, 1998 Pittsburgh, PA
May 1998	Update on Adenosine University of Pittsburgh Brain Trauma Research Center University of Pittsburgh, May 26, 1998 Pittsburgh, PA
July 1998	General Clinical Research Center Children's Hospital of Pittsburgh, July 14, 1998 Pittsburgh, PA
October 1998	Special Investigator Research Update Journal Club Children's Hospital of Pittsburgh, October 23, 1998 Pittsburgh, PA

June 1999 — MRI-in the Assessment of Experimentally Induced Traumatic Brain Injury in Rats
Pittsburgh NMR Center NIH Site Visit
Carnegie Mellon University, June 11, 1999
Pittsburgh, PA

October 1999 — CSF analysis of secondary mediators in neurotrauma
Brain Trauma Research Center
Department of Neurological Surgery, October 13, 1999
Pittsburgh, PA

RESEARCH:

Grants Received:

The role of granulocytes in reperfusion injury after brain ischemia.
Health Research Service Foundation (United Way)
$13,089 7/87 - 6/88 Principal Investigator

Cerebrovascular and cerebrometabolic effects of platelet-activating factor.
Western Pennsylvania Heart Association
$17,908 7/88 - 6/89 Principal Investigator

Polymorphonuclear leukocytes in the genesis of posttraumatic cerebral edema.
Children's Hospital of Pittsburgh Human Rights Committee Grant
$6,275 7/88 - 6/89 Principal Investigator

The effect of the PAF antagonist Ginkgo Biloba extract on posttraumatic cerebral edema in rats.
Willman Schwabe Pharmaceutical Corporation, Karlsruhedurlach, West Germany
$3,000 11/88 Principal Investigator

The effect of platelet-activating factor-receptor antagonists on posttraumatic cerebral edema in rats.
University of Pittsburgh Internal Grants Program
$10,000 7/89 - 6/90 Principal Investigator

Effect of activated polymorphonuclear leukocytes on cerebral blood flow in rats.
Western Pennsylvania Heart Association
$47,838 7/90 - 6/92 Principal Investigator

Regional cerebral blood flow after concussive head injury in adult and immature rats.
University of Pittsburgh Department of Anesthesiology and Critical Care Medicine
$6,715 7/90 - 6/91 Faculty Supervisor

Polymorphonuclear leukocytes in traumatic brain injury.
Sunny von Bulow Coma and Head Trauma Foundation
$35,000 9/90 - 8/91 Principal Investigator

Effect of hyponatremia on brain Ph function morphology (Sheldon Adler PI).
NIH-RO1 $736,936 Consultant (5% commitment)

Age related differences in blood brain barrier permeability after cerebral trauma in rats.
Children's Hospital Human Rights Committee Grant
$7,251 3/91 - 2/92 Faculty Supervisor

Effect of hypothermia on traumatic brain injury in immature rats.
University of Pittsburgh Department of Anesthesiology and Critical Care Medicine
$6826 2/93 - 1/94 Co-Principal Investigator

Role of inflammation in cerebrovascular failure after head injury.
Society of Critical Care Medicine, Established Investigator Grant
$225,000 7/93-6/96 Principal Investigator

Effect of leukocyte adhesion cell molecule antagonist on the acute inflammation response after traumatic brain injury in rats.
University of Pittsburgh Department of Anesthesiology and Critical Care Medicine
$7,998 1/94 - 12/94 Co-Principal Investigator

The effect of SCR-1 on posttraumatic neutrophil accumulation and markers of injury after cerebral trauma in rats.
University of Pittsburgh Department of Anesthesiology and Critical Care Medicine
$4,420 1/94 - 12/94 Co-Principal Investigator

The role of inflammation in cerebrovascular failure after head injury.
American Heart Association Pennsylvania Affiliate Fellowship Grant
$22,000 7/94 - 6/95 Mentor (Salary support of Dr. Robert Clark)

Perfusion MRI Assessment of Cerebral Blood Flow After Head Injury.
Laerdal Foundation
$12,290 1/95 - 12/95 Principal Investigator

Exsangguination Cardiac Arrest, Hypothermic Preservation, and Delayed Resuscitation.
Laerdal Foundation (S. Tisherman, PI)
$10,000 1/95 - 12/95 Consultant

University of Pittsburgh Brain Trauma Research Center
NIH Program Project (Dr. Donald Marion-Principal Investigator)
$3,051,980 4/01/95 - 3/31/00

Project #4 Neutrophils and the Acute Inflammatory Response to Traumatic Brain Injury.
$389,567 Principal Investigator

Core C RAT/SURGERY/IMAGING
$332,240 Principal Investigator

The Role of Adenosine in the Development of Cerebrovascular Failure Following Severe Head Injury in Children.
Children's Hospital of Pittsburgh, General Clinical Research Center Advisory Committee
$7,350 Co-Investigator and Mentor - (for Michael Bell, M.D.)

Application of Magnetic Resonance Imaging to Measure Cerebral Blood Flow, CO_2 Responsivity and Blood-Brain Barrier Integrity After Traumatic Brain Injury in Immature Rat.
Children's Hospital of Pittsburgh Research Advisory Committee Seed Grant
$9,940 7/1/95 - 6/30/96 Co-Investigator and Mentor-(Michael Forbes, M.D.)

Nitric Oxide-Mediated Cerebrovascular Failure After Brain Injury.
Schertz Fellowship Award, University of Pittsburgh, Dept. of Anesthesiology/CCM
$60,000 7/01/96 - 6/30/97 Mentor-(for Lisa Sinz, M.D.)

CSF Indexes of Inflammation and Tissue Injury to Pediatric Head Injury: Prognostic Implications.
Laerdal Foundation (S. DeKosky, PI)

$15,000	1/01/96 - 12/31/96	Co-Investigator

Nitric Oxide-Mediated Cerebrovascular Failure After Brain Injury.
American Heart Association Pennsylvania Affiliate

$69,996	7/01/96 - 6/30/98	Principal Investigator

Increasing Survival of Uncontrolled Hemorrhagic Shock in Rats: Oxygen Breathing and Hypothermia.
Geo-Centers/U.S. Department of Navy Grant

$332,996	3/15/96	Co-Investigator (5% committment)

Hypothermia in the Treatment of Severe Head Injury in Children.
Children's Hospital of Pittsburgh, General Clinical Research Center Advisory Committee
(P. David Adelson, M.D., PI)

$6,510	11/95 - 11/96	Co-Investigator

Emergency Interventions After Severe Traumatic Brain Injury in Rats: Effects of Neuropathology and Functional Outcome.
U.S. Army Medical Research and Material Command

$792,237	12/96 - 11/99	Principal Investigator

Cerebrovascular Response Following Severe Traumatic Brain Injury in Children.
Laerdal Foundation for Acute Medicine
(P. David Adelson, M.D., PI)

$11,500	7/96 - 6/97	Co-Investigator

Adenosine's Role in Experimental Traumatic Brain Injury in the Rat.
University Anesthesiology and Critical Care Medicine
Michael Bell, M.D., PI

$8051	7/96 - 6/97	Faculty Sponsor

The Role of Inducible Nitric Oxide Synthase in Delayed Neuronal Death After Traumatic Brain Injury.
Children's Hospital of Pittsburgh, Research Advisory Committee for Faculty Start-Up Projects
Robert Clark, M.D., PI

$75,808	7/95 - 6/97	Co-Investigator

The Role of Neuroprotective Genes after Traumatic Brain Injury.
NINDS-MCSDA
Robert S.B. Clark, M.D., PI

$409,320	12/01/96 - 12/31/01	Co-Sponsor

Severe Diffuse Traumatic Brain Injury in Immature Rats.
MCSDA-NINDS/KO8
P. David Adelson, M.D., PI

$409,320	12/01/96 - 11/30/01	Faculty Sponsor

Pathogenesis of Osmotic Induced Demyelination.
NIH-RO1
Sheldon Adler, M.D., PI

$858,487	05/31/97 - 05/31/99	Consultant [5%]

The Effect of Hypothermia on the Acute Inflammatory Response to Brain Injury.
Schertz Fellowship Award, University of Pittsburgh, Dept. of Anesthesiology/CCM
Michael Whalen, M.D., PI

$60,000	07/01/97 - 06/30/98	Faculty Sponsor

Quinolinic Acid, A Novel Mediator of Neurotoxicity after Brain Injury.
Laerdal Foundation for Acute Medicine

$6120	07/01/97 - 06/30/98	Principal Investigator

Cell Trafficking and Functions in Two Models of Cold Induction: Ultraprofound Hypothermia and Hibernation.
Navy Medical Research Institute
Florence Rollwagen, Ph.D., PI

$400,574	Co-Investigator

Augmenting Adenosine to Improve Outcome after Severe Head Injury
Laerdal Foundation for Acute Medicine
C. Robertson, M.D., PI

$10,000	01/01/98 - 12/31/98	Co-Principal Investigator

Reduction of Neuronal DNA Damage in Rats by Hypothermia after Traumatic Brain Injury.
Laerdal Foundation for Acute Medicine
Michael Whalen, M.D., PI

$9,310	07/01/98 06/30/99	Co-Investigator

Production of a Novel Macrophage-Derived Neurotoxin, Quinolinic Acid, in Brain after Severe Head Injury in Adults and Children.
University of Pittsburgh's Center for Injury Research and Control/CDC

$39,860	10/01/97 - 08/31/98	Principal Investigator

A Multidisciplinary NMR Center for Biomedical Research
Collaborative Research Project 3
Magnetic Resonance Imaging in the Assessment of Experimentally Induced Traumatic Brain Injury in Rats.
Project Leaders: Chien Ho, Ph.D., PI and Patrick M. Kochanek, M.D.

$5,945,043	07/01/98 - 06/30/03

Intercellular Adhesion Molecule-1 (ICAM-1) and Secondary Damage After Traumatic Brain Injury.
University of Pittsburgh Department of Anesthesiology and Critical Care Medicine
Michael Whalen, M.D., PI

$7,690	Faculty Sponsor/Co-PI

A PARS Inhibitor in Brain Trauma
Inotek Corporation's Small Business Innovative Research (SBIR) Grant
NIH
George Hasko, M.D., PI

$20,208	07/01/98 - 12/31/98	Collaborator

Effect of Novel AK Inhibitor on Outcome after CCI Plus Secondary Insult in Rats.
Metabasis, Inc.

$25,156	09/17/98 06/30/99	Principal Investigator

Caspase-Mediated Neuronal Death after Head Injury
NIH RO1
Robert S.B. Clark, M.D., PI

$917,678	04/01/99 - 03/21/03	Co-Investigtor [5%]

Programmed-Cell Death after Human Head Injury
Competitive Medical Research Fund (CMRF)
University of Pittsburgh Medical Center
Robert Clark, M.D., PI

$24,170	7/01/97 6/30/99	Co-Investigator

Relationship of CBF to Function after Severe TBI in Immature Rats Using Perfusion MRI.
CHP Seed Grant
P. David Adelson, M.D., PI

$10,400	01/01/99 12/31/99	Consultant

Training in Trauma and Sepsis Research (T32-GM-08516-04)
NIH/NIGMS
Timothy Billiar, M.D., PI

$137,896	7/01/94 - 6/30/99	Co-Investigator

Quinolinic Acid in Cerebrospinal Fluid Early After Severe Head Injury in Victims of Child Abuse
University of Pittsburgh CIRCL CDC
Donald Marion, M.D., PI of CIRCL Center

$219,468	09/01/98 08/31/03	Principal Investigator

Cylooxygenase 2 and Ischemic Neuronal Injury
NIH-RO1
Steven H. Graham, M.D, Ph.D., PI

	12/01/98 - 11/30/02	Consultant

Hypothermia for Severe TBI in Children
NIH-RO1
P. David Adelson, M.D., PI

$1,658,419	04/01/99 03/31/02	Consultant

Suppression of Traumatic Brain Edema with an Inhibitor of PARS.
Inotek/CDC
$33,020 07/01/99
11/30/99

Adenosine and Traumatic Brain Injury
NIH-RO1
$1,593,730 12/01/98 - Principal Investigator
11/30/03

CPC-211 (Dicholoracetate) in the Controlled Cortical Impact Model of Traumatic Brain Injury in Rats
Cypros Pharmaceutical Corporation
$46,053 Principal Investigator

General Clinical Research Center for Children's Hospital of Pittsburgh
NIH 5M01 RR00084-37
Donald Fischer, M.D., PI
$5,082,660 12/01/99 Associate Program Director (12.5% effort)
11/30/04 Effective 10/1/99

Grants Pending:

Stress Responses in Early Life Traumatic Brain Injury
MCSD -KO8
Elizabeth Gilles, M.D., PI (Ohio State University
$239,760 12/01/99 - Consultant
11/30/01

University of Pittsburgh Brain Trauma Research Center
NIH Program Project
Donald Marion, M.D., PI
$5,453,880 03/01/00
02/28/05

Project #3 iNOS and Traumatic Brain Injury
$633,143 03/01/00 Principal Investigator
02/28/05

Core C Animal Modeling and Outcome
$745,636 03/01/00 Principal Investigator
02/28/05

Training in Pediatric Neurointensive Care and Resuscitation Research
NIH Training Grant
$1,217,405 07/01/00 Principal Investigator
06/30/05

2. Seminars and invited lectureships related to your research:

1. Novel Pharmacologic Approaches to Brain Resuscitation After Ischemia. American Academy of Pediatrics, Washington, D.C., November 1-2, 1986.

2. Blood Elements as Mediators of Injury in Brain Ischemia, Society of Critical Care Medicine, Anaheim, California, May 26-29, 1987.

3. A Possible Role of Blood Elements in the Evolution of Cerebral Injury During the Postresuscitation Syndrome. International Symposium Reversibility of Clinical Death, University of Pittsburgh, Pittsburgh, Pennsylvania, May 2-6, 1987.

4. Reperfusion Brain Injury. Society of Critical Care Medicine, Orlando, Florida, May 31- June 3, 1988.

5. Blood Elements in the Evolution of Tissue Injury After Cerebral Injury. FASEB, American Physiological Society, New Orleans, Lousiana, March 19-24, 1989.

6. Quantitation of Posttraumatic Edema Formation and Granulocyte Accumulation in the Brain. Fourth International Symposium "New Frontiers of Biochemistry and Biophysics on Diagnosis and Treatment of Stroke, Neurotrauma, and Other Neurological Diseases", Florence, Italy, April 19-21, 1989.

7. Pathobiology of the Pediatric Arrest. Emergency Medicine Services - Children (EMS-C), Seattle, Washington, November 10, 1989.

8. New Directions in the Therapeutic Approach to Cerebral Trauma. EMS-C, Seattle, Washington, November 10, 1989.

9. Monitoring Cerebral Blood Flow in the Critically Ill: Monitoring Techniques. Society of Critical Care Medicine Meeting. San Francisco, California, May 31, 1990.

10. Role of Inflammation in Brain Injury: Novel Directions in the Therapeutic Approach to Cerebral Trauma. American Academy of Pediatrics Meeting. Boston, Massachusetts, October 5, 1990.

11. Novel Therapeutic Approaches to Cerebral Ischemia and Trauma: Inflammatory Response Model. Critical Care Grand Rounds, University of Virginia, Health Science Center. Charlottesville, Virginia, December 12, 1990.

12. The Role of PMNs in Ischemic and Traumatic Brain Injury. Athena Neurosciences Corporation. South San Francisco, California, May 9, 1991.

13. Asphyxial arrest. Care of the Critically Ill or Injured. Charleston, South Carolina, November 22, 1991.

14. Pediatric Neuro-intensive Care. Care of the Critically Ill or Injured. Charleston, South Carolina, November 23, 1991.

15. Neutrophils in Brain Injury: An update. Wyeth-Ayerst Corporation. Princeton, New Jersey, March 11-12, 1992.

16. Ischemic and Traumatic Brain Injury: Pathobiology and Cellular Mechanisms. Critical Care Pediatrics. University of Miami School of Medicine, Arnold Palmer Hospital for Children & Women, Lake Buena Vista, Florida, March 5-7, 1992.

17. New Directions in Neurointensive Care and Cerebral Resuscitation. Critical Care Pediatrics. University of Miami School of Medicine, Arnold Palmer Hospital for Children & Women, Lake Buena Vista, Florida, March 5-7, 1992.

18. Pediatric Neurointensive Care. Neurologic Critical Care SCCM Postgraduate Course. San Antonio, Texas, May 28, 1992.

19. Molecular Mechanisms of Ischemic and Traumatic CNS Injury. First World Congress of Pediatric Intensive Care. Baltimore, Maryland, June 24, 1992.

20. Cerebral Resuscitation. Pediatric Grand Rounds, Tod Children's Hospital, Youngstown, Ohio, December 17, 1992.

21. The Pittsburgh Head Injury Conference, Inflammatory and Neurotrophic Response to Traumatic Brain Injury, Pittsburgh, Pennsylvania, September 17, 1993.

22. Inflammation Response to Traumatic Brain Injury, Ohio State University, Department of Immunology Seminars, March 27, 1994.

23. Inflammatory Response to Traumatic Brain Injury, Johns Hopkins University, Department of Anesthesiology and Critical Care medicine, May 1994.

24. The Future of Resuscitation Pharmacology, First Joint Conference of American Heart Association and American Academy of Pediatrics on Pediatric Resuscitation, Washington, D.C., June 12, 1994.

25. Traumatic Brain Injury- Mechanisms and Management. 7th Annual Pediatric Critical Care Colloquium, Seattle, Washington, October 26-29, 1994.

26. Severe Traumatic Brain Injury in Children: Pathobiology, Management, and Controversies, SCCM's 1995 Current Concepts in Critical Care, San Francisco, California, January 30 - February 4, 1995.

27. Severe Traumatic Brain Injury in Children. Critical Care Medicine Research-in-Progress Conference, Emory University School of Medicine, Department of Pediatrics, March 16, 1995.

28. Inflammatory Responses to Traumatic Brain Injury. Basic Science Fellow's Conference, Emory University School of Medicine, Department of Pediatrics, March 16, 1995.

29. Magnetic Resonance Imaging in Assessment of Experimentally Induced Head Trauma in Rats. Advisory Committee Meeting, Pittsburgh NMR Center for Biomedical Research, Pittsburgh, Pennsylvania, June 2, 1995.

30. A Contemporary Approach to Traumatic Brain Injury in Children. Trauma Care and Injury Control...Making the Connection. 4th Annual Adult and Paediatric Symposium, Trauma Services, Victoria Hospital and The Southwest Area Emergency Health Services Committee, London, Ontario, Canada, June 15-16, 1995.

31. Pathophysiology of Brain Injury. American Academy of Pediatrics, Section on Emergency Medicine and Critical Care Medicine, San Francisco, California, October 14-18, 1995.

32. Inflammatory Response to Traumatic Brain Injury. AI duPont Institute, Wilmington, Deleware, November 7-8, 1995.

33. Basic Mechanisms of Brain Injury: An Overview and New Concepts. 2nd Annual Neuroscience Symposium, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, November 18, 1995.

34. Inflammatory Response. International Business Communications USA Conference, Philadelphia, Pennsylvania, December 15, 1995.

35. Inflammation Response to Brain Injury. Naval Medical Research Institute's Conference on Medical Research: Concepts for Far-Forward Casualty Care, Coolfont, Berkely Springs, West Virginia, January 17-19, 1996.

36. Inflammatory Response to Traumatic Brain Injury. The 1996 Advances in Acute Neurotrauma Conference, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, January 20-21, 1996.

37. Novel Therapeutic Approaches to CNS Injury. 34th Annual Symposium on Critical Care Trauma and Emergency Medicine, University of Southern California School of Medicine, Las Vegas Hilton Hotel, Las Vegas, Nevada, February 12-16, 1996.

38. Traumatic Brain Injury in Children. 34th Annual Symposium on Critical Care Trauma and Emergency Medicine, University of Southern California School of Medicine, Las Vegas Hilton Hotel, Las Vegas, Nevada, February 12-16, 1996.

39. Case Studies in Brain Injury. 34th Annual Symposium on Critical Care Trauma and Emergency Medicine, University of Southern California School of Medicine, Las Vegas Hilton Hotel, Las Vegas, Nevada, February 12-16, 1996.

40. Pediatric Neurointensive Care. 34th Annual Symposium on Critical Care Trauma and Emergency Medicine, University of Southern California School of Medicine, Las Vegas Hilton Hotel, Las Vegas, Nevada, February 12-16, 1996.

41. Acute Inflammatory Response to Traumatic Brain Injury. Pathophysiology of Secondary Brain Injury and Implications for Contemporary Treatment, University of Pittsburgh Medical Center, Sheraton Hotel at Station Square, Pittsburgh, Pennsylvania, May 17-18, 1996.

42. Inflammatory Process in the Pathobiology of Secondary Damage after Traumatic Brain Injury. Fifth Wiggers Bernard Conference on Shock, Sepsis and Organ Failure. Brain Damage Secondary to Hemorrhagic-Traumatic Shock, Brain Damage Secondary to Sepsis, Brain Damage Secondary to Traumatic Brain Injury. Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria, May 19-23, 1996.

43. Magnetic Resonance Imaging in Assessment of Experimentally Induced Traumatic Brain Injury in Rats, Advisory Committee Meeting, Pittsburgh NMR Center for Biomedical Research, Pittsburgh, Pennsylvania, June 18, 1996.

44. Acute Inflammatory Response to Traumatic Brain Injury, FASEB Summer Research Conference, Copper Mountain, Colorado, August 11-16, 1996.

45. Traumatic Brain Injury in Children: A Contemporary View. Tri-State Appalachian Alliance for Emergency Medical Services For Children-Enhance Program, Charleston, West Virginia, September 7, 1996.

46. Pharmacology of Suspended Animation. Naval Medical Research Institute-sponsored conference on Research Planning for Suspended Animation, Pittsburgh Holiday Inn, Pittsburgh, Pennsylvania, January 19-20, 1997.

47. Established Investigator Grant Lecture: Role of Inflammation After Severe Head Injury, 26th International Symposium of the Society of Critical Care Medicine, San Diego, California, February 6-10, 1997.

48. Controversies: Is Hyperventilation Important in Treating Brain Ischemia and Head Injury? Panel Member, 26th International Symposium of the Society of Critical Care Medicine, San Diego, California, February 6-10, 1997.

49. Inflammatory Response to Traumatic Brain Injury: Bench to Bedside. Combined Neurosurgery/Neurology Grand Rounds, Henry Ford Medical Center, Detroit, Michigan, February 19, 1997.

50. Inflammatory Response to Traumatic Brain Injury. Anesthesia Grand Rounds, Harvard Medical School, Children's Hospital, Boston, Massachusetts, March 26, 1997.

51. Role of Inflammation in Cerebrovascular Failure after Head Injury. TraumaCare '97, 10th Annual Trauma Anesthesia and Critical Care Symposium (ATACCS), Baltimore, Maryland, May 15-17, 1997.

52. Traumatic Brain Injury in Children - From Bench to Bedside. European Society for Pediatric Research, European Society for Paediatric Haematology and Immunology, European Society for Paediatric Infectious Diseases, Joint Meeting, Budapest, Hungary, August 31-September 3, 1997.

53. Inflammatory Response to Severe Traumatic Brain Injury in Humans. Sixth Vienna Shock Forum, Vienna, Austria, November 8-11, 1997.

54. Inducible NOS and Other Novel Mediators of Inflammation in Brain Trauma. Sixth Wiggers Bernard Conference on Nitric Oxide and its Inhibition in Shock, Sepsis and Organ Failure. Vienna, Austria, November 12-15, 1997.

55. Mechanisms and Pharmacology in Suspended Animation. Suspended Animation Investigators Meeting, Pittsburgh Holiday Inn, University of Pittsburgh, Pittsburgh, Pennsylvania, December 5-7, 1997.

56. Severe Traumatic Brain Injury in Children: Epidemiology, Pathophysiology, Monitoring, and Management. Society of Critical Care Medicine, Current Concepts in Pediatric Critical Care, San Antonio, Texas, February 2-3, 1998.

57. Cell Injury and Response: Neurons. Society of Critical Care Medicine, 27th Educational and Scientific Symposium, San Antonio, Texas, February 4-8, 1998.

58. Resuscitation and the Prevention of Secondary Damage after Severe Traumatic Brain Injury. Pan American Congress of Emergency and Disaster Medicine, San Jose, Costa Rica, March 2-6, 1998.

59. Inflammatory Response to Trauma, 18th International Symposium on Intensive Care & Emergency Medicine, Brussels, Belgium, March 17-20, 1998.

60. New Developments in Head Trauma, 18th International Symposium on Intensive Care & Emergency Medicine, Brussels, Belgium, March 17-20, 1998.

61. Head Trauma, 18th International Symposium on Intensive Care & Emergency Medicine, Brussels, Belgium, March 17-20, 1998.

62. Magnetic Resonance Imaging Assessment of Experimental Traumatic Brain Injury in Rats, Advisory Committee Meeting, Pittsburgh NMR Center for Biomedical Research, Pittsburgh, Pennsylvania, May 28, 1998.

63. Immune/Inflammatory Responses in Traumatic Brain Injury, Spinal Cord Injury and Ischemia. FASEB Summer Research Conference on the Neurobiology of Central Nervous System Injury. Wilsonville, Oregon, June 21-26, 1998.

64. Traumatic Brain Injury in Children - Bench to Bedside, 3rd International Symposium on the Advances in Management of Critically Ill Children, Buenos Aires, Argentina, June 25-27, 1998.

65. Novel Approaches to Cerebral Resuscitation, 3rd International Symposium on the Advances in Management of Critically Ill Children, Buenos Aires, Argentina, June 25-27, 1998.

66. Acute Cerebral Injury: Hypothermia, 3rd International Symposium on the Advances in Management of Critically Ill Children, Buenos Aires, Argentina, June 25-27, 1998.

67. Pediatric Neurointensive Care: Unique Aspects of Pediatric Brain Failure and Resuscitaiton, 3rd International Symposium on the Advances in Management of Critically Ill Children, Buenos Aires, Argentina, June 25-27, 1998.

68. Critical Care-Pediatric Practical Course 024. Congress of Neurological Surgeons Annual Meeting, Seattle, Washington, October 3-8, 1998.

69. Frontiers in Cerebral Resuscitation: Lessons Learned from Human Head Injury. 12th Annual Society for Pediatric Anesthesia Meeting, Orlando, Florida, October 16, 1998.

70. Neurosurgical Issues in the Pediatric Intensive Care Unit: Biochemical Derangements in the Evolution of Secondary Damage After Severe Traumatic Brain Injury (TBI) in Infants and Children. American Academy of Pediatrics 1998 Annual Meeting, San Francisco, California, October 17-21, 1998.

71. Frontiers in Cerebral Resuscitation - Lessons Learned from Head Injury. The Toronto Critical Care Medicine Symposium, Toronto, Ontario, Canada, October 29 - November 1, 1998.

72. Severe Traumatic Brain Injury in Infants and Children - From Bedside to the Bench - and Back. Distinguished Lecture Series, Pediatric Grand Rounds, Children's Hospital of Omaha, Omaha, Nebraska, November 12-13, 1998.

73. Several - Not So Crazy- Resuscitation Strategies for the Troubled Brain. Distinguished Lecture Series, Pediatric Research Conference, Children's Hospital of Omaha, Omaha, Nebraska, November 12-13, 1998.

74. Severe Traumatic Brain Injury in Infants and Children - From Bedside to the Bench- and Back. 1998 Grand Rounds-Taubin Lecture, Children's National Medical Center, Washington, DC, December 1-2, 1998.

75. Several -Not So Crazy- Resuscitation Strategies for the Troubled Brain. Pediatric Research Conference, Children's National Medical Center, Washington, DC, December 1-2, 1998.

76. Evolution of Secondary Damage after Traumatic Brain Injury: Studies in Controlled Cortical Impact. Pediatric Research Conference, NIH, Washington, DC, December 3, 1998.

77. Inflammatory Cascades in Neurotrauma. Mechanisms of Brain Injury: Lessons from the Bench. Proceedings of the 28th Educational & Scientific Symposium. Society of Critical Care Medicine, San Francisco, California, January 23-27, 1999.

78. Traumatic Brain Injury in Children: From Bench to Bedside. Gladys Fashena Lecture - Grand Rounds, University of Texas Southwestern Medical Center, Dallas, Texas, March 9-10, 1999.

79. Novel Therapeutic Approaches to Brain Injury. Pediatric Research Conference, University of Texas Southwestern Medical Center, Dallas, Texas, March 9-10, 1999.

80. Frontiers in Cerebral Resuscitation: Lessons learned from Studies in Experimental and Clinical Head Injury. Uppsalla, Sweden, April 29, 1999.

81. Molecular Biology and Brain: III Argentine Congress of Emergency and Critical Care in Pediatrics, Buenos Aires, Argentina, September 23-25, 1999.

82. Brain Protection, III Argentine Congress of Emergency and Critical Care in Pediatrics, Buenos Aires, Argentina, September 23-25, 1999.

83. What Are the Key Mechanisms of Secondary Damage after Traumatic Brain Injury? Seventh Vienna Shock Forum, Vienna, Austria, November 13 - 16, 1999.

84. Pediatric Neuro Critical Care: Developmental Considerations in TBI. 29th Educational and Scientific Symposium. Orland, Florida, February 11-15, 2000.

3. Other research related activities:

Editorial Board

Stroke - 1989, 1990, 1991
Critical Care Medicine - 1996 - present
Journal of Neurotrauma - 1996 - present
Pediatric Life Support International, Founding Editor - 1996
Critical Care Medicine, Scientific Editor - 1997 - present
New Horizons, Scientific Editor – 1998 - present

Ad Hoc Reviewer:
Journals -

Stroke, 1987, 1988, 1989, 1995
Critical Care Medicine, 1988, 1993 - present
Journal of Neurosurgical Anesthesia, 1988
Pediatric Pulmonology, 1992
Journal of Cerebral Blood Flow and Metabolism, 1993 - present
Brain Research, 1994, 1999-present
Journal of Neurotrauma, 1994 - present
Anesthesia and Analgesia, 1994, 1995
Molecular and Clinical Neuropathology, 1994, 1995, 1997
Journal of Neuroscience, 1995, 1998-present
Journal of Intensive Care Medicine, 1995, 1997
Experimental Neurology, 1995
Anesthesiology, 1996
Journal of Neurochemistry, 1997, 1998
Brain Research Bulletin, 1997
American Journal of Physiology: Heart & Circulatory Physiology, 1999 – present
PNAS-Proceedings of the National Academy of Sciences, USA, 1999-present
American Journal of Pathology, 1999 - present

Ad Hoc Reviewer:
Grants -

NIH/ADAMHA Peer Review Consultant, 1992
Western Pennsylvania Psychiatric Institute and Clinic, 1992 - 1994
PSI Foundation, Ontario, Canada, 1994
Children's Hospital of Eastern Ontario Research Institute, Ontario Canada 1994, 1995
University of Pittsburgh ADRC Seed Grant Proposal, 1995
Department of Veterans Affairs Merit, 1996
NIH NSD-A Study Section, 1997
The Wellcome Trust, 1998
The Hospital for Sick Children Foundation, 1998

Committees
Local -

Research Advisory Committee - Department of Anesthesiology and Critical Care Medicine, University of Pittsburgh, 1993 - present
Scientific Affairs Committee, Department of Anesthesiology and Critical Care Medicine, University of Pittsburgh, 1989 - present
GCRC Advisory Committee, Children's Hospital of Pittsburgh, 1991- present
GCRC Advisory Committee, University of Pittsburgh Medical Center, 1996 – September 1999
23rd Annual Meeting of ISOTT, Pittsburgh, PA, August 23-27, 1995
Anesthesia & CCM Newsletter, 1995 - present

Reappointment and Promotion Committee - Department of Anesthesiology and Critcal Care Medicine, University of Pittsburgh, 1995 – 1996, 1998-present
Research Advisory Committee - Children's Hospital of Pittsburgh, 1997 - present
Executive Steering Committee, Department of Anesthesiology and Critical Care Medicine, University of Pittsburgh, 1997 - present
Chairman, Scientific Affairs Committee, Department of Anesthesiology and Critical Care Medicine, University of Pittsburgh, 1998 - present
Health Sciences Animal Research Advisory Committee (HSARAC) ,1999 – present
Strategic Planning Committee - Children's Hospital of Pittsburgh, 1999 – present
Associate Director, GCRC Advisory Committee, Children's Hospital of Pittsburgh, 1999- present

National -

Program Committee - Society of Critical Care Medicine Meeting, 1990, 1991, 1992, 1993
Program Chairman - Society of Critical Care Medicine Meeting, 1994
Selection Committee - Young Investigator Award, SCCM, 1989, 1990, 1994
Abstract Reviewer - SPR (Section on Critical Care Medicine), 1993
Selection Committee - Laerdal Foundation Lecture, SCCM, 1994
Selection Committee - In Training Award SCCM, 1994
Continuing Education Committee - Society of Critical Care Medicine, 1994 - 1996
Critical Care Consultant - Tenth International Brain Edema Symposium, 1996
Program Committee - Neurotrauma Society Meeting, 1997, 2000
Program Committee - Sixth Vienna Shock Forum, 1997
Program Chairman - Sixth Wiggers Bernard Conference, 1997
American Board of Pediatrics - Sub-board in Pediatric Critical Care Medicine, 1998 – present
Chair, Credentials Committee – American Board of Pediatrics, Pediatric Critical Care Medicine Subboard - 2000

Other -

Multidisciplinary Critical Care Knowledge Assessment Program (MCCKAP) of the Society of Critical Care Medicine, Editorial Board Member, 1991, 1992, 1993, 1994
Field Tester for the American Board of Pediatrics Subspecialty Board Examination in Pediatric Critical Care Medicine, 1993
National Multi-Centered Animal Traumatic Brain Injury Study (NATBIS) planning committees participant, 1993, 1995
Consultant to the First International Conference on Pediatric Resuscitation, Washington, DC, June 1994
Consultant - Cypros Pharmaceutical Corporation, 1997

ARTICLES IN SUBMISSION

1. Adler S, Verbalis JG, Kochanek PM, Williams DS: Altered Cerebral Blood Flow in Normonatremic and Hyponatremic Rats Following Acute Increases in Plasma Sodium. Am J Physiol (in submission).

2. DeKosky ST O'Malley M, Goss JR, Kochanek PM, Burmeister LA: Hypothyroidism Attenuates the Nerve Growth Factor Response Following Traumatic Brain Injury in the Adult Rat. Endocrinology (in submission).

3. Ruppel RA, Kochanek PM, Adelson PD, Rose M, Wisniewski SR, Bell MJ, Clark RSB, Marion DW, Graham SH: Excitotoxicity after Severe Traumatic Brain Injury in Infants and Children: The Role of Child Abuse. J Pediatrics (in submission).

ARTICLES IN PREPARATION

1. Adelson PD, Robichaud RJ, Hamilton RL, Kochanek PM: Histopathologic Changes Following Diffuse Traumatic Brain Injury in the Immature Rat. (in preparation).

2. Adelson PD, Whalen M, Robichaud P, Carlos T, Kochanek P: Blood Brain Barrier Permeability and Acute Inflammation in Two Models of TBI in the Immature Rat. (in preparation).

3. Bell M, Adelson PD, Jackson E, Clark R, Mi Z, Carcillo J, Kochanek PM: Adenosine Concentrations in Cerebrospinal Fluid After Severe Traumatic Brain Injury in Children. Neurosurgery (in submission).

4. Bell MJ, Robertson CS, Kochanek PM, Goodman JC, Gopinpath S, Carcillo JA, Clark RSB, Marion D, Mi Z, Jackson E: Interstitial Brain Adenosine and Xanthine Increase During Jugular Venous Oxygen Desaturations in Humans After Traumatic Brain Injury. Crit Care Med (in preparation).

5. Hickey RW, Ferimer H, Alexander HL, Garman RH, Callaway CW, Hicks S, Safar P, Graham SH, Kochanek PM: Beneficial Cerebral Effects of Permissive Hypothermia Late After Asphyxia in Rats. (in preparation).

6. Kochanek PM, Hendrich KS, Dixon CE, Schiding JK, Williams DS, DeKosky ST, Graham SH, Marion DW, Ho C: Perfusion Magnetic Resonance Imaging at One Year After Controlled Cortical Impact in Rats. J Neurotrauma. (in preparation).

7. Kochanek PM, Hendrich K, Schiding J, Whalen M, Williams D, Ho C: Sequential MRI Assessement of Cerebral Blood Flow and Blood-Brain Barrier Permeability Early After Traumatic Brain Injury in Rats. (in preparation).

8. Whalen MJ, Doughty LA, Carlos TM, Wisniewski SR, Kochanek PM, Carcillo JA: Intercellular Adhesion Molecule-1 are Increased in the Plasma of Children with Sepsis-Induced Multiple Organ Failure. (in preparation).

9. Whalen TM, Carlos TM, Wisniewski SR, Clark RSB, Melick J, Marion DW, Kochanek PM: Effect of Neutropenia and GCSF-Induced Neutrophilia on Blood-Brain Barrier Permeability and Brain Edema after Traumatic Brain Injury in Rats. Crit Care Med (in preparation).

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Augmented neuronal death in CA3 hippocampus following hyperventilation early after controlled cortical impact

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Minimizing secondary injury after severe traumatic brain injury (TBI) is the primary goal of cerebral resuscitation. For more than two decades, hyperventilation has been one of the most often used strategies in the management of TBI. Laboratory and clinical studies, however, have verified a post-TBI state of reduced cerebral perfusion that may increase the brain's vulnerability to secondary injury. In addition, it has been suggested in a clinical study that hyperventilation may worsen outcome after TBI.

Object. Using the controlled cortical impact model in rats, the authors tested the hypothesis that aggressive hyperventilation applied immediately after TBI would worsen functional outcome, expand the contusion, and promote neuronal death in selectively vulnerable hippocampal neurons.

Methods. Twenty-six intubated, mechanically ventilated, isoflurane-anesthetized male Sprague–Dawley rats were subjected to controlled cortical impact (4 m/second, 2.5-mm depth of deformation) and randomized after 10 minutes to either hyperventilation ($PaCO_2 = 20.3 \pm 0.7$ mm Hg) or normal ventilation groups ($PaCO_2 = 34.9 \pm 0.3$ mm Hg) containing 13 rats apiece and were treated for 5 hours. Beam balance and Morris water maze (MWM) performance latencies were measured in eight rats from each group on Days 1 to 5 and 7 to 11, respectively, after controlled cortical impact. The rats were killed at 14 days postinjury, and serial coronal sections of their brains were studied for contusion volume and hippocampal neuron counting (CA1, CA3) by an observer who was blinded to their treatment group.

Mortality rates were similar in both groups (two of 13 in the normal ventilation compared with three of 13 in the hyperventilation group, not significant [NS]). There were no differences between the groups in mean arterial blood pressure, brain temperature, and serum glucose concentration. There were no differences between groups in performance latencies for both beam balance and MWM or contusion volume (27.8 ± 5.1 mm^3 compared with 27.8 ± 3.3 mm^3, NS) in the normal ventilation compared with the hyperventilation groups, respectively. In brain sections cut from the center of the contusion, hippocampal neuronal survival in the CA1 region was similar in both groups; however, hyperventilation reduced the number of surviving hippocampal CA3 neurons (29.7 cells/hpf, range 24.2–31.7 in the normal ventilation group compared with 19.9 cells/hpf, range 17–23.7 in the hyperventilation group [25th–75th percentiles]; $*p < 0.05$, Mann–Whitney rank-sum test).

Conclusions. Aggressive hyperventilation early after TBI augments CA3 hippocampal neuronal death; however, it did not impair functional outcome or expand the contusion. These data indicate that CA3 hippocampal neurons are selectively vulnerable to the effects of hyperventilation after TBI. Further studies delineating the mechanisms underlying these effects are needed, because the injudicious application of hyperventilation early after TBI may contribute to secondary neuronal injury.

KEY WORDS • **head injury** • **hyperventilation** • **alkalosis** • **hippocampus** • **rat**

TRAUMATIC brain injury (TBI) is often complicated by malignant intracranial hypertension,[32] which is associated with high mortality rates and has been managed using a combination of therapies including osmotherapy, diuretics, sedation, neuromuscular blockade, optimization of cerebral perfusion pressure, and hyperventilation.[6,12,32,38,51] Hyperventilation therapy has been an integral part of the clinical armamentarium in the management of severe TBI for more than 20 years:[11] this therapy rapidly reduces cerebral blood flow (CBF) and cerebral blood volume in areas of the brain with intact CO_2 autoregulation, providing one option in the management of TBI complicated by malignant intracranial hypertension.[1,34,42]

In recent studies, however, researchers have defined a state of reduced CBF early after TBI in humans[3,31] and animals,[5,20,25,46,56,57] particularly in the first 8 hours after TBI. Some authors have hypothesized that the brain is more

vulnerable to secondary injury during this period and that additional reduction of CBF by hyperventilation may attenuate the delivery of important energy substrates.[7,11,30,39,47,48] Yoshida and Marmarou[58] reported that hyperventilation produced relative ischemia in cat brain after fluid-percussion injury and demonstrated an increase in brain lactate and inhibition of recovery of the ratio of phosphocreatine to inorganic phosphate. Muizelaar, et al.,[40] also demonstrated a loss of brain interstitial bicarbonate buffer after sustained prophylactic hyperventilation in rabbits. It has been reported that hyperventilation after TBI in animals and humans can reduce CBF to what traditionally have been considered ischemic levels.[10,24,42] However, defining the ischemic threshold in injured tissue is problematic.[22,33] Muizelaar, et al.,[39] reported that prolonged hyperventilation after TBI in humans may worsen functional outcome, raising questions regarding the appropriate indications and timing for the optimum application of hyperventilation after TBI. Recently published guidelines for the management of severe head injury[6] state that "in the absence of intracranial hypertension, hyperventilation ($PaCO_2 \leq 35$ mm Hg) therapy should be avoided during the first 24 hours after severe TBI. . . ," although "hyperventilation therapy may be necessary for brief periods where there is acute neurologic deterioration. . . ." Consistent with these guidelines, in the setting of acute neurological deterioration, aggressive hyperventilation is used by both emergency and critical care personnel. In addition, in the initial stabilization of the brain-injured patient, aggressive hyperventilation (appropriate in the setting of impending herniation, or iatrogenic) occasionally occurs in both the prehospital and acute care settings. The specific impact of hyperventilation during this early low-flow period remains to be determined. Despite the availability of well-characterized rodent models of TBI, which reproduce the early posttraumatic reduction in CBF, the effect of aggressive hyperventilation on histopathological and functional outcome has not, to our knowledge, been investigated.

Using a rat model of focal percussive contusion, we hypothesized that aggressive hyperventilation, beginning immediately after TBI and continuing for 5 hours, would worsen functional outcome, expand the contusion, and promote neuronal death in selectively vulnerable hippocampal neurons.

Materials and Methods

Animals and Study Groups

All experimental protocols used in this report were approved by the Animal Care and Use Committee of the University of Pittsburgh. Twenty-six virus-free Sprague–Dawley rats weighing 346 ± 5 g were studied. Food and water were continuously available in their home cages. After TBI the rats were randomly assigned to one of two groups of 13 animals, one receiving normal ventilation ($PaCO_2$ = 30–40 mm Hg) and one receiving hyperventilation ($PaCO_2$ = 15–25 mm Hg).

Surgery and Brain Trauma Model

Anesthesia was induced using 4% isoflurane in N_2O/O_2 (2:1). The rats were endotracheally intubated and mechanically ventilated. The isoflurane concentration was reduced to 2% followed by sterile surgical placement of a femoral arterial catheter for continuous mean arterial blood pressure (MABP) and arterial blood gas monitoring. Intramuscular injections of penicillin (100,000 U) and gentamicin (10 mg/kg) were given to minimize the risk of infection. Pancuronium bromide was administered at dosages of 0.1 mg/kg/hour via the arterial line to control ventilation. The rats' core temperature was monitored using a rectal probe.

After stereotactically guided head positioning, an incision was made and the scalp was retracted, exposing the left parietal bone. A craniotomy was made using a high-speed dental drill aided by a binocular operating microscope. A burr hole was made 5 mm anterior and 2 mm lateral to the bregma in the left side of the skull and a temperature probe (0.009-in outer diameter) was inserted through the burr hole and 2 mm into the left parietal cortex. The bone flap was left in place and the isoflurane was reduced to 1% followed by a 30-minute equilibration period. The brain temperature was maintained at 37 ± 0.5°C. Normal arterial blood gas levels were achieved in all rats and PaO_2 was maintained at greater than 70 mm Hg.

The TBIs were produced using a controlled cortical impact device as recently described[9,25] with minor modifications. Fifteen minutes before controlled cortical impact, an arterial blood sample was obtained for measurement of arterial blood gas levels, glucose concentration, and hematocrit. The bone flap was then removed and a vertical controlled cortical impact (4 m/second impactor velocity, 2.5-mm deformation depth) was delivered onto the exposed dura overlying the left parietal cortex. The bone flap was replaced and sealed with dental cement and the scalp was sutured.

Study Design

The study protocol was designed to mimic the aggressive use of hyperventilation (as opposed to normal ventilation) in the immediate posttrauma period in the prehospital as well as early hospital setting. Ten minutes after controlled cortical impact, rats were randomized to either the normal ventilation group (13 animals, $PaCO_2$ range 30–40 mm Hg) or the hyperventilation group (13 animals, $PaCO_2$ range 15–25 mm Hg). The ventilator was adjusted to maintain normocarbia or hypocarbia for 5 hours after controlled cortical impact. Arterial blood gas readings were obtained at 30 minutes post–controlled cortical impact, then hourly. The MABP was recorded every 30 minutes after controlled cortical impact. Brain and rectal temperatures were recorded every 15 minutes.

At 5 hours after controlled cortical impact, anesthesia was discontinued. Temperature probes and the femoral artery catheter were removed and the rat was weaned from mechanical ventilation in the course of 1 hour and underwent extubation. The time to extubation was recorded. After extubation, supplemental O_2 was administered for 30 minutes. When it had fully recovered, the rat was returned to its cage with full access to food and water.

Functional Outcome and Behavior Assessment

Beam Balance. Vestibulomotor function was tested using the beam balance test[14] in eight rats from each group. One hour before surgery, the rat was placed lengthwise on a 1.5-cm-wide beam suspended above the ground. The time the rat remained on the beam was recorded (up to 60 seconds). The rat was then removed from the beam and the procedure was repeated. Rats were considered trained when they remained on the beam for three consecutive periods of 60 seconds. Beam balance tests were also performed daily on Days 1 to 5 postinjury. Three trials were recorded and averaged each day for each rat.

Morris Water Maze. Cognitive function was tested in the same eight rats from each group using a standard variation of the Morris water maze (MWM) paradigm.[15,35] A pool 180 cm in diameter and 60 cm deep was painted black and filled with water to a depth of 28 cm. A clear Plexiglas platform 10 cm in diameter and 26 cm high (2 cm below the water surface) was used as the hidden goal platform. The pool was located in a 2.5 × 2.5-m room with numerous extra-maze cues (for example, posters, pipes, bookcase) that remained constant throughout the experiment. Testing started 7 days after controlled cortical impact to avoid confounding effects of motor deficits. The rats underwent four trials per day for 5 consecutive days to assess spatial memory performance. The rats started each trial once from each of the four possible start locations

TABLE 1

*Physiological values in two groups of rats treated with hyperventilation or normal ventilation after TBI**

Value	Normal Ventilation		Hyperventilation	
	Baseline	Postrandomization	Baseline	Postrandomization
pH	7.39 ± 0.01	7.37 ± 0.01	7.38 ± 0.01	7.53 ± 0.01†
$PaCO_2$ (mm Hg)	36.7 ± 1.1	34.9 ± 0.3	37.2 ± 0.9	20.3 ± 0.7†
PaO_2 (mm Hg)	165 ± 6	167 ± 4	168 ± 4	180 ± 3†
base deficit (mmol/L)	2.7 ± 3.4	4.2 ± 0.7	−0.6 ± 0.9	4.8 ± 0.6
serum glucose (mg%)	189 ± 9	174 ± 6	158 ± 10	152 ± 9
hct (%)	36 ± 2.3	35 ± 0.6	32.3 ± 1.5	35 ± 0.6
time to extubate (min)	NA	28 ± 6	NA	29 ± 5
brain temperature (°C)	36.7 ± 0.1	37 ± 0	36.6 ± 0.1	37 ± 0
rectal temperature (°C)	36.5 ± 0.6	37 ± 0	37.1 ± 0.1	37.1 ± 0.1
MABP (mm Hg)	129 ± 4	123 ± 4	129 ± 8	128 ± 3

* All values are expressed as mean ± SEM. Abbreviations: hct = hematocrit; NA = not applicable.
† $p < 0.05$ at 30 minutes postrandomization compared with baseline.

(north, south, east, and west); the order of the starting location was randomized. The goal platform was positioned 45 cm from the outside wall and was placed in either the northeast, southeast, southwest, or northwest quadrant of the maze. The location of the platform was kept constant for each rat. Rats were manually placed in the pool facing the wall and were given a maximum of 120 seconds to find the hidden platform. If the rats failed to find the platform within 120 seconds, they were placed there by the researcher. All rats were allowed to remain on the platform for 30 seconds before being placed in a heated incubator between trials. There was a 4-minute intertrial interval. All data were recorded by means of a video tracking system.

Histopathological Studies

At 14 days after controlled cortical impact (after completion of all of the functional outcome testing), the rats were anesthetized with 5% isoflurane and killed by perfusion fixation using 10% buffered formalin. Their brains were removed and postfixed at 4°C for a minimum of 1 week, and then cryoprotected in sucrose and cut with a cryotome into 10-μ coronal sections at 1-mm increments from the occipital to the frontal lobe and stained with Cresyl violet.

Contusion Volume. We used a computerized image analysis system to outline the margin of the contusion and the sectional area of the contusion at each 1-mm increment was calculated by an observer (M.L.F.) who was blinded to the treatment group. Contusion volume in each rat was calculated as the sum of these sections.

Hippocampal Cell Counting. Neuronal loss in hippocampal regions CA1 and CA3 pyramidal layers was quantified.[8] A coronal section cut from the dorsal hippocampus underlying the area of contusion, approximately 2.6 mm posterior to the bregma, was used for analysis in each rat. The regions were visualized at × 100 magnification, then localized and counted at × 400 by an observer (R.S.B.C.) blinded to treatment group. Only complete cells with a clearly defined body and nucleus were counted. Surviving pyramidal CA1 and CA3 hippocampal neurons were counted in six separate × 400 fields for each region in both hemispheres. Sections were excluded if the boundary of the contusion extended into the pyramidal layers of the hippocampus or if fixation artifacts precluded accurate counting. Data are reported as the average number of surviving neurons per high-power field for the CA1 and CA3 hippocampal regions in both the ipsilateral and contralateral hemispheres.

Statistical Analysis

Survival was compared between groups using Fisher's exact test. Between group comparisons of physiological parameters, beam balance, and MWM latencies were made using one- or two-way analysis of variance (ANOVA) for repeated measures where appropriate and post-hoc tests with appropriate correction for multiple comparisons. Contusion volume was normally distributed and was compared between groups using Student's t-test. Hippocampal neuronal survival in CA1 and CA3 was not normally distributed and was compared between groups using the Mann–Whitney rank-sum test. Significance was defined at a probability level of less than 0.05.

Sources of Supplies and Equipment

Pancuronium bromide and gentamicin were purchased from Elkins-Sinn, Cherry Hill, NJ, and penicillin was acquired from Upjohn, Kalamazoo, MI. The stereotactic head positioning system was obtained from David Kopf, Tjunga, CA. The temperature probe was purchased from Physitemp Corp., Clifton, NJ. The video tracking system (Poly-Trak) was acquired from San Diego Instrument, Inc., San Diego, CA, and the image analysis system (MCID) was from Imaging Research, St. Catherines, Ontario, Canada.

Results

Physiological Parameters

Baseline and 30-minute postrandomization physiological data are presented for all measured parameters in Table 1. After randomization, there was a marked increase in pH and decrease in $PaCO_2$ in the hyperventilation group (compared with baseline, $p < 0.05$). Hyperventilation was also associated with a small increase (12 mm Hg) in PaO_2 compared with baseline ($p < 0.05$). This difference was attributable to the increased minute ventilation and mean airway pressure in the hyperventilation group. At no time were any of the rats hypoxemic ($PaO_2 < 100$ mm Hg). The entire time course of $PaCO_2$, arterial pH, MABP, and brain temperature after TBI is given for both groups in Fig. 1. The $PaCO_2$ and pH levels differed between groups at all time points after randomization ($p < 0.05$). The MABP and brain temperature were similar in both groups.

Five of 26 rats died during the 14-day study, with all deaths occurring on the day of injury. Two rats remained unresponsive postinjury and were unable to demonstrate any spontaneous respiratory effort for 1 hour after discontinuation of anesthesia and were therefore killed. Three rats developed pulmonary edema and/or respiratory distress and died soon after extubation. There were no differences in mortality between groups (two of 13 in the normal ventilation group compared with three of 13 in the hyperventilation group). There were no differences between groups in time to extubation (Table 1).

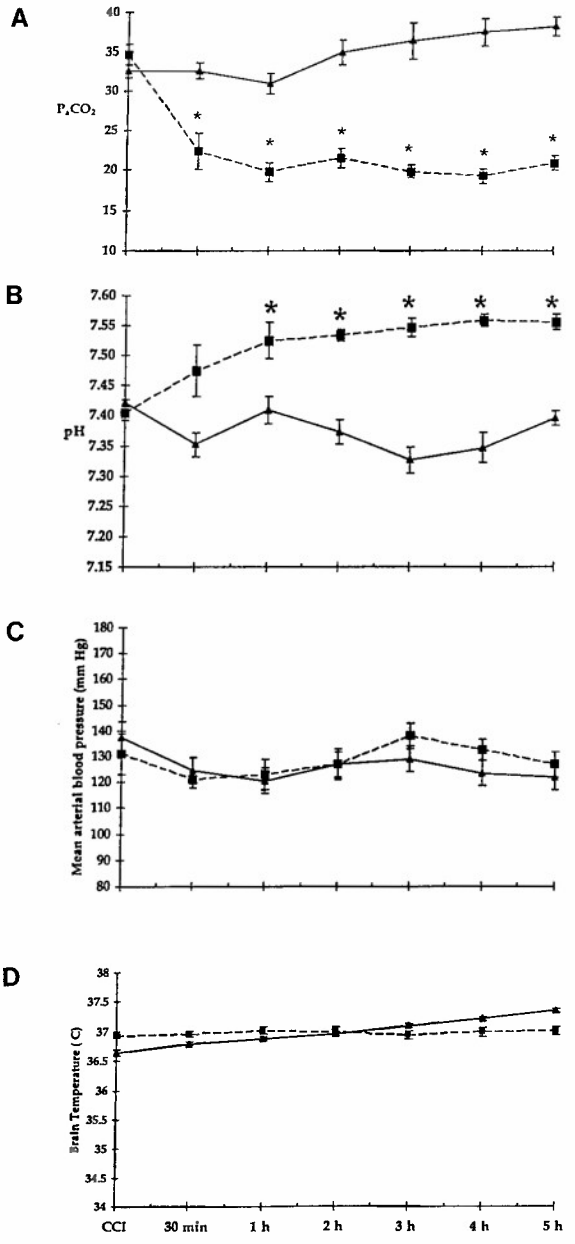

FIG. 1. Graphs showing time course of (A) $PaCO_2$ (mm Hg), (B) arterial pH, (C) MABP (mm Hg), and (D) brain temperature (°C) in all rats treated with either normal ventilation (*triangles* w/ *solid line*, 13 animals) or hyperventilation (*squares* w/ *broken line*, 13 animals) after controlled cortical impact. *$p < 0.05$ for normal ventilation compared with hyperventilation. Data are expressed as the mean ± standard error of the mean (SEM).

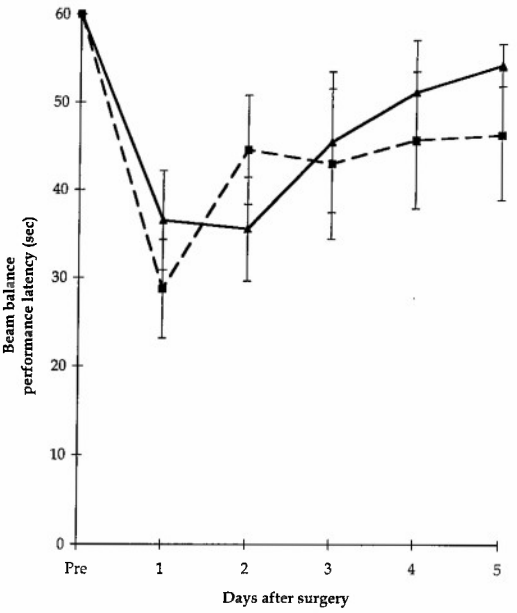

FIG. 2. Graph showing mean beam balance performance latencies (mean ± SEM, in seconds) in rats before and on Days 1 to 5 after controlled cortical impact (4 m/second, 2.5-mm cortical deformation depth). Repeated-measures ANOVA revealed no difference in duration of balance maintained between the two groups (*triangles* = normal ventilation [eight rats]; *squares* = hyperventilation [eight rats]).

Functional Outcome Assessment

Beam Balance. There was no difference between groups in motor performance latencies over time ($F_{1,15} = 0.17$, $p < 0.69$, Fig. 2). Maximum impairment of performance occurred on Days 1 or 2 in both groups, and eventually returned to baseline. Beam balance performance did not differ significantly between normal ventilation and hyperventilation groups.

Morris Water Maze. There was no difference between normal ventilation and hyperventilation groups in the time needed to find the hidden platform in the MWM test ($F_{1,15} = 0.50$, $p < 0.50$, Fig. 3). In addition, there was a statistically nonsignificant tendency ($t_{13} = 1.77$, $p < 0.065$) for the rats in the hyperventilation group to swim slower than the rats in the normal ventilation group (30.8 ± 1.0 compared with 35.4 ± 2.1 cm/second).

Histopathological Studies

Contusion Volume. At the injury level selected for this study, the contusion was generally restricted to the parietal cortex beneath the impact site. Contusion volume in both groups is shown in Fig. 4. There was no difference between groups (27.8 ± 5.1 mm^3 in the normal ventilation group compared with 27.8 ± 3.3 mm^3 in the hyperventilation group) in this outcome parameter.

Hippocampal Cell Counting. Figure 5 shows the number of surviving neurons/hpf in the CA1 and CA3 regions of the dorsal hippocampus ipsilateral to the contusion. There were no differences in the number of surviving CA1 hippocampal neurons between groups after controlled cortical impact. There was, however, a further reduction in the number of surviving CA3 neurons in the hyperventilation group after controlled cortical impact compared with the normal ventilation group (normal ventilation 29.7, range 24.2–31.7 neurons/hpf, compared with hyperventilation 19.9, range 17–23.7 neurons/hpf; median [25th–75th percentiles], $p < 0.05$). Neuronal cell counts in the CA1 and CA3 regions of the hemisphere contralateral to the contusion did not differ in either the normal ventilation or hyperventilation groups (CA1 counts = 55.3, range 52.1–59

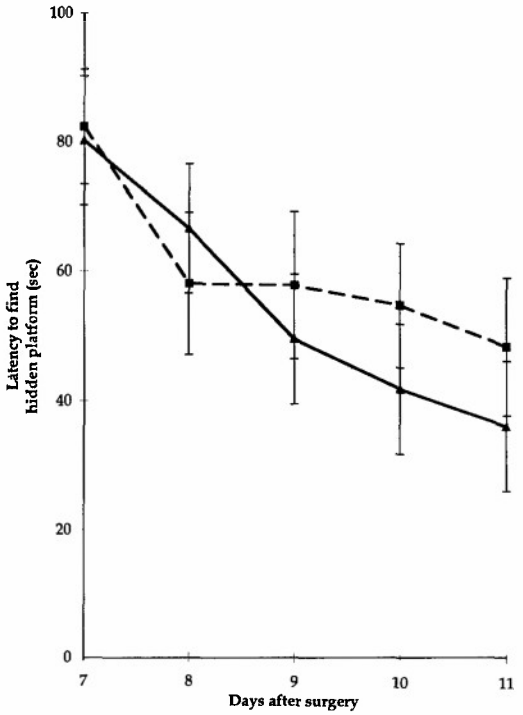

FIG. 3. Graph showing MWM performance latency to find a hidden platform (mean ± SEM, in seconds) by rats on Days 7 to 11 after controlled cortical impact. There was no difference between groups (*triangles* = normal ventilation [eight animals]; *squares* = hyperventilation [eight animals]) when performances were compared using ANOVA with repeated measures.

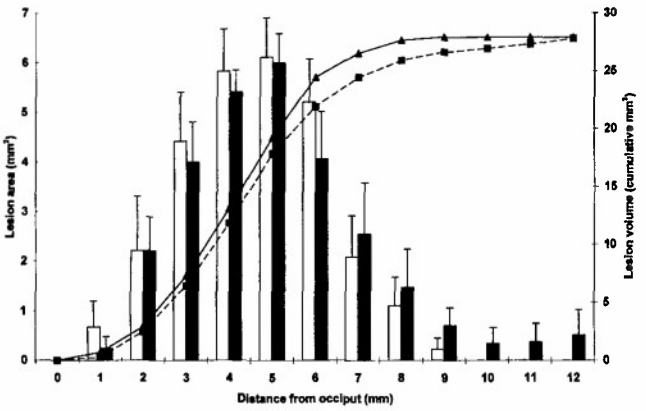

FIG. 4. Bar graph depicting mean lesion area (*left y-axis*, mm^2) compared with distance from occiput (mm) measured 14 days after controlled cortical impact (*open bars*, normal ventilation [11 rats]; *closed bars*, hyperventilation [10 rats]). Contusion volume (mm^3) was calculated as the sum of these areas in each group and is depicted as the cumulative volume *(right y-axis)* in the normal ventilation *(triangles)* and hyperventilation *(squares)* groups. There was no difference between groups in contusion volume (normal ventilation, 27.8 ± 5.1 mm^3 compared with hyperventilation, 27.8 ± 3.1 mm^3, mean ± SEM).

[normal ventilation] and 57.3, range 51.3–59 [hyperventilation]; CA3 = 40, range 36.6–41.2 [normal ventilation] and 38, range 33–41.7 [hyperventilation]).

Discussion

In a model of controlled cortical impact–induced focal contusion in rats, aggressive hyperventilation for 5 hours after TBI augments neuronal death in the CA3 region of the hippocampus ipsilateral to the contusion. However, hyperventilation did not worsen motor function or cognitive outcome, as assessed using standard beam balance and MWM paradigms, respectively, and did not increase contusion volume.

Hippocampal CA3 neurons are selectively vulnerable to delayed neuronal death after TBI.[2,8,19,49,52,53] Theories about the mechanisms underlying this process remain speculative. Potential mechanisms include ischemia, TBI-induced excitotoxicity, apoptosis, and inflammation.[8,19,49]

Yamakami and McIntosh[56,57] reported reduced CBF as early as 15 and 30 minutes after TBI. Using a piglet model of TBI, Pfenninger, et al.,[46] reported CBF reduction as early as 5 minutes post-TBI. Some flow levels were in the range consistent with ischemia. We have previously demonstrated that the hippocampus and cortex ipsilateral to the impact show marked flow reduction (at least 60%) at 2 hours after TBI in the controlled cortical impact model.[25] Cerebral blood flow approaches ischemic levels in the core of the contusion at 2 hours postinjury. Although we have not evaluated the reactivity status of the cerebral circulation to changes in $PaCO_2$ at 2 hours after TBI in this model, we have reported that CO_2 reactivity is impaired, although still present (62–71% of baseline) in and around the contusion at 24 hours after controlled cortical impact in rats.[16]

Hyperventilation rapidly reduces cerebral blood volume and intracranial pressure (ICP).[11] In some studies, this intervention has been associated with CBF values consistent with ischemia or brain tissue hypoxia.[10,11,42,48] After global cerebral ischemia in dogs, hyperventilation did not increase neuronal death;[55] however, the brains were assessed at 8 hours after reperfusion, and neuronal death may be delayed. Although ischemia may be considered a contributing mechanism in the observed augmented neuronal death, ischemia alone is an inadequate explanation for our findings in light of the preservation of CA1 neurons. Although CA1 neurons are known to be selectively vulnerable to ischemic injury,[23] they were not affected by hyperventilation in this paradigm. Furthermore, in our model, CA1 neurons are more proximal to the point of impact in the cortex compared with CA3 neurons. The lack of CA1 neuronal death in light of ischemic and (presumed) anatomical vulnerability weighs against ischemia and primary injury as putative mechanisms of neuronal death in the hippocampus in this model. One limitation in this study is that neuronal counting using traditional histological methods may underestimate cell loss because of a loss of hippocampal volume.[52] We did not use stereological methods in this study. However, CA1 neuronal counts did not differ between groups and were equivalent to those observed in sham-injured animals studied in our laboratory in prior published[8] and unpublished work. In addition, comparisons were only made between injured groups within this study.

Hyperventilation produces cerebral vasoconstriction

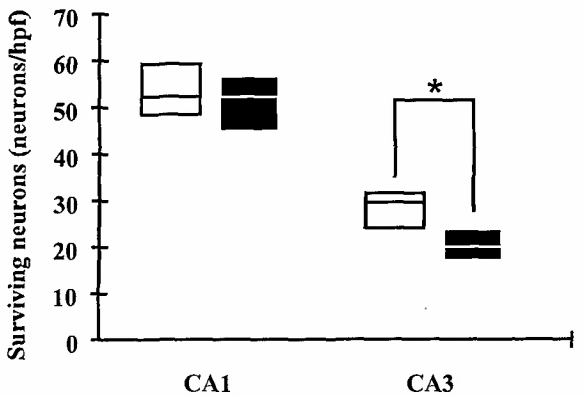

FIG. 5. Box plots representing the number of surviving CA1 and CA3 hippocampal neurons in coronal brain sections cut from the center of the lesion in the hemisphere ipsilateral to the contusion. Cells were counted 14 days postinjury. The median line is placed within the shaded 25th to 75th percentile range. There was a reduction in the number of surviving CA3 hippocampal neurons after injury in normal ventilation *(open boxes)* compared with hyperventilation *(solid boxes)* groups (29.7 cells/hpf, range 24.2–31.7 compared with 19.9 cells/hpf, range 17–23.7). *$p < 0.05$, Mann–Whitney rank-sum test.

and alkalosis.[40] Alkalosis exacerbates *N*-methyl-D-aspartate receptor–mediated neurotoxicity.[17,18,21,43] As a result of aggressive hyperventilation, the rats in our study were quite alkalotic as indicated by arterial pH measurements. Although we did not measure brain pH, a decrease in $PaCO_2$ immediately reduces brain interstitial pH.[40] Although alkalosis appears to have deleterious effects on neurons, acidosis has been shown to have both beneficial and detrimental effects. Giffard, et al.,[17] and Takadera, et al.,[54] reported a neuroprotective effect of acidosis via an attenuation of the *N*-methyl-D-aspartate receptor activation in vitro. Rosner and Becker[50] reported a deleterious effect of tissue acidosis after experimental TBI in cats. The spatial distribution of brain pH around the contusion and in the hippocampus has not been determined for either normal ventilation or hyperventilation conditions in our model.

Finally, the potential effects of hyperventilation on other mechanisms such as posttraumatic seizures or axonal injury may contribute to the enhanced vulnerability of CA3 neurons. The lateralization of the deleterious effects also raises the possibility that spreading wave depression may be a component of the neurotoxic milieu after TBI in this model of focal contusion.[20] It could also be the case that the combined effect of alkalosis and further flow reduction by hyperventilation is deleterious in regions vulnerable to excitotoxicity such as CA3. Early, aggressive, or prophylactic hyperventilation, therefore, in the context of reduced CBF, may potentiate excitotoxic mechanisms and augment neuronal death.

Aggressive hyperventilation in the early low-flow period did not worsen functional outcome or expand the contusion, failing to support a significant portion of our initial hypothesis. Ultimate contusion size, in controlled cortical impact or other models of focal contusion, is relatively refractory to manipulation by a variety of interventions;[4,8] however, application of hypothermia, particularly prior to injury, reduces contusion volume resulting from controlled cortical impact and lateral fluid-percussion injury.[13,44] Although we chose rather aggressive hyperventilation in an attempt to produce a maximum effect, we did not test the effect of hyperventilation on a milder contusion, which may be more manipulable to secondary insults. The contusion penumbra has not been clearly defined in either of the standard rodent TBI models (controlled cortical impact or fluid–percussion) for any level of injury. It is possible that selectively vulnerable CA3 hippocampal neurons are the only potential target for a deleterious effect of hyperventilation in our model. However, the effect of hyperventilation on the survival of neurons in the dentate gyrus or hilus (all vulnerable to TBI)[9,29] was not assessed.

Hippocampal damage and memory deficits are common after TBI in humans.[26,28] This study did not reveal any added effect of hyperventilation on functional outcome deficits as measured by beam balance and MWM latencies. A number of factors may have contributed to this. Our sample size may have limited statistical power; however, this sample size was adequate to detect the exacerbation of functional deficits by the addition of 30 minutes of moderate hypoxemia (PaO_2 40 mm Hg) in our model.[8] Second, the cognitive deficits in this model are modest compared with those detailed in previous reports.[15] Bilateral hippocampal damage may be necessary to create more marked functional deficits.[36,37] In addition, CA3 damage may not mediate post-TBI memory deficits, as manifested in MWM test results. Finally, the specific functional outcome paradigm may not have the necessary sensitivity to detect subtle functional deficits. For example, more demanding MWM paradigms have been used by other investigators.[27,52] However, in support of the testing strategy used, our hypothesis was that hyperventilation would worsen functional deficits.

This study does not completely address the uncommon situation in which, soon after severe head injury, marked intracranial hypertension is observed. Hyperventilation may in fact be life saving in its ability to impede herniation. Similarly, we did not measure ICP or titrate ventilation to control cerebral perfusion pressure, and we evaluated only one level of hyperventilation and injury severity. We did not attempt to model the clinical scenario of optimum titration of ventilation when ICP is increased. In the clinical setting, some investigators have demonstrated a wide variety of beneficial effects of hyperventilation under those conditions, such as homogenization of CBF, normalization of cerebral glucose uptake, and improvement in autoregulation.[12,41,42] Rather, we chose the worst-case scenario, aggressive hyperventilation during the early posttrauma period when flow is already low and excitotoxicity is peaking.[45] However, our study does show that hyperventilation is associated with a tangible risk to vulnerable neurons in the controlled cortical impact model. To our knowledge, this is the first in vivo study demonstrating that hyperventilation can augment neuronal injury after TBI, suggesting that there is indeed a tradeoff associated with this intervention.

Conclusions

We have demonstrated that aggressive, early hyperven-

tilation after TBI augments neuronal death in CA3 hippocampus. The further reduction of CBF with hyperventilation during the low CBF state immediately after severe TBI, coupled with alkalosis, may increase the vulnerability of selected neurons to traumatic injury. Further studies are needed to delineate the relative contributions of these mechanisms to the observed effects. The results of this study reinforce that meticulous attention is necessary to prevent secondary injury after TBI, and a risk in the use of hyperventilation is demonstrated.

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Therapeutic Hypothermia After Traumatic Brain Injury or Hemorrhagic Shock: From Mild Cooling to Suspended Animation

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Objectives:

1. To familiarize the reader with contemporary studies on the application of resuscitative hypothermia in the treatment of traumatic brain injury and hemorrhagic shock.
2. To describe the potential mechanisms for the beneficial effects of hypothermia in these settings.
3. To present some recent findings from both laboratory and clinical studies of resuscitative hypothermia conducted at the University of Pittsburgh.
4. To discuss possible side effects and limitations of the application of therapeutic hypothermia.
5. To discuss future directions for novel applications of hypothermia in combination with pharmacologic interventions.

Historical Perspective

One of the earliest reports of the potential beneficial effects of hypothermia in the treatment of traumatic brain injury was described by Charles Phelps in 1897 in his classic textbook Traumatic injuries of the brain and its membranes! It is fitting that this monograph was assembled on the 100th anniversary of this remarkable description.

> *"The shaving of the head, which had been advised as a means of facilitating diagnosis, is at the same time a measure of treatment... The essential advantage... to be derived from this procedure is that it permits the effective application of the ice-cap, which next to trephination, ...is most nearly a directly curative resource... It is contraindicated in hemorrhages and cerebral lacerations when uncomplicated by serious contusion; but, as those lesions are constantly thus complicated, it may be held a proper resort when such symptoms are manifest, without regard to exact diagnosis."*

In the early 1940s, Fay[2,3] examined the deliberate application of hypothermia in traumatic brain injury, and this was followed by several additional series of case reports and uncontrolled trials between 1943 and 1979 by other pioneers in this field including Woringer et al,[4] Sedzimir,[5] Lazorhes and Campan,[6] and Rosomoff[7] in traumatic brain injury, Albin et al[8] in spinal cord injury, Bigelow et al[9] and Swan et al[10] in cardiothoracic surgery, Rosomoff et al[11] in focal cerebral ischemia, Siebke et al[12] and Conn et al[13] in near drowning, Wolfe,[14] Benson et al,[15] Ravitch and Safar[16] in cardiopulmonary arrest, and Rush et al[17] in the application of deep hypothermia for total circulatory arrest. Although remarkable effects were suggested in many of these reports, they failed to demonstrate convincingly that hypothermia was beneficial and did not result in the widespread application of resuscitative hypothermia. These reports were complicated by a number of difficulties including variation in depth and duration of hypothermia, and failure to include concurrent normothermic controls. In addition, reports of potential infectious complications in patients treated with the sustained application of moderate hypothermia[18] tempered enthusiasm for further studying resuscitative hypothermia in a controlled fashion.

Laboratory studies supporting the application of therapeutic hypothermia in traumatic brain injury and hemorrhagic shock

In the mid 1980s there was renewed interest in the laboratory investigation of the deliberate application of therapeutic hypothermia for protection (induced before the insult) or resuscitation (induced after the insult). This work was focused predominantly in models of global cerebral ischemia in rats and monkeys,[19-23] cardiopulmonary arrest[24-28] and near drowning in dogs.[29] Central to this resurgence in interest in hypothermia was the development of three novel concepts: 1) that remarkably mild hypothermia (a temperature reduction of between 3° and 5°C) was effective in reducing secondary brain damage,[19-30] 2) that the duration of mild hypothermia necessary for a beneficial effect might be transient - as short as 1 or 2 hours[19,28] and 3) that brain temperature, not body temperature, was the critical therapeutic target.[19] The chance discovery of the efficacy of mild, transient hypothermia in these studies revived the importance of hypothermia research because mild and transient hypothermia are safer and easier to induce than the previously tried moderate, sustained hypothermia. It is important to define the approximate temperature ranges commonly used to describe specific depths of therapeutic hypothermia. Generally accepted definitions of these ranges are mild (34° to 36°C), moderate (28° to 32°C), deep (15° to 25°C), and profound (< 15°C) hypothermia.[31]

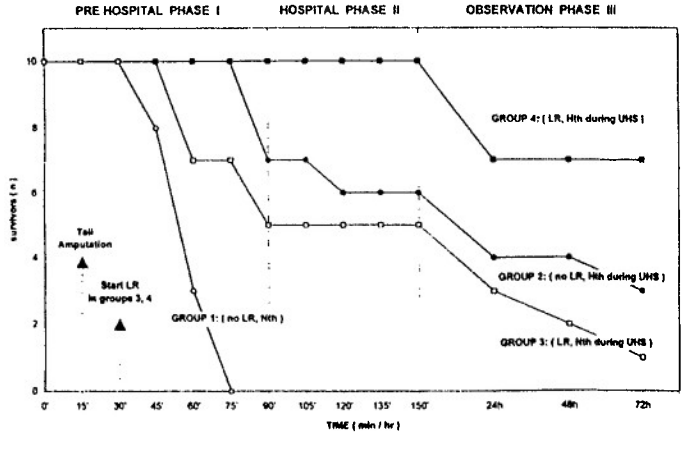

Figure 1: Survival after uncontrolled hemorrhagic shock (UHS) in rats from the study of Kim et al.[41] The insult in all groups is comprised of a volume controlled initial hemorrhage followed by tail amputation. Treatments include normothermia (Nth, Group 1), hypothermia (Hth, Group 2, 30°C applied between 15 min. and 120 min.), normothermia plus lactated Ringers (LR) fluid resuscitation (Nth + LR, Group 3), or hypothermia plus fluid resuscitation (Hth + LR, Group 4.) Survival to 72 hours was maximal in rats treated with hypothermia plus LR. Reprinted from the Journal of Trauma with permission.

Specific investigation of the application of therapeutic hypothermia in the treatment of traumatic brain injury was renewed by the report of Clifton et al[32] who obscrved an inverse correlation between functional outcome and brain temperature (between 30° and 40°C). This was followed by a series of reports from several laboratories further defining the beneficial effect of hypothermia in a wide variety of models (both rodent and canine) of traumatic brain injury.[33-37]

Recent controlled laboratory studies of the utility of resuscitative hypothermia in models of hemorrhagic shock developed from the initial work of Crippen et al in our center[38] and of Meyer and Horton.[39] This resuscitative effect was demonstrated in models of both controlled[38,40] and uncontrolled[41] hemorrhagic shock (Figure 1), and with both mild and moderate hypothermia.[42,43] In controlled laboratory studies addressing an additional hemorrhagic shock-related application of deliberate hypothermia, Tisherman et al[44,45] investigated the application of deep and profound hypothermic circulatory arrest to enable resuscitative surgery that would otherwise be impossible. Our series of studies into "suspended animation" has culminated so far in the study by Capone et al[46] who reported complete recovery of the brain in dogs after normothermic hemorrhagic shock of 1 hour followed by profound hypothermic circulatory arrest of 1 hour. This application of resuscitative hypothermia is being further developed as a possible novel therapeutic approach to the management of pulscless battlefield casualties, specifically, "suspended animation" for transport and repair of otherwise lethal extracranial wounds. "Suspended animation" could be induced and reversed by portable cardipulmonary bypass[47] and followed by subsequent delayed resuscitation.[48]

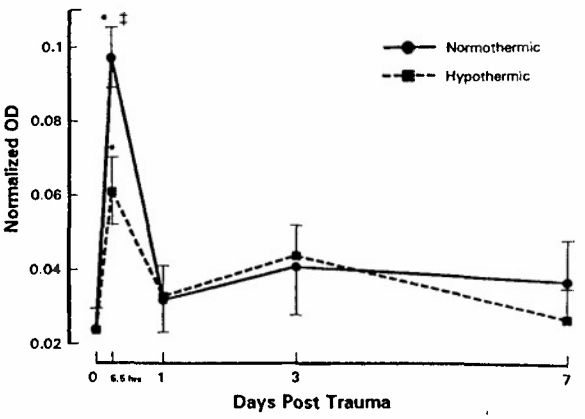

Figure 2. Desitometric analysis of RNA gel blot hybridizations for IL-1β message before, and at serial times after experimental cerebral contusion in rats, from the work of Goss et al.[65] Filled circles represent data from rats maintained at brain temperature 37°C, while solid squares represent data from rats maintained at a brain temperature of 32°C for 4 hours after injury. A marked increased in IL-ß message was observed at 5.5 hours after injury which was partially attenuated by hypothermia. Reprinted from the Journal of Neurotrauma with permission.

Why hypothermia: Proposed mechanisms for the beneficial effects of deliberate hypothermia in traumatic brain injury and hemorrhagic shock

Laboratory and clinical trials in cerebral resuscitation from ischemic or traumatic brain injury have repeatedly highlighted the tremendous challenge involved in demonstrating reproducible efficacy, in a wide variety of injury models or injury types, when a single therapeutic agent is used.[49-51] The complex, multifactorial nature of the cascades of secondary damage purported to occur in both ischemic and traumatic brain injury strongly suggests the need for multimodal therapies.[23,48-52] A similar multifactorial pathogenesis is proposed in the evolution of visceral damage after hemorrhagic shock.[31] A great deal of evidence suggests that hypothermia favorably and simultaneously influences a large number of secondary injury mechanisms including; energy failure,[53] oxidant injury,[54,55] delayed neuronal death,[19,56] excitotoxicity,[56] intracranial hypertension[57] edema formation,[35,57] cytoskeletal protein degradation,[58] blood-brain barrier permeability,[59] IL-1ß production[60] (Figure 2), and neutrophil accumulation.[61] It is very likely that some critical combination of beneficial effects on these mechanisms is responsible for the success of therapeutic hypothermia in experimental and clinical trials.

Clinical investigation of therapeutic hypothermia in traumatic brain injury

Although there is a much larger body of laboratory data supporting the use of mild, transient, resuscitative hypothermia in ischemic rather than traumatic brain injury, clinical application of deliberate hypothermia has been spearheaded in controlled trials after traumatic brain injury. Uncontrolled trials of moderate hypothermia in patients after traumatic brain injury looked promising[57,62] but were abandoned because of management problems. Marion et al[63] reported a beneficial effect of moderate (32°C), transient (24 hours) hypothermia on intracranial hypertension in adults with severe closed head injury. A reduction in the need for other therapies for control of intracranial hypertension was observed. Clifton et al[64] reported a reduction in the incidence of posttraumatic seizures in adults treated with moderate hypothermia for 48 hours after severe head injury. A trend toward improved outcome was also observed. Similarly, Shiozaki et al[65] reported efficacy of mild hypothermia in controlling refractory intracranial hypertension in patients with severe traumatic brain injury. Most recently, Marion et al[66] demonstrated that moderate (32°C), transient (24 hours) hypothermia improved functional outcome as measured with the Glasgow outcome scale at 6 months after severe traumatic brain injury in 82 patients randomized to either hypothermia or normothermia. This beneficial effect extended to 12 months in the subgroup of patients with admission Glasgow coma score of 5 to 7 (Table 1). In addition, reductions in IL-ß and glutamate concentrations were demonstrated in cerebrospinal fluid samples from hypothermic vs normothermic patients, suggesting the possibility of beneficial effects of hypothermia on posttraumatic inflammation and excitotoxicity, respectively. Remarkably, a significant reduction in cerebral metabolic rate for oxygen was not observed,[63,66] suggesting that this beneficial effect was not due to a simple reduction in cerebral oxidative metabolic demands. A multicenter randomized controlled clinical trial of 48 hours of hypothermia vs normothermia in the treatment of human head injury is currently underway.

Potential limitations and complications of the application of deliberate hypothermia

Hypothermia is associated with potentially limiting side effects. Suppression of acute inflammation[67] and an increased infection risk[15,18] are concerns. These complications appear to be importantly related to the duration of hypothermia and the underlying condition that is being treated. In traumatic brain injury, Marion et al[66] and Clifton et al[64] did not observe increases in the incidence of infection with 24 hour and 48 hour

TABLE 1 GLASGOW OUTCOME SCORES IN THE HYPOTHERMIA AND NORMOTHERMIA GROUPS AT 3, 6, AND 12 MONTHS

Glasgow Outcome Scores	At 3 Months		At 6 Months		At 12 Months	
	Hypothermia	Normothermia	Hypothermia	Normothermia	Hypothermia†	Normothermia
All Patients						
1. (Death)	8 (20)	9 (21)	8 (20)	10 (24)	9 (23)	10 (24)
2. (Vegetative state)	6 (15)	11 (26)	3 (8)	7 (17)	3 (8)	8 (19)
3. (Severe disability)	11 (28)	5 (36)	7 (18)	11 (26)	3 (8)	8 (19)
4. (Moderate disability)	8 (20)	4 (10)	7 (18)	8 (19)	9 (23)	5 (12)
5. (Mild or no disability)	7 (18)	3 (7)	15 (38)	6 (14)	15 (38)	11 (26)
Total	40	42	40	42	39	42
P Value‡	0.12		0.05		0.18	
Patients with coma score 5 to 7						
1. (Death)	2 (9)	5 (19)	2 (9)	6 (23)	2 (9)	6 (23)
2. (Vegetative state)	2 (9)	7 (27)	1 (5)	3 (12)	1 (5)	4 (15)
3. (Severe disability)	6 (27)	9 (35)	3 (14)	8 (31)	3 (14)	6 (23)
4. (Moderate disability)	6 (27)	3 (12)	4 (18)	6 (23)	5 (23)	2 (8)
5. (Mild or no disability)	6 (27)	2 (8)	12 (55)	3 (12)	11 (50)	8 (31)
Total	22	26	22	26	22	26
P Value‡	0.01		0.01		0.04	

*Percentages may not add to 100 because of rounding

†One patient was lost to follow-up

‡P values are comparisons of all five outcomes in the hypothermia and normothermia groups.

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applications of hypothermia, respectively. However, longer applications of hypothermia may have considerable risk.[18] In addition, application of mild hypothermia in settings not associated with ischemia but associated with considerable infection risk (such as elective abdominal surgery in patients with malignancies) increases infection rates.[68]

Coagulopathy is suggested as another potential complication of hypothermia. However, in the studies of severely head injured patients by Marion et al,[63,66] platelet counts and prothrombin times did not differ significantly between groups, and no difference in posttrauma intracranial hematomas or other hemorrhagic complications were noted despite the fact that some of the patients had multiple trauma. Cardiac arrhythmias were also not observed. The threshold for these complications appears to be temperatures below 30°C.[69,70] On the other hand, a recent report[71] suggested that morbid cardiac events after non-cardiac surgery were more common in mildly hypothermic patients compared to those who remained normothermic.

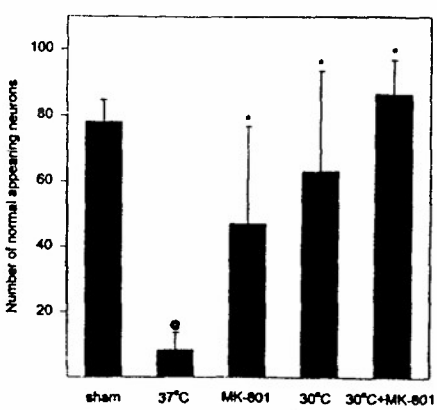

Figure 3. Bar graph from the work of Dietrich et al[75] showing the number of normal appearing neurons in striatum at 2 months after sham operation or cerebral ischemia in rats treated with normothermia (37°C), the glutamate receptor antagonist MK-801, hypothermia (30°C), or the combination of hypothermia plus MK-801. Neuronal survival was maximal after treatment with the combination of moderate hypothermia and MK-801. Reprinted from the Journal of Cerebral Blood Flow and Metabolism with permission.

Although the systemic complications appear relatively minimal for the transient (24 hour) application of mild or moderate hypothermia, one area of investigation that deserves further study is that of the effect of hypothermia on regenerative and endogenous defense mechanisms in brain. Goss et al[60] reported that 4 hours of moderate hypothermia resulted in a sustained inhibition of nerve growth factor production in brain after experimental contusion in rats. Nerve growth factor is an important homeostatic molecule in the central nervous system that upregulates antioxidant defenses and prevents apoptosis. The ramifications of this effect of hypothermia on brain parenchyma is currently under investigation.

Finally, another potential limitation of resuscitative hypothermia may be that it produces a temporary rather than sustained effect —i.e., delays rather than ameliorates damage. This possibility was first suggested in classic studies of the effect of hypothemia on acute inflammation,[67,72] and was reintroduced in work by Dietrich et al[73] in models of global cerebral ischemia, where brief episodes (1-3 hours) of hypothermia only delayed death of neurons in selectively vulnerable brain regions. Recent work by Colbourne et al,[74] however, suggests that longer durations of hypothermia may produce permanent benefit.

Future directions

Some of the most intriguing recent work in the therapeutic application of hypothermia in laboratory studies involves the combination of hypothermia with other therapies. Dietrich et al[75] reported that combination of 3 hours of moderate hypothermia with sustained administration of the glutamate antagonist MK-801 produced a synergistic beneficial effect on neuronal survival in a model of global cerebral ischemia (Figure 3). Similar reports have been suggested for the combination of hypothermia and other therapies.[76] Additional promising strategies that will require further study include the combination of hypothermia with either growth factors,[60] anti-inflammatory agents or flow promoting treatments.[60,77]

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No Long Term Benefit from Hypothermia after Severe Traumatic Brain Injury with Secondary Insult in Rats

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ABSTRACT

Objectives: To evaluate the effect of application of transient, moderate hypothermia on outcome following experimental traumatic brain injury (TBI) with a secondary hypoxemic insult.

Design: Prospective, randomized study.

Setting: University-based animal research facility.

Subjects: Male Sprague-Dawley rats.

Interventions: All rats were subjected to severe traumatic brain injury (TBI) followed by 30 min of moderate hypoxemia, associated with mild hypotension. Rats were randomized to three groups: a) normothermia (37 ± 0.5 °C); b) immediate hypothermia (32 ± 0.5 °C initiated after trauma, before hypoxemia); and c) delayed hypothermia (32 ± 0.5 °C after hypoxemia). The brain temperature was controlled for 4 h after TBI and hypoxemia.

Measurements and Main Results: Animals were evaluated after TBI for motor and cognitive performance using beam balance (days 1-5 after TBI), beam walking (days 1-5 after TBI) and Morris Water Maze (days 14-18 after TBI) assessments. On day 21 after TBI, rats were perfused with paraformaldehyde and brains were histologically evaluated for lesion volume and hippocampal neuron counts. All three groups showed marked deficits in beam balance, beam walking and Morris Water Maze performance. However, these deficits did not differ between groups. There was no difference in lesion volume between groups. All animals had significant hippocampal neuronal loss on the side ipsilateral to injury, but this loss was similar between groups.

Conclusions: In this rat model of severe TBI with secondary insult, moderate hypothermia for 4 hours post-trauma failed to improve motor function, cognitive function,

lesion volume or hippocampal neuronal survival. Combination therapies may be necessary in this difficult setting.

Key Words: traumatic brain injury; hypothermia; hypoxemia

INTRODUCTION

Secondary insults after experimental traumatic brain injury (TBI) have been shown to exacerbate disturbances in key physiologic parameters, including hypoperfusion, energy failure, cerebral edema, and EEG suppression (1-3). In addition, animals subjected to a secondary hypoxemic insult after TBI have worse motor and histologic outcomes than those subjected to TBI alone (1, 3, 4). Following severe TBI, patients often experience a variety of secondary systemic insults related to extracerebral traumatic injury. As many as 20 - 50 % of patients presenting with severe TBI have experienced a period of hypoxemia (5-7). Autopsy findings of head injured patients (8) demonstrate evidence of ischemic neuronal death throughout the brain. Similarly, clinical studies have demonstrated higher morbidity and mortality among head injured patients who had experienced a secondary insult, specifically hypoxemia or hypotension (7). Often, the most severely devastated patients are those who experience the combination of TBI with hypoxemia and hypotension.

Hypothermia has been used as a successful treatment modality following brain injury in many experimental models and clinical settings. Neuroprotective effects of hypothermia in animal models include attenuation of release of excitatory amino acids (9, 10, 11), reduction in hydroxyl radicals (9) and inflammatory mediators (12, 13), and reduction in disruption of the blood-brain barrier (14). In the setting of experimental TBI, hypothermia improves outcome (15, 16). In models of fluid percussion injury (15) and controlled cortical impact (CCI) (16) reductions in both functional and motor deficits are observed in animals treated with moderate hypothermia after TBI when compared to normothermic animals. Transient, moderate hypothermia applied following global or

focal ischemic insult in animal models has improved histologic outcomes (17-19). Clinical studies have similarly demonstrated improvements in functional outcomes (20) and ICP (21, 22) in patients treated with moderate hypothermia.

Despite the importance of secondary insults to clinical outcome after TBI, the variety of experimental models of TBI and secondary insult that have been developed, and the success of hypothermia in both clinical and experimental TBI, the effect of the application of hypothermia in the setting of TBI with secondary insult has not been studied. We hypothesized that moderate hypothermia would improve outcome after CCI with secondary insult in rats.

MATERIALS AND METHODS

This study was approved by the University of Pittsburgh Animal Care and Use Committee. The care and handling of animals were in accord with National Institute of Health guidelines.

Experimental protocol

Virus-free male Sprague-Dawley rats (329-460 g) were studied. The animals were allowed free access to food and water before and after surgery. All surgical procedures were performed using aseptic technique.

Anesthesia was induced in a plastic jar with 4% isoflurane (Anaquest, Memphis, TN) in O_2. The trachea was intubated with a 14-gauge angiocatheter and the lungs were mechanically ventilated with 2% isoflurane/66% N_2O/balance O_2. A femoral arterial catheter (PE-50) was inserted for continuous monitoring of blood pressure and arterial blood sampling. Pancuronium bromide (0.1 mg/kg/h, Elkins-Sinn, Cherry Hill, NJ) was given for immobilization. A rectal probe was inserted to monitor core temperature.

Traumatic Brain Injury Model

The head was fixed in a sterotactic device (David Kopf, Tujunga, CA) and a midline scalp incision was made to expose the parietal bone. A craniotomy was made over the left parietal cortex with a dental drill, using the coronal and interparietal sutures as margins. The intact dura and bone flap were left in place until immediately before trauma. A temperature probe (0.009 inch outside diameter, Physiotemp Corp., Clifton, NJ) was inserted through a burr hole into the left parietal cortex 5 mm anterior to the bregma and 2 mm lateral to the sagittal suture. Rats were equilibrated under anesthesia (1.1% isoflurane/66 % N_2O/balance O_2) at a brain temperature of 37 ± 0.5°C for 30 min

before TBI. Fifteen minutes before trauma an arterial blood sample (0.5 ml) was obtained to verify normal arterial blood gas (ABG) tensions, serum glucose and hematocrit.

TBI was performed using the CCI device (23, 24), with minor modifications to the procedure previously described (25). Briefly, after removal of the bone flap, injury was produced using a device with a 6-mm metal impactor tip that is pneumatically driven in the vertical plane at a predetermined depth, velocity, and duration of brain deformation. For all studies, a depth of penetration of 2.5 mm, a velocity of 4.0 ± 0.2 m/sec, and a duration of deformation of 50 msec was used. Following trauma, the bone flap was replaced and sealed with dental cement (Koldmount, Vernon Banshoff Co., Albany, NY) and the scalp incision was closed.

Secondary Insult

Beginning 1 min after CCI, all rats underwent a 30 min period of hypoxemia as previously described (4). Air and oxygen were blended to achieve an FiO2 of 11% (1.1% isoflurane/74% N_20/19% air/6% O_2). This produced a PaO_2 range of 46-51 and MAP range of 50-74. Arterial blood gas (ABG) samples were obtained in all rats at 10 and 25 min during the hypoxemic period.

Hypothermia

Rats were randomized into three groups: normothermia, immediate hypothermia and delayed hypothermia. Hypothermia (temp = 32°C) was achieved by the external application of ice packs to the head to lower brain temperature over a 15 min period. This temperature was maintained for 4 h and the brain was rewarmed over 1 h. ABG samples were obtained every hour during the hypothermia period. Physiologic parameters (MAP,

brain temperature, rectal temperature) were recorded every 30 min during hypothermia and during rewarming.

The immediate hypothermia group (n=10) had brain cooling initiated immediately after trauma, coincident with the onset of hypoxemia. The delayed hypothermia group (n=10) had cooling initiated upon the completion of hypoxemia, 30 min after TBI. The normothermia group (n=19) had brain temperature maintained at 37 ± 0.5°C throughout the experimental period. At the end of the experiment, after completion of rewarming, anesthesia was discontinued. Rats were extubated, placed in 100% oxygen for an additional 30 min, then returned to their cages, where they were allowed free access to food and water.

Motor Function Assessments

Gross vestibulomotor function was assessed using a beam-balance task (24, 26). The rats were trained by three trials prior to TBI to obtain a baseline measurement. Beam-balance latency (up to 60 sec) was measured on days 1-5 after TBI.

Fine vestibulomotor function and coordination were assessed using a beam-walking task (27). Performance was assessed by measuring the rat's latency to traverse the beam and enter a goal box. Beam-walking latency was measured on days 1-5 after TBI.

Cognitive Assessment (Morris Water Maze)

Water-maze testing started on day 14 postinjury. The hidden platform task assesses the rat's ability to learn spatial relations between distal cues and the escape platform. Performance is impaired by cortical and hippocampal lesions. We used a variant of the Morris water maze (28). Rats were given 120 sec to find the hidden platform. If the rat failed to find the platform within 120 sec, it was placed on the

platform by the experimenter. Rats were given four swimming trials per day for 5 consecutive days. Water maze tests were given on days 14-18 after TBI. The last 2 days of testing consisted of a visible platform task in which the platform was raised 2 cm above the water surface. This visible task controls for potential non-specific visual or motor deficits.

Lesion Volume Analysis

At 21 d after TBI, rats were anesthetized and perfused with 500 ml of 4% buffered formaldehyde. Brains were removed and post-fixed for a minimum of 1 wk at 4°C and cryoprotected in sucrose. Coronal sections (10-μm) were prepared through the entire brain at 1-mm intervals from the occiput. Sections were stained with cresyl violet. In the serial sections taken at 1-mm intervals, the margins of both the contusion and the total left hemisphere were outlined by a blinded observer using image analysis (Image Research). Contusion and hemispheric areas were measured. Contusion volume was calculated and expressed as mm^3.

Hippocampal Cell Counts

Neuronal loss in hippocampal CA1 and CA3 pyramidal layers was quantified using a method previously described by Clark et al (4). A coronal section through the dorsal hippocampus underlying the area of contusion was used for analysis. This location was approximately 2.6 mm posterior to bregma. The regions were visualized at 400X magnification by a blinded observer. CA1 and CA3 hippocampal neurons were counted in six separate fields for each region in both injured and uninjured hemispheres. Only complete cells with a defined cell body and intact nucleus were counted. Hippocampal

neuron survival was reported as the average number of surviving neurons per high power field ipsilateral to injury.

Statistical Analysis

All data are presented as mean ± SEM. Because of the large number of physiologic variables recorded, comparisons of physiologic data were made using a multiple regression analysis, evaluating the effect of treatment and time for each variable. Beam balance, beam walking and Morris water maze data were analyzed using repeated-measures analysis of variance (ANOVA) using GB-STAT statistical software. Lesion volumes and hippocampal neuron counts were compared using The Kruskal-Wallis test and Dunn's test. A significance level of $p < 0.05$ was used for all tests.

RESULTS

Physiologic Variables

Physiologic data (including brain and rectal temperature, MAP, arterial pH and blood gasses, blood glucose and hematocrit) from all animals that survived the experimental protocol are presented in Table 1. There were no differences between groups in pH, $PaCO_2$, and hematocrit. As expected, the groups differed in brain and rectal temperatures ($p < 0.05$), and this difference was seen between groups when controlling for time. Brain temperature remained at 37 ± 0.2 °C in the normothermia group. Brain temperature decreased from 37.2 ± 0.1 °C before trauma to 32.0 ± 0.1 °C by 25 min of cooling in the immediate hypothermia group. Brain temperature decreased from 37.1 ± 0.1 °C before trauma to 32.1 ± 0.1 °C by 25 min of cooling in the delayed hypothermia group.

Groups differed in MAP when controlling for time ($p < 0.05$). Initial MAP in the normothermia group was 91-92 and decreased to 62-63 mm Hg during hypoxemia, returning to a post-insult level of 84-85 mm Hg. The immediate hypothermia group started with a baseline MAP of 103-104, decreased to 68-74 during hypoxemia, and returned to 91-94 mm Hg post-insult. The delayed hypothermia group had the lowest overall MAP, starting with a baseline of 84-85, decreasing to 50-54 during hypoxemia and returning to 71-76 mm Hg post-insult.

Groups also differed in PaO_2 and glucose when controlling for time (both $p < 0.05$). The differences between groups appeared modest and unlikely to be clinically significant. Glucose levels were also different between groups over time. The normothermia group had initial glucose levels of 182 mg/dl and decreased to levels of 148 mg/dl at 3 h post-insult. The immediate and delayed hypothermia groups had more stable glucose levels

Survival

Survival rate to 21 d for completion of motor and cognitive testing was 79 % for the normothermia group, 80 % for the immediate hypothermia group and 62 % for the delayed hypothermia group. Motor performance, cognitive performance, lesion volume analysis and hippocampal neuron counts are reported on all animals that survived to cognitive testing at 20 d and were able to swim for water maze testing.

Motor Performance

All three groups showed a decrease in beam balance performance and an increase in beam walking latency following trauma. However, there was no difference in beam balance duration (Figure 1) or beam walking latency (Figure 2) between normothermic and hypothermic groups.

Cognitive Performance (Morris Water Maze)

All three groups showed a marked latency in finding the submerged platform on days 14-18 following trauma. However, there was no difference between groups in performance in the water maze (Figure 3). The groups were similar in discovery of the visible platform on days 19-20 following trauma (Figure 3).

Lesion Volume Analysis

Lesion volumes (mm^3) measured at 21 d after TBI are shown in Table 2. There appeared to be a reduction in lesion volume in the hypothermia vs normothermic groups. However, this reduction in lesion volume was not statistically different from normothermia. Lesion area at various distances from the occiput is shown in Figure 4.

Hippocampal Neuron Counts

Surviving hippocampal neuron counts are shown in Table 3. Both normothermic and hypothermic animals had significant hippocampal neuronal loss on the side ipsilateral to injury. For comparison, average neuron counts in CA1 and CA3 hippocampus on the side contralateral to injury were 48-56 cells/hpf. There were no significant differences in the number of surviving CA1 or CA3 hippocampal neurons in the hypothermic versus normothermic groups.

DISCUSSION

To our knowledge, this is the first study to evaluate the effect of hypothermia on severe experimental TBI with secondary insult. Surprisingly, no difference in motor performance, cognitive performance, lesion volume or hippocampal neuronal survival was observed with the application of moderate hypothermia after severe TBI with secondary insult in a rat model. Also unexpectedly, both the immediate hypothermia group, with hypothermia initiated after trauma and before secondary hypoxemia, and the delayed hypothermia group, with hypothermia applied after both brain trauma and hypoxemia, demonstrated similar functional and histologic deficits when compared to each other and to the normothermia group.

Beneficial effects of hypothermia on histopathologic outcome following TBI have been demonstrated by several investigators. The timing of this histologic evaluation appears to be important. Dietrich et al (29) showed a reduction in cortical contusion volume and frequency of necrotic cortical neurons in rats that received 3 hours of immediate post-trauma hypothermia (30°C) following parasagittal fluid percussion injury. These animals were evaluated at 3 days post-trauma. However, in the same model, investigators found no difference in hippocampal CA1, CA3, CA4 or dentate neuronal survival in rats receiving post-trauma hypothermia compared to normothermic animals when brains were analyzed at 8 weeks following TBI (30). In models of ischemic brain injury, transient application of therapeutic hypothermia has also shown a temporary beneficial effect on hippocampal neuronal survival. Early evaluation revealed decreased hippocampal CA1 cell loss, but this protection by post-trauma hypothermia (30°C) was not seen in the animals evaluated at 2 months following ischemia insult (31). In our

model, severe TBI was followed by 30 min of hypoxemia. All animals showed a reduction in systemic blood pressure during the hypoxemic period, likely highlighting an ischemic component to the secondary insult.

The amount of tissue loss following experimental TBI varies greatly dependent on the model. This model of severe CCI followed by 30 minutes of hypoxemia produced lesion volumes of 50 to 65 mm^3. This is much larger than contusion volumes seen in other traumatic injury models, such as lateral fluid percussion (2.14 mm^3) (27), or in similar CCI models without hypoxemia (~30 mm^3), (5). The severe insult produced in this model might explain the failure of post-trauma hypothermia to show a significant reduction in lesion volume. However, other experimental TBI models applying hypothermia after injury have also failed to reduce necrotic volumes (30, 32). Cherian et al showed increasing sizes of contusion volume as the degree of secondary insult (bilateral carotid occlusion) increased following CCI in rats (33). In addition, it is likely that the lesion volume observed at 21 days post-injury is the result of damage by many different mechanisms operating in the early and late post-trauma phases. Clark et al (4) has demonstrated cells with either necrotic or apoptotic phenotypes in various brain regions following TBI in a similar model with hypoxemia. It is unclear if earlier assessment would have revealed more hippocampal protection with post-traumatic hypothermia in this model. However, our goal is to favorably influence long-term outcome. Hypothermia as a single treatment modality, and applied for only 4 hours following TBI, might be unable to reduce overall lesion volumes in such a model.

Previous experimental studies applying moderate hypothermia after TBI have demonstrated protection against motor and spatial memory deficits after both CCI (16)

and fluid percussion injury (30, 34). However these models did not include a period of hypoxemia or any other secondary insults after trauma. This secondary hypoxemic insult worsens histologic outcome and could, therefore, worsen behavioral outcome following TBI. In a similar model of CCI with secondary hypoxemia, rats demonstrated progressively worse motor function (beam-balance latency) with increasing amounts of post-trauma hypoxemia (4). This trend was seen even in rats who received mild hypoxemia (PaO_2 = 58-63 mm Hg) following CCI. In a fluid percussion injury model, Ishige et al (1) showed significantly worse neurological status scores in rats that underwent impact injury followed by a 30 minute period of hypoxemia (PaO_2 = 35-40 mm Hg) versus those injured without secondary hypoxemia. These neurologic deficits were also not observed in rats that received hypoxemia alone.

Clinical studies of patients with head injury have also reported marked worsening of outcome parameters in the setting of TBI with secondary insult such as hypoxemia or hypotension. In an analysis of 717 patients from the Traumatic Coma Data Bank, Chestnut et al (7) found that hypoxia and hypotension were independently associated with increases in morbidity and mortality from severe head injury. This study showed a marked shift towards vegetative/dead outcomes in patients who endured hypoxia ($PaO_2 \leq$ 60 mm Hg) or hypotension (systolic BP $\leq$ 90) during the pre-hospital or resuscitation period. This is especially relevant to our model of TBI because rats underwent a planned 30 min period of hypoxemia, which was also associated with a decrease in systemic blood pressure. The difference in MAP between groups may have caused additional experimental differences. The delayed hypothermia group had an overall lower MAP trend, and may have experienced more significant secondary ischemic flows.

Clinical studies applying hypothermia after TBI have yielded a variety of positive results. In a phase II study of moderate hypothermia, Clifton et al found a reduction in incidence of post-traumatic seizures (35). Shiozaki et al (22) documented improved control of intracranial pressure (ICP) with the application of mild (34°C) hypothermia after conventional therapies had failed to control ICP (22). Most recently, Marion et al (20) demonstrated faster recovery of functional outcome with the application of moderate hypothermia (32°C) after severe TBI in 82 patients randomized to either normothermia or hypothermia for 24 hours after injury. Relevant to our findings, these clinical trials showed important distinctions in the subset of very severely injured patients. Marion's study included all patients with initial GCS $\leq$ 8, but the beneficial effect of hypothermia only extended to 12 months in the subset of patients with initial GCS = 5-7 (20). In Shiozaki's study, the subset of patients admitted with GCS scores of 3-4 had a much lower incidence of favorable outcome at 6 months after injury (only 1 patient out of 22, 4.5%) versus the group with GCS scores of 5-7 (11 patients out of 40, 27.5%), despite the application of mild hypothermia (22). Importantly, Marion et al excluded patients with hypoxia or hypotension.

Our model of CCI with hypoxemia results in a very severe injury relative to other models. In addition, during the 30 min period of hypoxemia, the rats experience a significant drop in their mean arterial pressure. In a recent experimental TBI model (36), post-traumatic application of moderate hypothermia (30°C for 3h) resulted, after rewarming, in a lower cerebral perfusion without a corresponding decrease in cerebral glucose utilization, creating a state of metabolism to blood flow mismatch. This may be especially important in the clinical setting in which severely head-injured patients undergo

a secondary insult of hypoxia and/or hypotension prior to initiation of treatment for their head injury. Analysis of trauma patients has consistently revealed worse outcomes in patients who experienced sustained hypotension in the pre-hospital setting versus those who remained normotensive. Specifically, Chestnut et al (7) showed hypotension was associated with a 150% increase in mortality rate. Wald et al (37) also found that prehospital hypotension doubled the incidence of adverse outcome (37). In a review of pediatric trauma patients, Pigula et al (38) found that hypotension significantly increased the mortality rate. As a result, patients with significant secondary insult have been excluded from evaluation in clinical trials (20). This subset clearly represents a population in which currently available interventions may have limited efficacy --even those proven to be effective in the sets of TBI alone.

There are many mechanisms of cell injury and death after TBI. Investigators have shown additional pathophysiologic mechanisms operating when secondary insults were added to the already vulnerable, traumatically injured brain (1, 3, 33, 39). Recent experimental investigations have focused on the period following trauma, during which secondary insults may potentiate neuronal damage, utilizing a wide variety of therapies. However, many of these therapies have been tested in models of TBI where oxygenation and ventilation of animals following TBI are controlled. Given that many head-injured patients present with preceding hypoxemia, it may be important to reassess these therapies in models imitating this clinical setting. It is possible that proven treatments may be less effective in a model with applied hypoxemia and accompanying hypotension. Novel therapies targeting this complex clinical scenario have yet to be developed.

In conclusion, in this rat model of severe CCI with hypoxemia, moderate hypothermia for 4 hours post-trauma failed to improve hippocampal neuron survival, lesion volume, motor function or cognitive function. Combination therapies or development of novel therapies may be necessary to see significant improvement in outcome in this difficult setting.

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FIGURE LEGENDS

Figure 1. Beam balance latency in normothermia (closed circles), immediate hypothermia (open circles), and delayed hypothermia (triangles) on days 1-5 after CCI. Values are mean ± SEM.

Figure 2. Beam walking latency in normothermia (closed circles), immediate hypothermia (open circles), and delayed hypothermia (triangles) on days 1-5 after CCI. Values are mean ± SEM.

Figure 3. Morris Water Maze performance in normothermia (closed circles), immediate hypothermia (open circles), and delayed hypothermia (triangles) on days 14-18 after CCI. Visible platform latency in all groups on days 19-20 after CCI. Values are mean ± SEM.

Figure 4. Lesion area at various distances from the occiput (mm) in normothermia (closed circles), immediate hypothermia (open circles), and delayed hypothermia (triangles) groups. Values are mean ± SEM.

Table 1. Physiologic data

Group	Brain T (°C)	Rectal T (°C)	MAP (mm Hg)	pH	$PaCO_2$ (mm Hg)	PaO_2 (mm Hg)	Glucose (mg/dl)	HCT (%)
37°C (n = 19)								
Baseline	37.1 ± 0.1[a]	37.3 ± 0.1	91 ± 3	7.44 ± 0.01	39 ± 1	169 ± 4	182 ± 10	40 ± 0
Insult	37.0 ± 0.1	37.3 ± 0.0	92 ± 3	-	-	-	-	-
10 min	37.1 ± 0.1	37.1 ± 0.1	63 ± 4	7.43 ± 0.01	40 ± 1	46 ± 1	-	-
25 min	37.0 ± 0.0	37.1 ± 0.1	62 ± 3	7.42 ± 0.01	40 ± 1	47 ± 1	173 ± 13	39 ± 0
1 h	37.1 ± 0.0	37.2 ± 0.1	84 ± 2	7.43 ± 0.01	38 ± 1	160 ± 3	145 ± 4	39 ± 0
3 h	37.1 ± 0.1	37.2 ± 0.0	85 ± 3	7.42 ± 0.01	37 ± 1	172 ± 3	148 ± 4	38 ± 0
32°C - Immediate (n = 10)								
Baseline	37.2 ± 0.1	37.3 ± 0.1	103 ± 2	7.45 ± 0.01	40 ± 1	157 ± 3	171 ± 8	41 ± 1
Insult	37.2 ± 0.0	37.3 ± 0.1	104 ± 3	-	-	-	-	-
10 min	32.5 ± 0.2	32.5 ± 0.2	74 ± 5	7.44 ± 0.01	37 ± 1	51 ± 1	-	-
25 min	32.0 ± 0.1	32.1 ± 0.1	68 ± 3	7.45 ± 0.01	38 ± 1	50 ± 1	208 ± 22	39 ± 0
1 h	31.9 ± 0.1	32.0 ± 0.1	91 ± 4	7.45 ± 0.01	38 ± 1	201 ± 5	166 ± 11	39 ± 0
3 h	32.1 ± 0.1	32.0 ± 0.1	94 ± 3	7.42 ± 0.01	38 ± 0	193 ± 6	182 ± 15	39 ± 0
32° - Delayed (n = 10)								
Baseline	37.1 ± 0.1	37.3 ± 0.1	85 ± 2	7.43 ± 0.01	39 ± 1	176 ± 7	196 ± 14	41 ± 1
Insult	37.1 ± 0.1	37.3 ± 0.1	84 ± 2	-	-	-	-	-
10 min	36.9 ± 0.1	37.1 ± 0.1	54 ± 3	7.42 ± 0.01	40 ± 1	47 ± 2	-	-
25 min	36.9 ± 0.1	37.1 ± 0.1	50 ± 2	7.41 ± 0.01	39 ± 1	47 ± 1	173 ± 10	39 ± 0
1 h	31.9 ± 0.1	31.9 ± 0.1	76 ± 2	7.43 ± 0.00	38 ± 1	205 ± 3	188 ± 11	39 ± 0
3 h	31.9 ± 0.1	32.0 ± 0.1	71 ± 5	7.41 ± 0.01	36 ± 0	215 ± 3	182 ± 9	37 ± 1

T = Temperature, MAP = Mean arterial pressure, HCT = Hematocrit

[a] Values are Mean ± SEM

Table 2: Lesion Volume

Group	Lesion Volume[a] (mm^3)		
Normothermia	65.3	±	6.9[b]
Immediate HT	50.2	±	8.2
Delayed HT	53.7	±	7.9

HT, hypothermia
[a] $p = 0.32$ by ANOVA
[b] Values are Mean ± SEM

Table 3. Hippocampal neuronal survival

Group	CA1 Neurons[a] (cells/hpf)	CA3 Neurons[a] (cells/hpf)
Normothermia	19.4 ± 4.2[b]	19.8 ± 4.6
Immediate HT	13.2 ± 8.7	15.6 ± 7.3
Delayed HT	13.7 ± 5.8	18.5 ± 7.3

HT = hypothermia, hpf = high-powered field
[a] ipsilateral to injury
[b] values are Mean ± SEM

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January 7, 2000

Dr. John A. Jane, Sr.
Editor
Journal of Neurosurgery
1224 West Main Street, Suite 450
Charlotte, VA 22903

Dear Dr. Jane,

Enclosed please find our manuscript entitled "Isoflurane improves long-term neurologic outcome vs fentanyl after traumatic brain injury in rats" which we are respectfully submitting for publication in the Journal of Neurosurgery. This manuscript has not been submitted to any other journal. Please note that Dr. Kimberly Statler received the 1999 Women in Neurotrauma Research award for this work at the 1999 meeting of the National Neurotrauma Society.

We thank you in advance for consideration of our work.

Sincerely,

Patrick M. Kochanek, M.D.

cc: Kimberly Statler, M.D.

ISOFLURANE IMPROVES LONG-TERM NEUROLOGIC OUTCOME VS FENTANYL AFTER TRAUMATIC BRAIN INJURY IN RATS

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Running title: Isoflurane vs fentanyl after TBI in rats

Support: US Army DAMD Grant 17-97-1-7009 (P.M. Kochanek)

Abstract

Despite routine use of fentanyl in patients after traumatic brain injury (TBI), it is unclear if it is the optimal sedative/analgesic agent. Isoflurane anesthesia is commonly used in experimental TBI. We hypothesized that isoflurane would be neuroprotective vs fentanyl after TBI in rats. Rats underwent controlled cortical impact (CCI) and received 4 h of N_2O:O_2 (2:1) and either fentanyl (10 μg/kg iv bolus, 50 μg/kg/h infusion) or isoflurane (1% by inhalation) with controlled ventilation. Shams underwent identical preparation, but no CCI. Functional outcome (beam balance, beam walking, and Morris water maze [MWM] tasks) was assessed over 20 days. Lesion volume and hippocampal neuron survival were quantified on d 21. Additional rats underwent identical CCI and anesthesia with intracranial pressure (ICP) monitoring, and brain water content was assessed.

Motor and MWM performances were better in injured rats treated with isoflurane vs fentanyl ($p < 0.05$), but did not differ between shams. Damage to CA1 hippocampus was attenuated in isoflurane-treated rats ($p < 0.05$). Fentanyl-treated rats had higher mean arterial blood pressure (MAP) and cerebral perfusion pressure (CPP) after injury ($p < 0.05$); however, ICP and brain water were similar between treatment groups.

Isoflurane improved functional outcome and attenuated damage to CA1 hippocampus vs fentanyl in rats subjected to CCI. Isoflurane may be neuroprotective vs fentanyl by augmenting cerebral blood flow and/or reducing excitotoxicity, not by reducing ICP or brain water content. Alternatively, fentanyl may be detrimental. Isoflurane may mask beneficial effects of novel

agents tested in experimental TBI models. Additionally, fentanyl may not be the optimal sedative/analgesic agent early after TBI in humans.

Key words: sedation, analgesia, anesthesia, head injury, narcotics, opioids

Introduction

In current clinical practice, opioids are routinely administered after traumatic brain injury (TBI). Fentanyl is one of the first-line agents because of its short half-life and low incidence of hypotension. Despite standard clinical use, it remains unclear if fentanyl represents the optimal sedative/analgesic agent in the acute period following TBI. Unlike the clinical arena, opioids are rarely used in experimental TBI. In fact, most models of TBI use isoflurane or pentobarbital anesthesia.

Much of the study of opioids in TBI has focused on the actions of endogenous opiates, such as dynorphin, and specific opiate receptor effects.[17,29,30,34,51] Although mu receptor agonists, such as morphine and fentanyl, have been shown to have some beneficial effects after central nervous system injury,[29,30] recent studies in cerebral ischemia and focal cryogenic lesion suggest that isoflurane may be neuroprotective compared to fentanyl.[37-39,48] In rats subjected to global cerebral ischemia, isoflurane reduced neuronal damage and improved motor function compared to fentanyl.[37] Similarly, after focal ischemia, rats anesthetized with isoflurane had smaller infarct volumes than those receiving fentanyl. Lesion volumes in rats treated with fentanyl were similar to those in unanesthetized control rats.[48] Isoflurane has been reported to enhance post-insult cerebral blood flow (CBF), produce widespread increases in brain surface PO_2 and reduce edema in a rabbit model of focal cryogenic lesion. Conversely, both CBF and regional PO_2 were decreased, and edema was increased, in rabbits anesthetized with fentanyl.[38,39] Using a fluid-percussion model in cats, DeWitt et al, addressed the question of whether fentanyl was

detrimental in TBI and found that fentanyl produced no adverse effects compared to vehicle. However, in that study, fentanyl was administered to cats already anesthetized with isoflurane.[3]

To our knowledge, isoflurane has not been directly compared to fentanyl in a contemporary model of TBI with long-term functional outcome and histologic assessment. We hypothesized that isoflurane would be neuroprotective compared to fentanyl when administered early after TBI. To test our hypothesis, we directly compared fentanyl and isoflurane anesthesia in a controlled cortical impact (CCI) model of TBI.

Materials and Methods

Virus-free, mature male (280 - 400g) Sprague-Dawley rats were used in this study. The rats had free access to food and water before and after surgery. All studies were approved by the University of Pittsburgh Animal Care and Use Committee. All surgical procedures were performed using aseptic technique.

Outcome Protocol

Rats were initially anesthetized with N_2O:O_2 (2:1) and 4% isoflurane (IsoFlo, Abbott Laboratories, North Chicago, IL) via a nose cone and then endotracheally intubated with a 14-guage angiocatheter and mechanically ventilated. Anesthesia was maintained for the duration of surgical preparation with 2 - 2.5% isoflurane and N_2O:O_2 (2:1). Pancuronium bromide (0.1 mg/kg/h, Elkins-Sinn, Cherry Hill, NJ) was given intravenously for muscle relaxation. Femoral venous and arterial vessels were cannulated for continuous blood pressure measurement, blood

sampling, and administration of medications. A rectal probe was inserted to monitor core temperature. The rat was then placed in a stereotaxic frame (David Kopf, Tujunga, CA) and a left parietal craniotomy (7mm x 8mm) was performed using a high-speed dental drill. The dura and bone flap were left in place until immediately before CCI. A burr hole was drilled into the left frontal bone for temperature probe (2.28-mm outside diameter, Physiotemp Corp., Clifton, NJ) placement into the frontal lobe. Continuously monitored physiologic parameters included arterial blood pressure and rectal and brain temperatures. Parameters monitored intermittently included blood glucose, hematocrit, and arterial blood gas samples, which were assessed every 15 minutes for the initial hour and every 30 minutes thereafter. Throughout the experiment, $PaCO_2$ was controlled at 35 - 45 mm Hg. This protocol produced a PaO_2 of greater than 70 mm Hg in all preparations. Both brain and rectal temperatures were maintained at 37.0 ± 0.5 °C.

Rats were allowed to stabilize for 5 min after completion of surgical preparation and then randomized to receive either fentanyl or isoflurane anesthesia. In the fentanyl group (n = 9), isoflurane was discontinued and 10 μg/kg of fentanyl (50 μg/ml, Elkins-Sinn, Cherry Hill, NJ) was administered intravenously, followed by a continuous intravenous infusion at 50 μg/kg/h. In the isoflurane group (n = 9), inspired isoflurane concentration was reduced to 1%, and normal saline, the vehicle for fentanyl-treated rats, was administered to match the volume received by fentanyl infusion. Both anesthetic groups continue to receive N_2O:O_2 (2:1). After 30 min of equilibration, TBI was induced by CCI using a pneumatic-driven piston device that has been shown (with isoflurane anesthesia) to deliver a reliable and reproducible degree of injury with a mortality rate of less than 5%.[12,26] In pilot studies comparing isoflurane and fentanyl using our

standard CCI model (6-mm tip, 4 m/s velocity, 50 msec duration of deformation and 2.5-mm deformation depth), all of the fentanyl-treated rats developed pulmonary edema and died early after injury. Thus, to compare the effect of isoflurane vs fentanyl on long-term outcome in our model, our standard injury was reduced (6 mm tip, 4 m/s velocity, 50 msec duration of deformation and 2.0-mm deformation depth). After CCI, the bone flap was replaced and sealed with dental cement, and the scalp incision was closed. Anesthesia was continued for 4 h in the isoflurane group and 3.5 h in the fentanyl group to facilitate similar extubation times.[37] At the end of the anesthetic period, rats received 100% oxygen, were allowed to awaken and resume spontaneous breathing, and were then extubated and returned to their cages. Sham rats underwent identical preparation and anesthesia, but no CCI ($n = 6$ per anesthetic group).

Motor function, including beam balance and beam walking tasks, was tested by an observer blinded to group assignment on days 1 - 5 after injury.[12,13] Briefly, in the beam balance task, the rat was placed on a suspended, narrow wooden beam (1.5-cm wide) and the time that the rat remained on the beam was recorded (up to 60 sec). For beam walking, the rat was placed at one end of the beam and a dark, quiet chamber was located at the other end. An adverse stimulus of loud white noise was applied and the time for the rat to escape across the beam into the chamber was recorded (up to 60 sec). Rats were trained with three trials before CCI or sham injury, which also served as baseline values.

Morris Water Maze (MWM) testing was performed using an acquisition paradigm on days 14 - 20 after injury.[15] Briefly, on post-injury days 14 - 18, the rat was placed into a pool (2-m diameter) and required to locate a hidden platform in order to escape the water. On post-injury days 19 and 20, the platform was raised so that the surface was visible 5-cm above the water

level. Latency to find the platform was used to compare performances. Swim speed was measured on post-injury day 20 to insure that rats in all experimental groups exhibited equal motivation and motor function.

Lesion volume and hippocampal neuron survival were assessed on day 21 after injury.[9,26] Rats were re-anesthetized and then perfused with heparinized saline followed by 4% paraformaldehyde. Brains were removed, post-fixed and cryoprotected. Serial coronal sections (10-μm) were made at 1-mm intervals through the entire brain. Sections were mounted on slides and stained with cresyl violet. The areas of both tissue loss and the entire uninjured hemisphere were determined by an observer blinded to experimental group using an image analysis system (MCID, Imaging Research, St. Catherines, Ontario, Canada). Lesion volume was reported in cubic mm, as a percentage of uninjured hemisphere, and as area (mm^2) vs distance from the occiput (mm). Surviving hippocampal neurons were counted under 400X magnification in the entire anatomic CA1 and CA3 hippocampal regions in a coronal section taken 5-mm from the occiput by an observer blinded to experimental group (KS). Neuronal counts were reported as the mean number of surviving neurons per 400X field.

ICP Protocol

Based both on results of the outcome protocol showing that fentanyl-treated rats had higher MAP throughout the experiment and on a recent report that increased MAP may exacerbate injury after TBI,[27] ICP and brain water were monitored in a separate cohort of rats (n

= 9 per anesthetic group) subjected to either fentanyl or isoflurane anesthesia and CCI in an identical paradigm to that used to assess functional outcome.

Surgical preparation, randomization, anesthetic administration, and CCI were identical to the outcome protocol, with minor exceptions. Specifically, an intraparenchymal ICP monitor (Codman microsensor transducer, outer diameter 1.0-mm, Johnson and Johnson, Raynham, MA) was inserted through a burr hole in the frontal bone into the contralateral (right) frontal cortex at the time of craniotomy. After CCI, anesthesia was continued and ICP was monitored for 4 h in both anesthetic groups. Cerebral perfusion pressure (CPP) was calculated as the difference between MAP and ICP. Rats were killed by decapitation at the end of the anesthetic period. Brains were immediately removed and a 3-mm coronal slice was made through the center of the contusion using a rat brain slicer. Per cent brain water was determined in the coronal slice using the wet-dry weight method.[26] The section was weighed immediately, dried in an oven at 110° C for 48 hours and then reweighed. Brain water content was determined in both the injured and the homologous region of the uninjured hemispheres.

As an additional control, a separate cohort of rats (n = 3) was subjected to CCI and allowed to recover without anesthesia. These rats were prepared for CCI under isoflurane anesthesia as described, allowed to recover a tail-pinch response and then subjected to CCI. Arterial MAP was monitored via an indwelling femoral arterial catheter for 4 h during recovery without anesthesia.

Statistical Analysis

Physiological parameters ($PaCO_2$, PaO_2, glucose, hematocrit, MAP, ICP, CPP) and beam balance, beam walking, and MWM latencies were assessed by two-way analysis of variance for repeated measures. Swim speed, brain water content, and hippocampal neuronal survival were compared by one-way analysis of variance. Appropriate post-hoc tests corrected for multiple comparisons were applied. Time to extubation and lesion volume were compared between treatment groups using unpaired student's t-test. A p value < 0.05 was considered statistically significant. Values are expressed as mean ± SEM.

Results

Outcome Protocol

Average time to extubation did not differ after injury between isoflurane and fentanyl treatment groups (269 ± 7 min vs 275 ± 15 min, $p = 0.29$) Physiologic values, including $PaCO_2$, PaO_2, blood glucose and hematocrit did not differ between anesthetic groups. In contrast, MAP was higher in injured rats treated with fentanyl compared to their isoflurane counterparts ($p < 0.05$) during the entire posttrauma period (Figure 1). Similarly, MAP was higher in shams treated with fentanyl vs isoflurane ($p < 0.05$) during the entire duration of anesthesia (Figure 1). Fentanyl-treated rats had a MAP of approximately 150 mm Hg compared to approximately 105 mm Hg in the isoflurane groups.

Rats anesthetized with isoflurane performed better on beam balance and beam walking tasks after TBI compared to their fentanyl counterparts ($p < 0.05$, Figure 2). Following injury,

isoflurane-anesthetized rats also performed better than their fentanyl-treated counterparts during MWM testing with a hidden platform ($p < 0.05$, Figure 3). Motor and MWM performances did not differ between sham groups. All experimental groups showed improved MWM performance during visible (vs hidden) platform testing ($p < 0.05$ for all groups) and had similar swim speeds (Figure 3), indicating that all rats had similar motivation and motor ability during MWM testing. However, performance on the visible platform paradigm of the MWM was better in isoflurane vs fentanyl treated rats after TBI ($p < 0.05$, Figure 3). This suggests that the difference in MWM performance may not be solely attributable to cognitive deficits.

Lesion volume, expressed as mm^3 or as percent of uninjured hemisphere, at 21 days after TBI did not differ significantly between isoflurane and fentanyl treatment groups (Figure 4A,B). In contrast, neuron counts in the injured CA1 hippocampus were markedly greater in isoflurane-treated rats ($p < 0.05$, Figure 4C). Neuron counts in the injured CA3 hippocampus, however, did not differ significantly between treatment groups (Figure 4D). In the uninjured hemisphere, neuron counts in both CA1 and CA3 hippocampus did not differ between isoflurane- and fentanyl-treated rats, and were similar to shams (data not shown).

ICP Protocol

Physiologic values, including $PaCO_2$, PaO_2, glucose and hematocrit, did not differ between anesthetic groups. As in the outcome protocol, MAP was higher in rats treated with fentanyl compared to their isoflurane counterparts ($p < 0.05$, Figure 5A). ICP was similar in both anesthetic groups; however, there was a trend toward higher ICP in rats anesthetized with isoflurane by 3 - 4 h after TBI (Figure 5B). This strongly suggests that the higher MAP in

fentanyl vs isoflurane treated rats did not exacerbate intracranial hypertension. As expected from the difference in MAP, CPP was significantly higher in the fentanyl treatment group (Figure 5C). Brain water content, assessed at 4 h after injury, was higher in the injured vs uninjured hemisphere ($p < 0.05$) for both anesthetic groups (Figure 6). However, brain water in either the injured or uninjured hemisphere did not differ between isoflurane- and fentanyl-treated rats, indicating that edema was not exacerbated in the fentanyl group.

Average MAP during the 4 h post-injury observation period did not differ significantly between fentanyl-treated rats and those recovering from CCI without anesthesia (157 ± 6.2 mm Hg vs 147 ± 7.1 mm Hg, NS). In contrast, isoflurane-anesthetized rats had lower MAP (105 ± 5.5 mm Hg) compared to both fentanyl-treated rats and rats recovering without anesthesia ($p <$ 0.05 vs both groups, one-way ANOVA).

Discussion

Isoflurane anesthesia administered to rats subjected to CCI improved performance on both motor and MWM tasks and attenuated damage to CA1 hippocampus after TBI. Although fentanyl-treated rats had higher MAP and CPP than their isoflurane counterparts, this was not accompanied by increased ICP or brain water during the first 4 h after TBI. This suggests that the improved functional outcome in rats anesthetized with isoflurane may be a direct result of either beneficial actions of isoflurane, and/or detrimental effects of fentanyl.

The pathophysiology of TBI includes a primary injury caused by the mechanical disruption of tissue and various secondary injuries mediated, at least in part, by post-insult

hypoperfusion, ischemia, and excitotoxicity.[7,18,22,26,35] Isoflurane anesthesia may be neuroprotective vs fentanyl by decreasing excitotoxicity and/or augmenting cerebral blood flow.

Following TBI, interstitial levels of excitatory amino acids (EAAs), such as glutamate, are increased due to direct tissue injury and secondary ischemia. EAAs stimulate NMDA, AMPA/kainate, and metabotropic receptors, leading to neuronal membrane depolarization, cellular swelling, calcium influx, and ultimately, neuronal death.[35] Although models of cerebral ischemia have shown conflicting effects of isoflurane on glutamate levels in blood and brain interstitial fluid,[44,49] isoflurane has been shown to inhibit glutamate receptors, reduce NMDA-mediated calcium influx and delay neuronal injury induced by cerebral ischemia.[4,45]

To attribute the potential neuromodulatory actions of isoflurane on neuronal injury to only glutamate toxicity[4] and glutamate signaling transduction,[11] however is an oversimplification since isoflurane has many neural actions that could contribute to neuroprotection. Some include the inhibition of some voltage sensitive potassium channels,[23] the activation of specific receptor-coupled and voltage sensitive potassium channels,[31,52,54] the uncoupling of muscarinic receptors,[1,36,42] the enhancement of GABA A channels,[28] and the reduction of intracellular calcium stores and inhibition of IP3 sensitive intracellular calcium release.[21] Potential detrimental actions such as the enhancement of NMDA linked nNOS activation have also been documented;[46] however, the potential beneficial actions of isoflurane on excitotoxic cascades far outweigh the potential detrimental actions.

Additionally, isoflurane is a potent cerebral vasodilator. Studies of CBF after experimental TBI have shown significant reductions in both local and global CBF early (0.5 – 4 h) after injury.[7,18] Effects are greatest near the impact site, but global reductions in CBF are seen

as well.[18] In clinical studies, early post-traumatic hypoperfusion has been strongly correlated with poor outcome.[5,32] Although the effects of fentanyl on CBF have been subjected to limited study, Safo et al.[47] have reported that CBF was markedly reduced in rats treated with fentanyl (100 μg/kg iv) vs control rats anesthetized with N_2O. In addition, using perfusion MRI in normal rats anesthetized with doses identical to those used in this study, we have shown that CBF is 2 - 3 times higher in rats treated with isoflurane vs fentanyl (unpublished data). By promoting CBF, isoflurane may help attenuate post-traumatic hypoperfusion, reducing secondary injury and improving recovery. The selective neuroprotection of CA1, but not CA3, hippocampus in isoflurane- vs fentanyl-treated rats is consistent with this concept. Additionally, another CBF promoting strategy, L-arginine, has recently been shown to improve outcome after TBI.[8,10] Augmentation of CBF with an associated increase in cerebral blood volume therefore offers a potential explanation for both improved functional and histological outcome and the tendency toward higher ICP and brain water seen in isoflurane- vs fentanyl-treated rats in this study. Indeed, the combination of CBF promotion and reduced excitotoxicity may be particularly beneficial.

Alternatively, fentanyl may be detrimental after TBI. Opioids generally suppress neuronal excitability, however, mu receptor agonists, such as fentanyl, may contribute to hippocampal neuron excitation.[6,40,41] In fact, high-dose fentanyl (25 - 100 μg/kg in humans and 400 μg/kg in rats) has been associated with subcortical seizures.[14,24,33,43,47,50] Although fentanyl exhibits low affinity for kappa receptors,[43] kappa receptor antagonists have been shown to

improve both neurological outcome after spinal cord injury and CBF after fluid-percussion injury in cats.[14,33]

Other factors may contribute to improved outcome in isoflurane- vs fentanyl-treated rats. These include the disparity in both MAP and CPP and possible differences in the depth of anesthesia between treatment groups. Although increased MAP can have detrimental effects after TBI, in our study, ICP and brain water were not significantly different in isoflurane vs fentanyl treatment groups at the end of the 4 h treatment period. This suggests that the higher MAP associated with fentanyl vs isoflurane anesthesia had no acute detrimental effects on intracranial hypertension or brain edema. Additionally, MAP did not differ significantly between rats treated with fentanyl and those allowed to recover without anesthesia. The values for MAP observed in both isoflurane and fentanyl groups were within the reported range of cerebral autoregulation (50 - 170 mm Hg) for normotensive rats.[19,20,53] The disparity in MAP is therefore unlikely to have contributed importantly to the observed difference in functional outcome. Although a recent study using the CCI model in rats suggests that increased MAP and CPP may exacerbate injury after TBI, in that study, systemic hypertension was induced by large doses of dopamine,[27] that may have produced detrimental effects after injury which were independent of blood pressure.[2] Additionally, our study does not address the important detrimental effect of hypotension following clinical TBI, particularly with multiple trauma. The blood pressure supporting effects of fentanyl (vs other sedative agents) may be beneficial in this section.

Although comparison of anesthetic depth between inhalation and intravenous agents is difficult, differences in anesthetic depth are unlikely to explain the observed difference in outcome seen between isoflurane- and fentanyl-treated rats. The dose of isoflurane used (1% by

inhalation), in combination with $N_2O:O_2$ (2:1) represents approximately 1.2 minimal alveolar concentration.[16] The dose of fentanyl falls in the range between the ED50 for purposeful movement and complete blockade of this response[25] and is similar to standard doses used in rat models of central nervous system injury.[3,37-39,48] Additionally, fentanyl-treated rats did not exhibit signs of increased stress vs isoflurane-treated rats, such as higher blood glucose, suggesting that anesthesia was adequate. Finally, rats in both treatment groups emerged from anesthesia similarly in our paradigm. After discontinuation of fentanyl, the rats were fully alert and exhibited similar activity to isoflurane-treated rats following extubation.

The theoretical advantages of isoflurane vs fentanyl are compelling; however, explanations for the observed improvement in neurologic outcome after TBI in isoflurane-anesthetized rats remain to be determined. What has become clearer is that anesthetic agents may have considerable impact upon outcome following TBI.

The results of this study suggest two important potential ramifications. First, isoflurane may not represent the optimal anesthetic in experimental TBI since it may mask potential benefits of novel therapies. Second, despite common use, fentanyl may not be the optimal sedative/analgesic agent to administer to patients in the acute phase after severe head injury. Although we do not suggest that isoflurane represents a clinically applicable therapy for the initial stabilization and treatment of patients after TBI, further study of the mechanistic differences between isoflurane and fentanyl anesthesia is warranted. Defining the factors responsible for improved outcome with isoflurane may help to direct the clinical application of more optimal sedative/analgesic agents and possibly to identify novel therapies. Finally, more

comprehensive comparisons of clinically relevant sedative/analgesic agents are needed in experimental TBI.

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Figure 1: MAP vs time after injury, outcome protocol. MAP in both fentanyl-treated injured (open circles) and sham (open triangles) rats was approximately 50 mm Hg higher than in isoflurane-treated rats at all time points (injured shown by closed circles and shams by closed triangles). * $p < 0.05$, isoflurane vs fentanyl at each time point after injury, § $p < 0.05$, isoflurane vs fentanyl at all time points, including baseline, in shams.

Figure 2: **A.** Beam balance latency vs time in days after injury. Sham rats (triangles) had similar latencies throughout the 5-day testing period. After injury, beam balance latency was shorter for both isoflurane (closed circles) and fentanyl (open circles) treatment groups vs sham; however, isoflurane-anesthetized rats recovered more quickly (vs fentanyl). * $p < 0.05$, injured vs sham. **B.** Beam walking latency vs time in days after injury. Again, performance was impaired after injury in both anesthetic groups vs shams. Although isoflurane-treated rats recovered by post-injury day 3, fentanyl-treated rats failed to regain normal function by the end of the 5-day testing period. * $p < 0.05$, injured vs sham.

Figure 3: **A.** Latency to find a platform vs time after injury in an acquisition paradigm of the MWM. Sham rats anesthetized with isoflurane (closed triangles) or fentanyl (open triangles) had similar performances throughout the testing period. During the first few days of testing with a hidden platform, injured rats in both fentanyl (open circles) and isoflurane (closed circles) treatment groups had impaired performance vs sham. By the third day of testing, latencies to find the hidden platform were similar in injured isoflurane-anesthetized rats and shams. In contrast, longer latencies persisted in injured fentanyl-treated rats throughout the 5-day hidden

platform testing period. Latencies in all experimental groups improved during visible (vs hidden) platform testing; however, shams and injured isoflurane-anesthetized rats performed better than injured fentanyl-treated rats on both days of visible platform testing. * $p < 0.05$, injured vs sham; § $p < 0.05$, isoflurane vs fentanyl. **B.** Swim speed, tested on day 20 after injury, did not differ between experimental groups.

Figure 4: **A.** Lesion volumes, measured on post-injury day 21, did not differ significantly between isoflurane (solid) and fentanyl (open) treatment groups. **B.** Lesion volume, expressed as area (mm2) vs distance (mm) from occiput, did not differ significantly between treatment groups. **C.** Neuron counts in injured CA1 hippocampus were greater in isoflurane- vs fentanyl-treated rats. Neuron counts in uninjured CA1 hippocampus were similar in both treatment groups. * $p < 0.05$, injured vs uninjured. **D.** CA3 hippocampus neuron counts in either injured or uninjured hemisphere did not differ significantly between isoflurane- and fentanyl-treated rats.

Figure 5: **A.** MAP vs time after injury, ICP protocol. MAP was approximately 40 mm Hg higher in rats treated with isoflurane (closed squares) vs fentanyl (open squares) throughout the observation period. * $p < 0.05$, fentanyl vs isoflurane vs fentanyl. **B.** ICP vs time after injury. Initial ICP was approximately 4 mm Hg in both isoflurane (open squares) and fentanyl (closed squares) treatment groups. ICP progressively increased, reaching 10 - 18 mm Hg by 4 h after injury. Although ICP was similar between anesthetic groups, isoflurane-anesthetized rats exhibited a trend toward higher ICP after injury (vs fentanyl treated rats) that did not reach significance. **C.** CPP vs time after injury. CPP was increased in rats treated with fentanyl (open

squares) vs isoflurane (closed squares), * $p < 0.05$, isoflurane vs fentanyl at all time points except 3.5 and 4h.

Figure 6: Brain Water 4 h after TBI. Brain water in the injured hemisphere was increased compared to the respective non-injured hemisphere in both isoflurane- and fentanyl-anesthetized rats; however, brain water did not differ between anesthetic groups. * $p < 0.05$, isoflurane vs fentanyl.

Figure 1

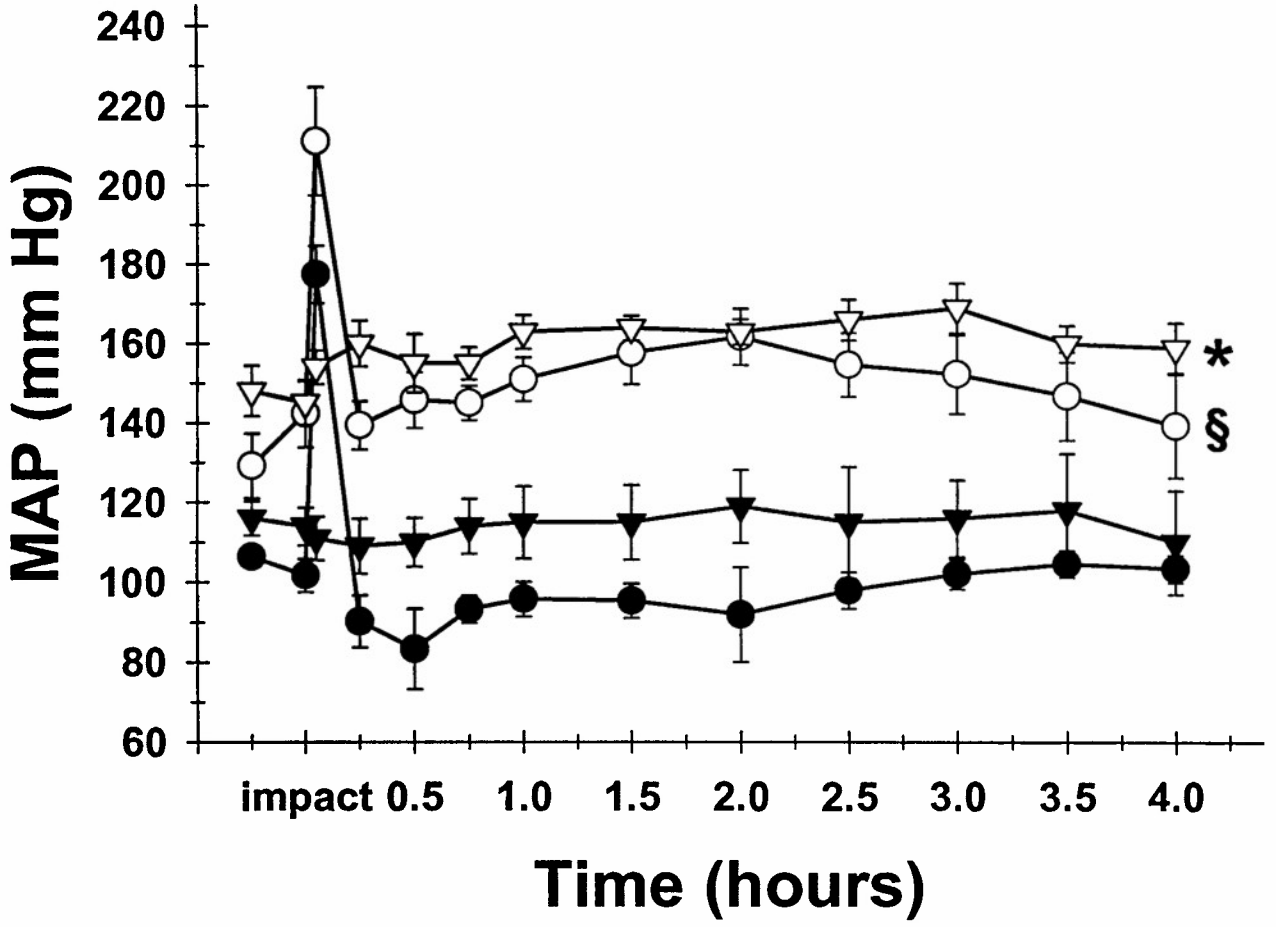

MAP (mm Hg)
240
220
200
180
160
140
120
100
80
60
impact
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Time (hours)
*
§

Figure 2A

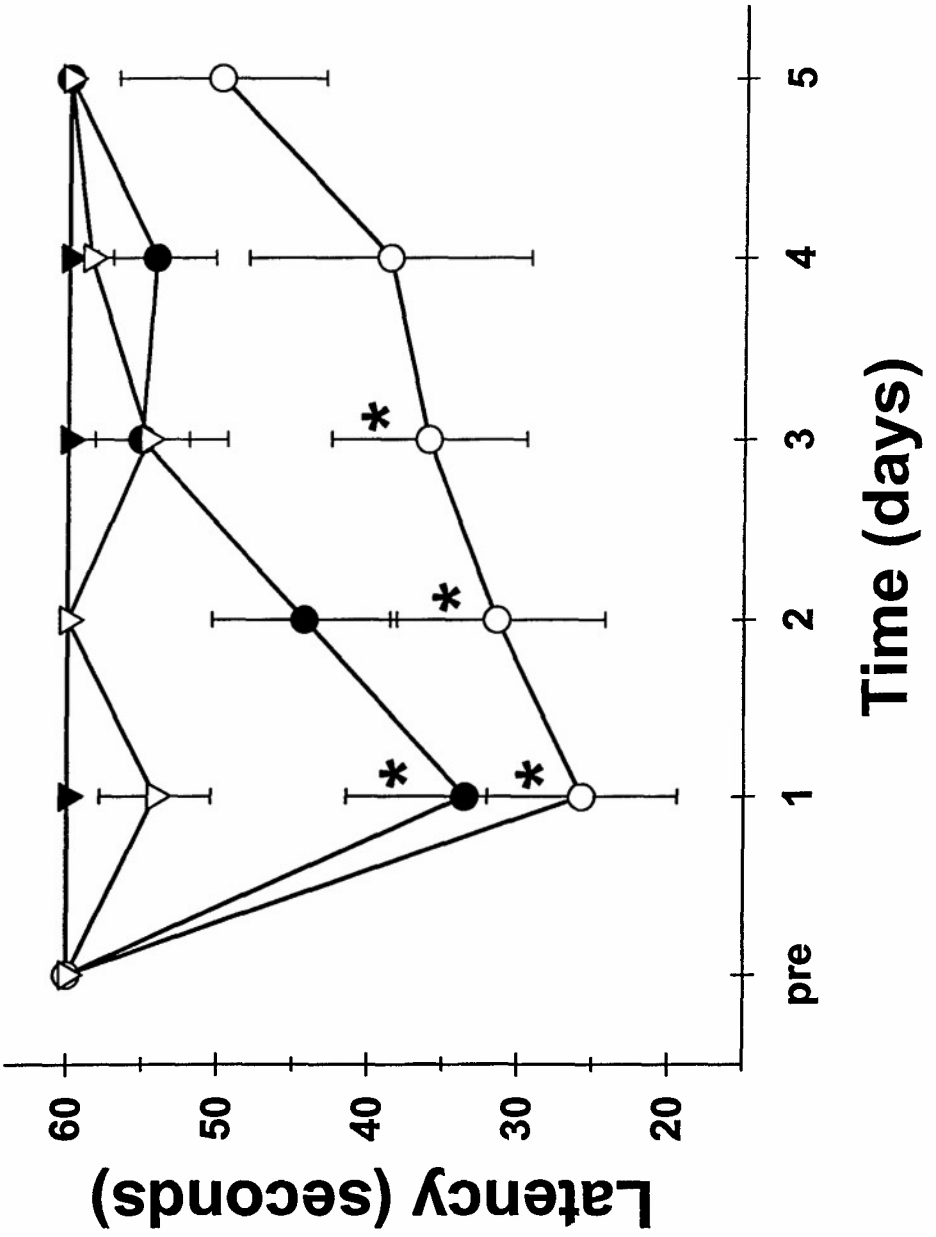


Figure 2B

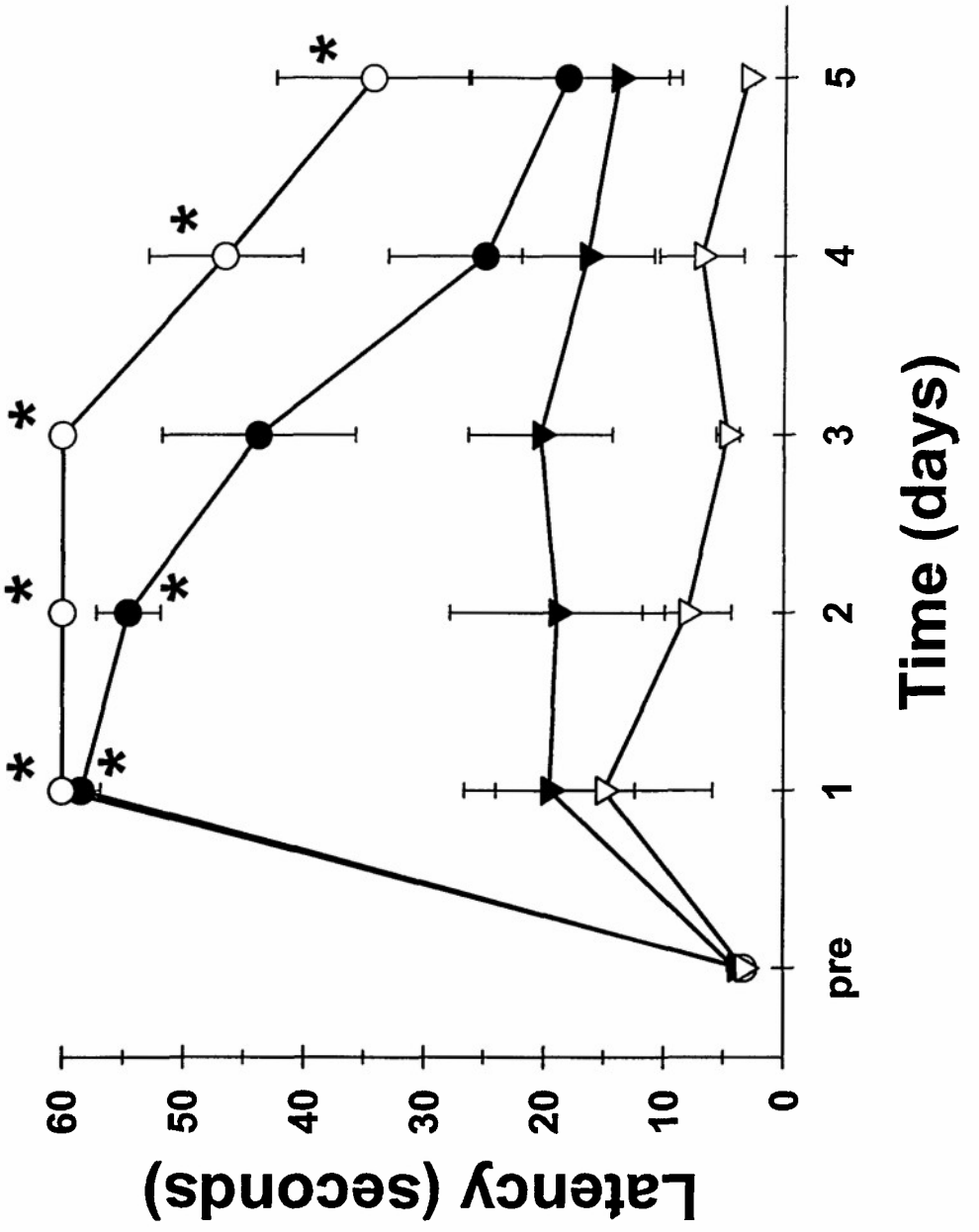


Figure 3A

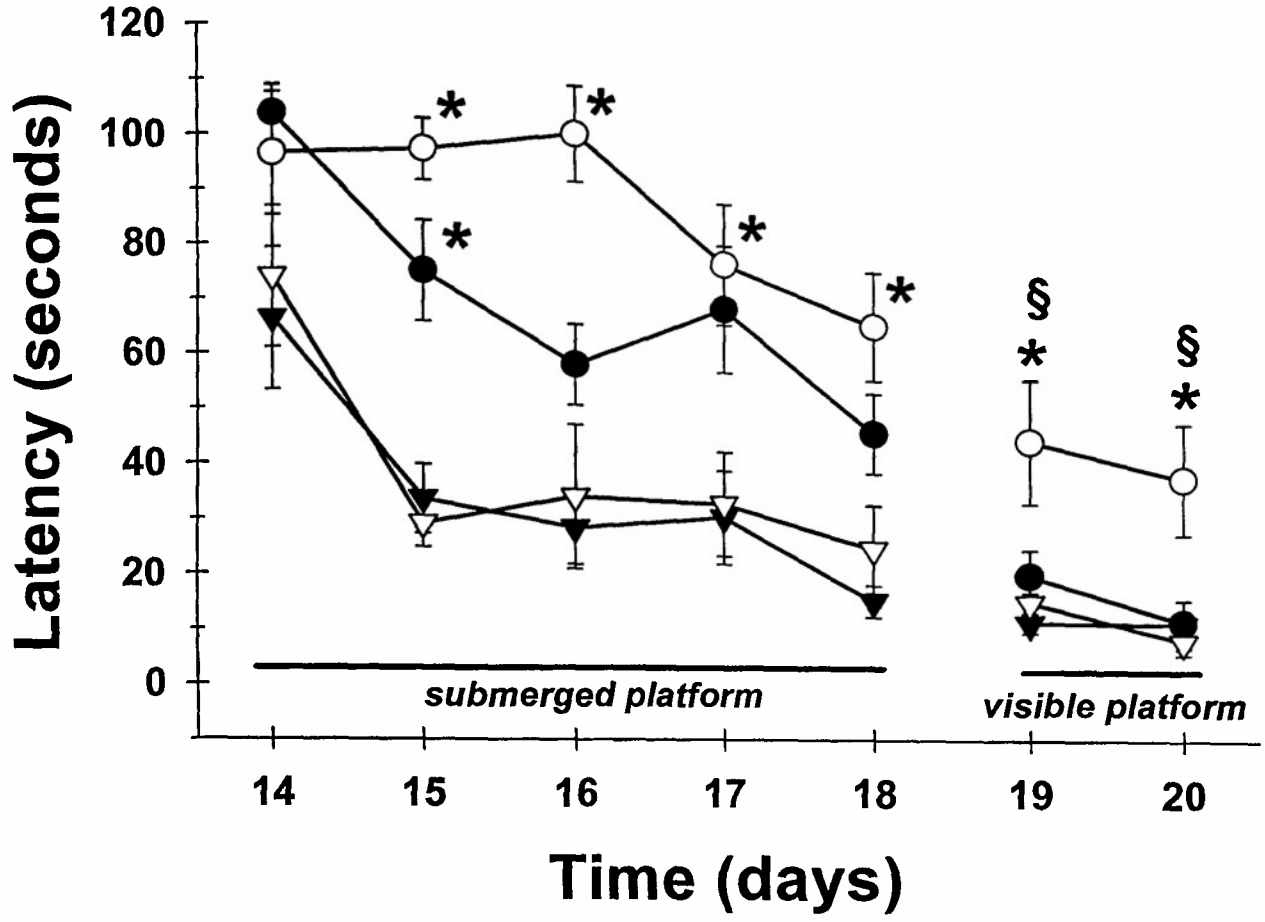

Latency (seconds)
120
100
80
60
40
20
0
*
§
submerged platform
visible platform
14
15
16
17
18
19
20
Time (days)

Figure 3B

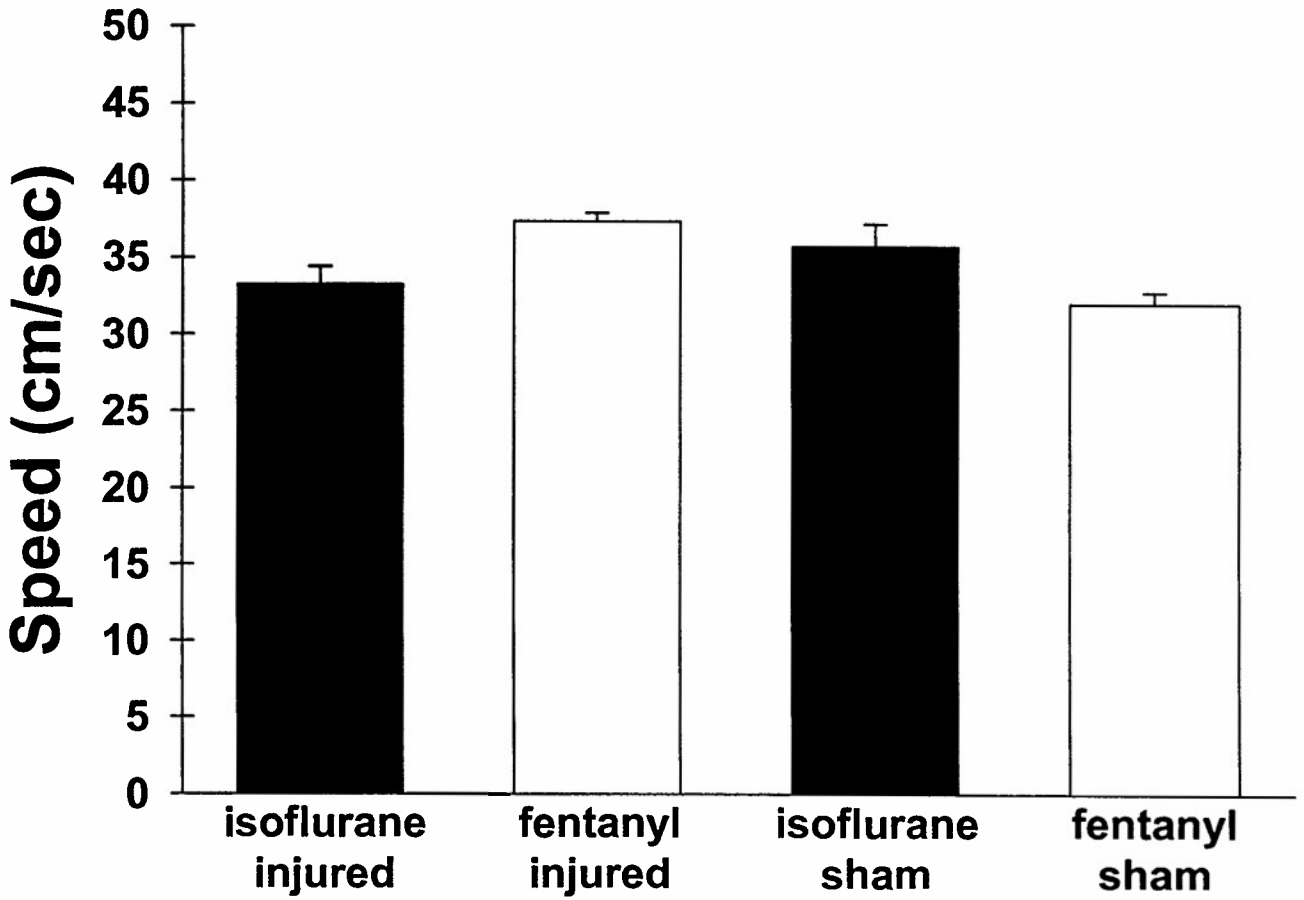

Speed (cm/sec)
50
45
40
35
30
25
20
15
10
5
0
isoflurane injured
fentanyl injured
isoflurane sham
fentanyl sham

Figure 4A

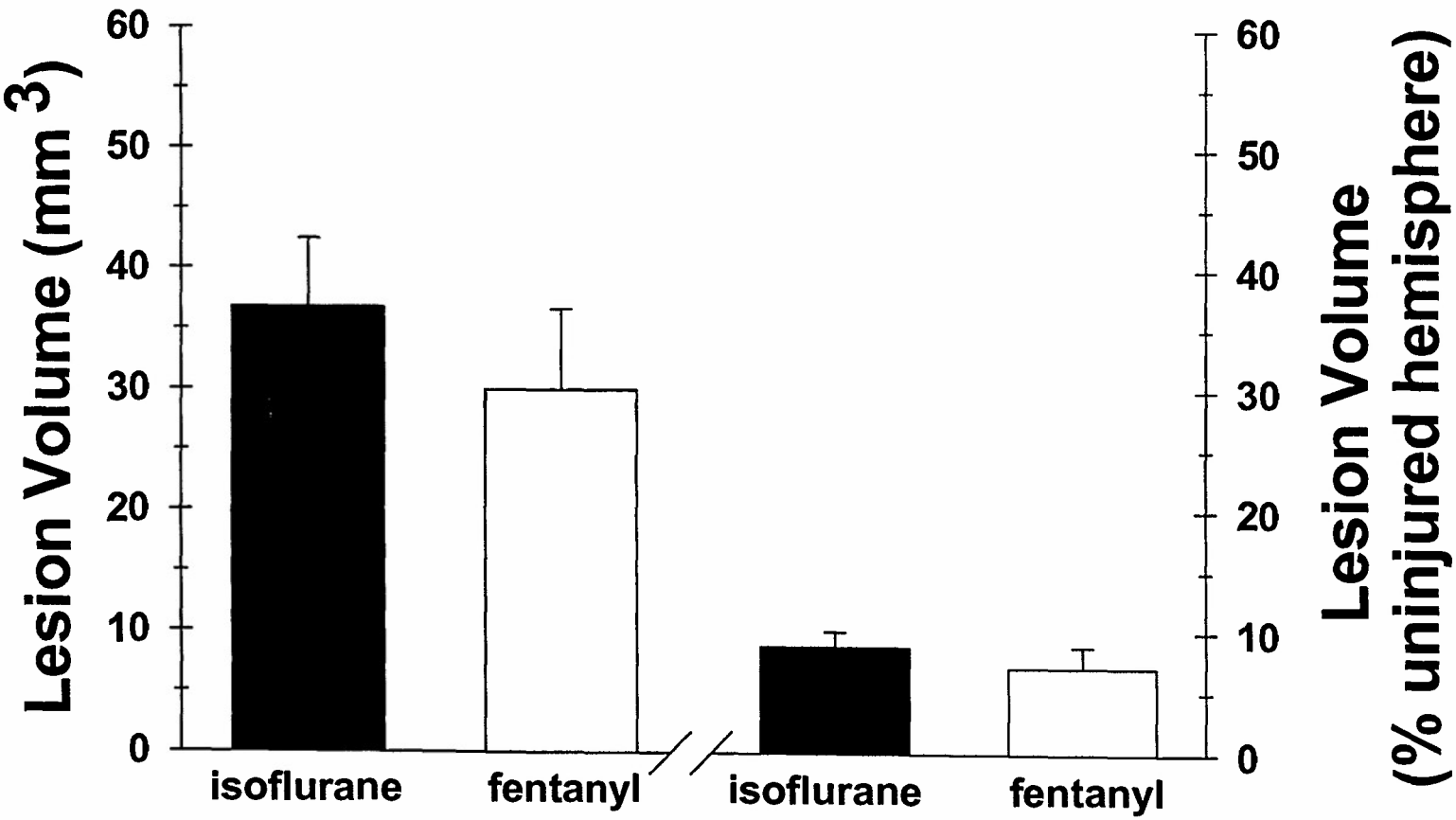

Lesion Volume (mm 3)
60
50
40
30
20
10
0
isoflurane
fentanyl
isoflurane
fentanyl
Lesion Volume
(% uninjured hemisphere)

Figure 4B

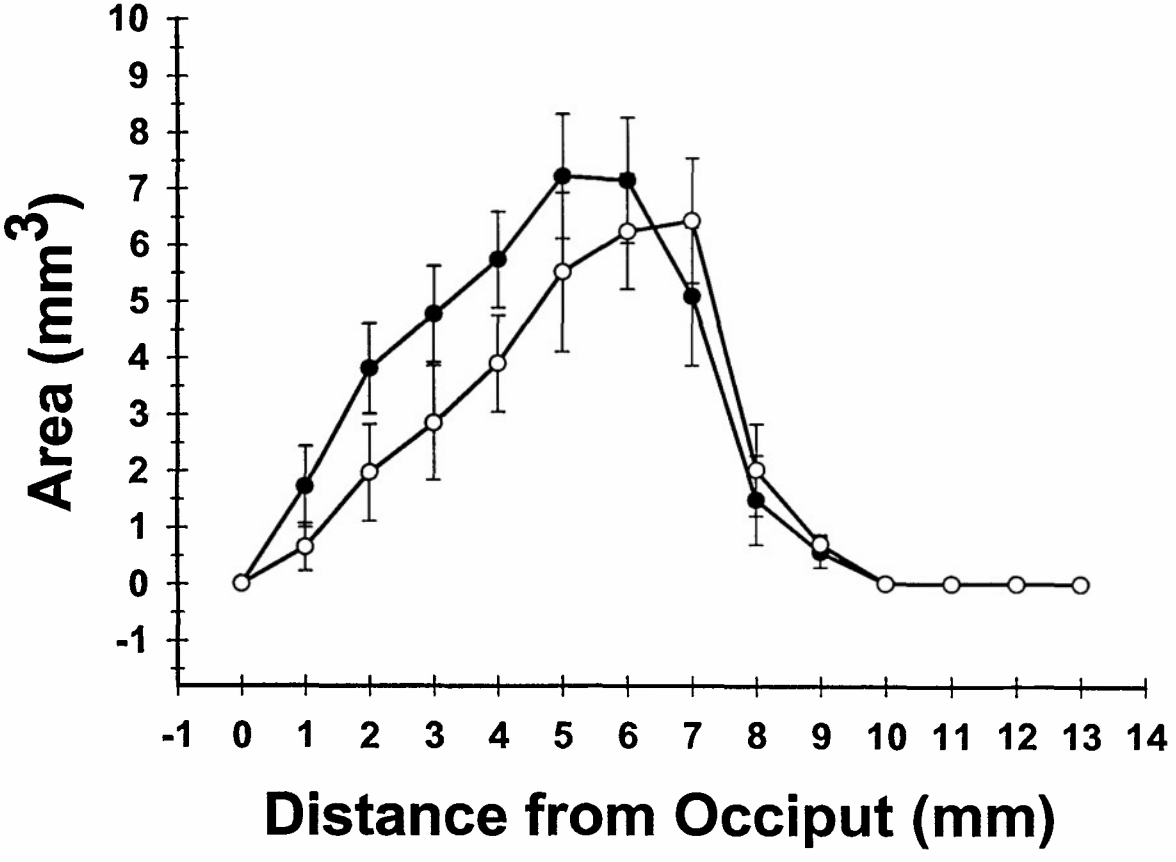

Area (mm3)
10
9
8
7
6
5
4
3
2
1
0
-1
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Distance from Occiput (mm)

Figure 4C

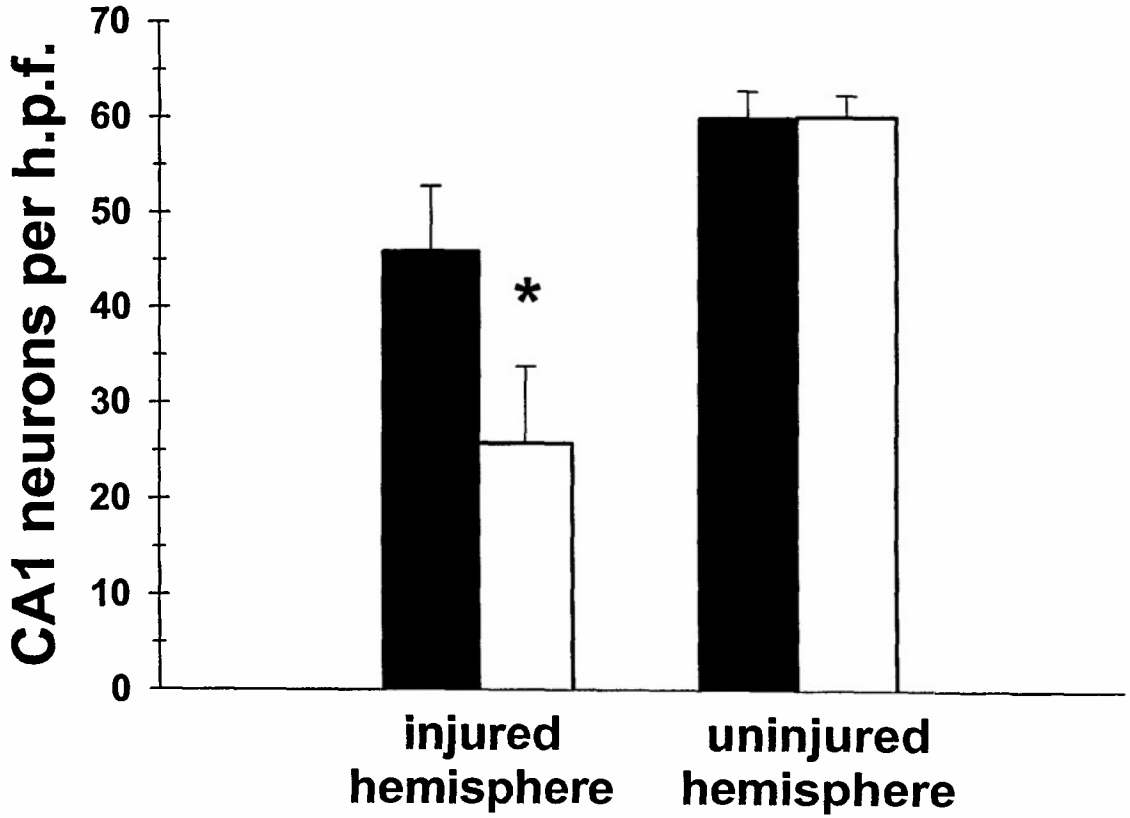

CA1 neurons per h.p.f.
70
60
50
40
30
20
10
0
*
injured hemisphere
uninjured hemisphere

Figure 4D

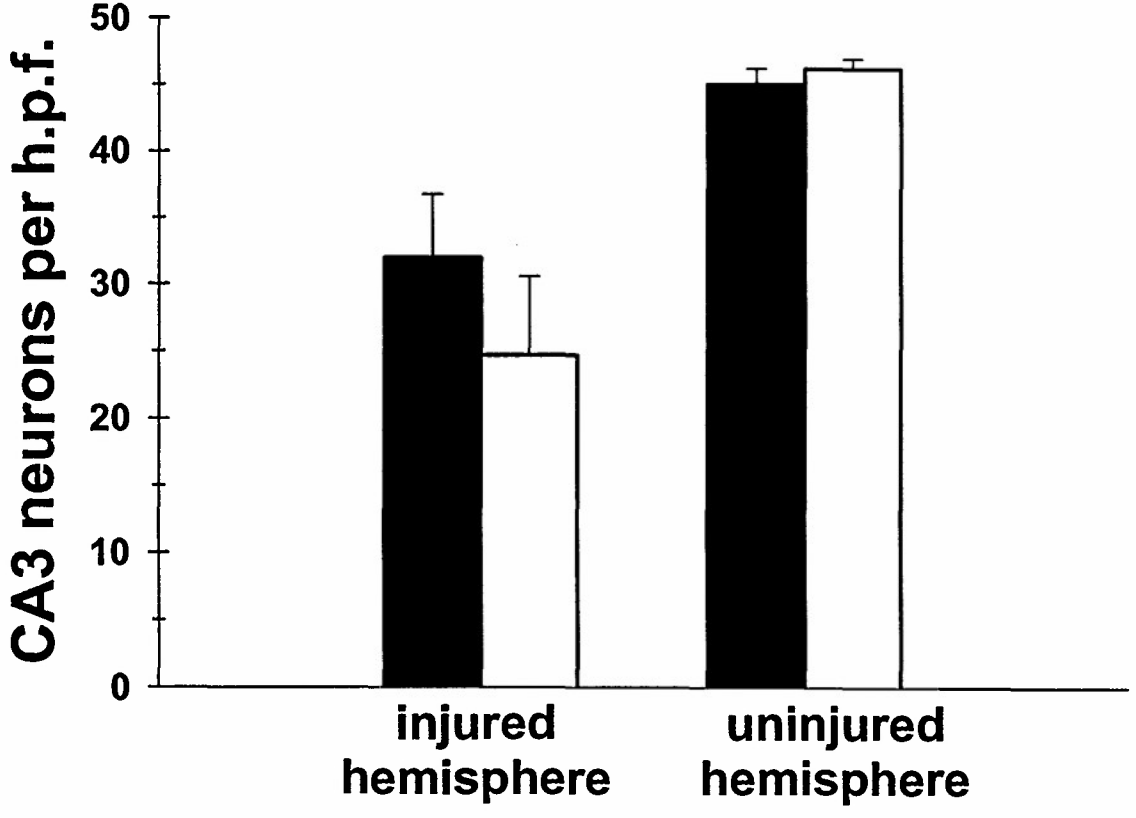

CA3 neurons per h.p.f.
50
40
30
20
10
0
injured hemisphere
uninjured hemisphere

Figure 5A

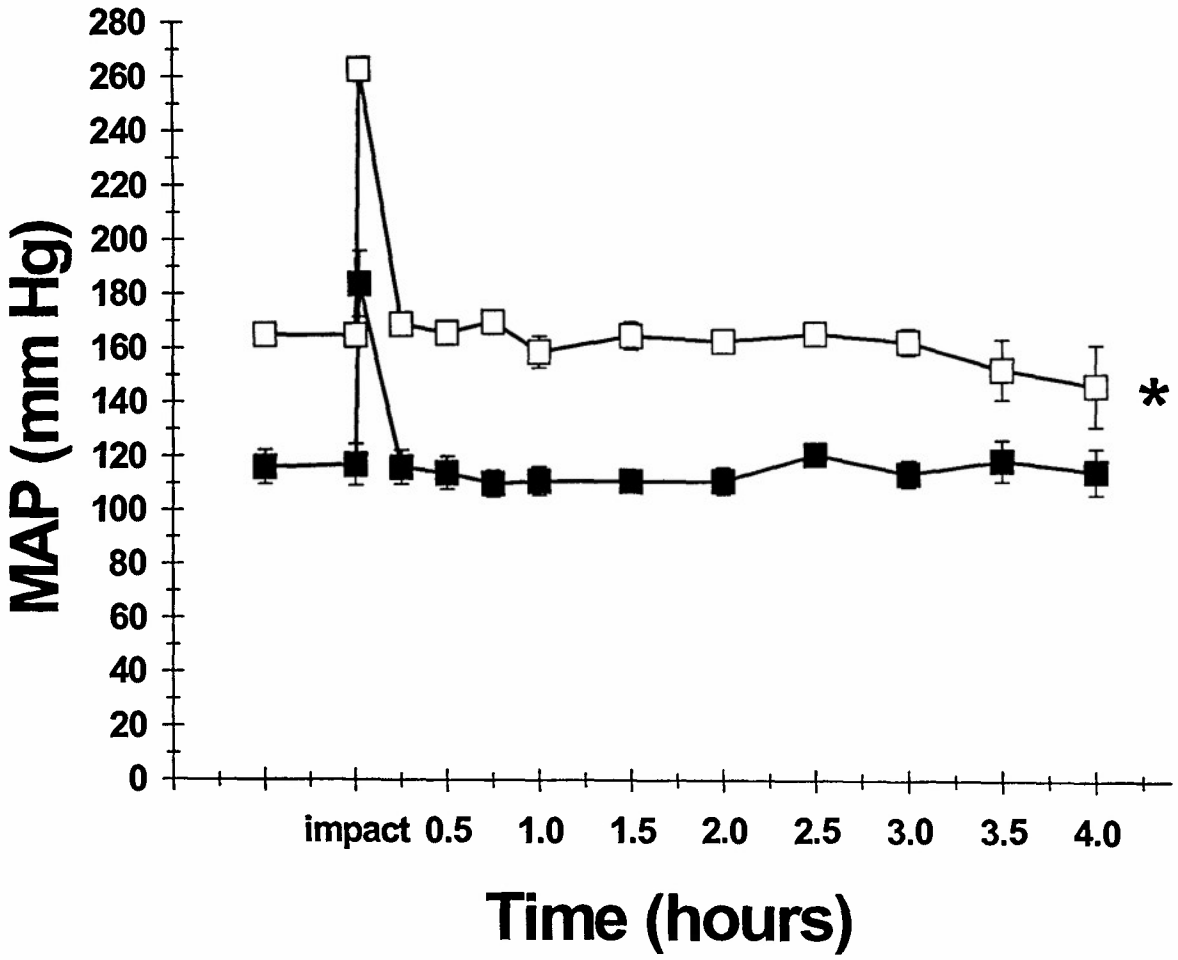

MAP (mm Hg)
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
impact
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Time (hours)
*

Figure 5B

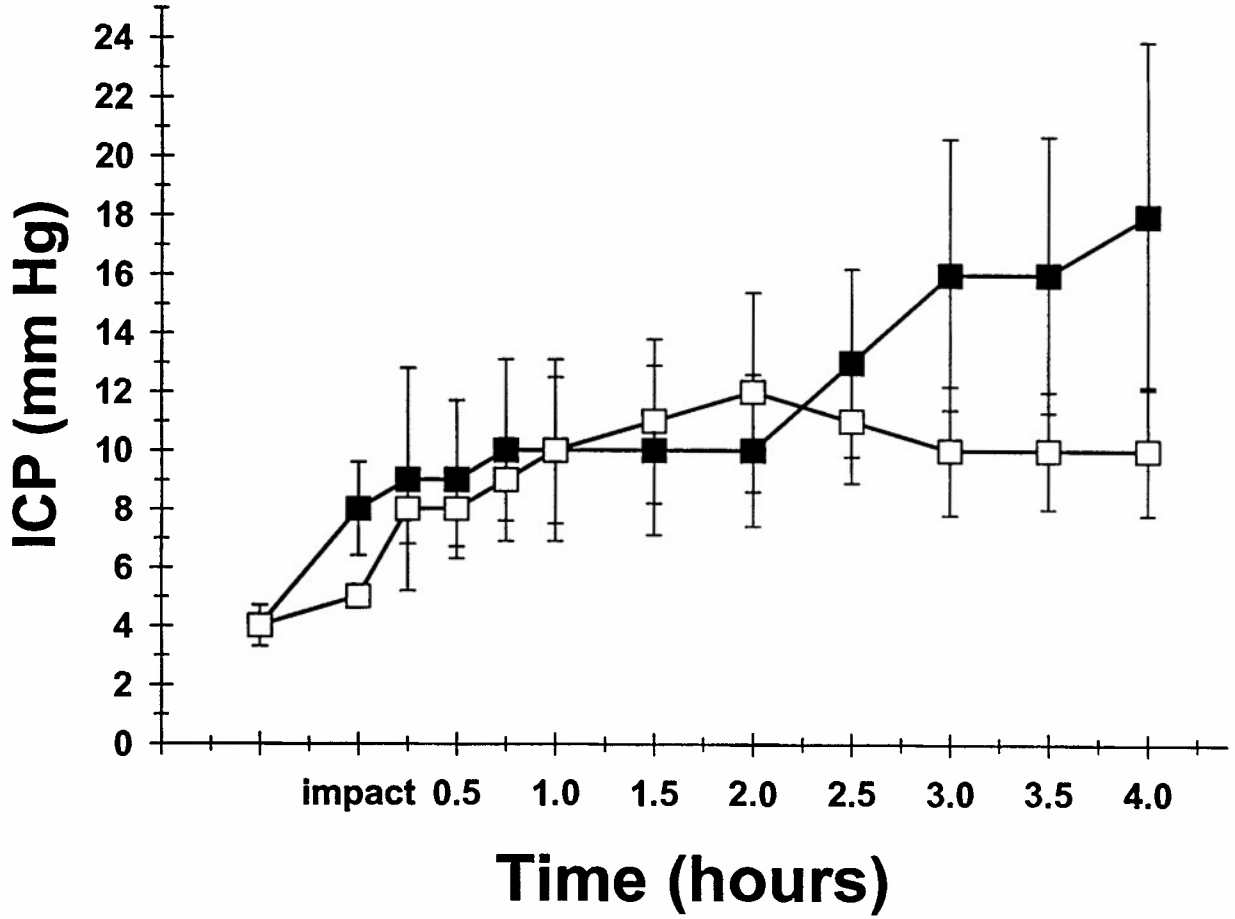

ICP (mm Hg)
24
22
20
18
16
14
12
10
8
6
4
2
0
impact
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Time (hours)

Figure 5C

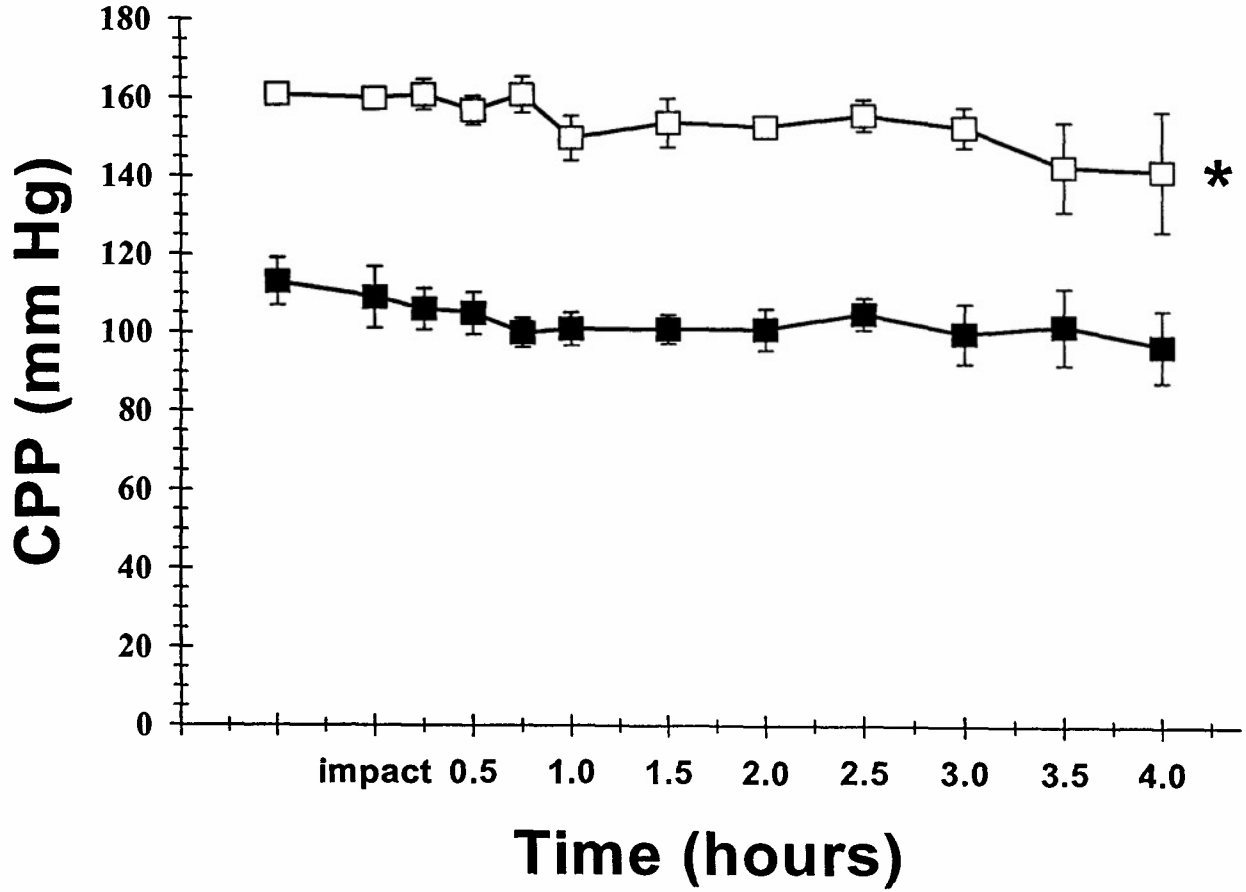
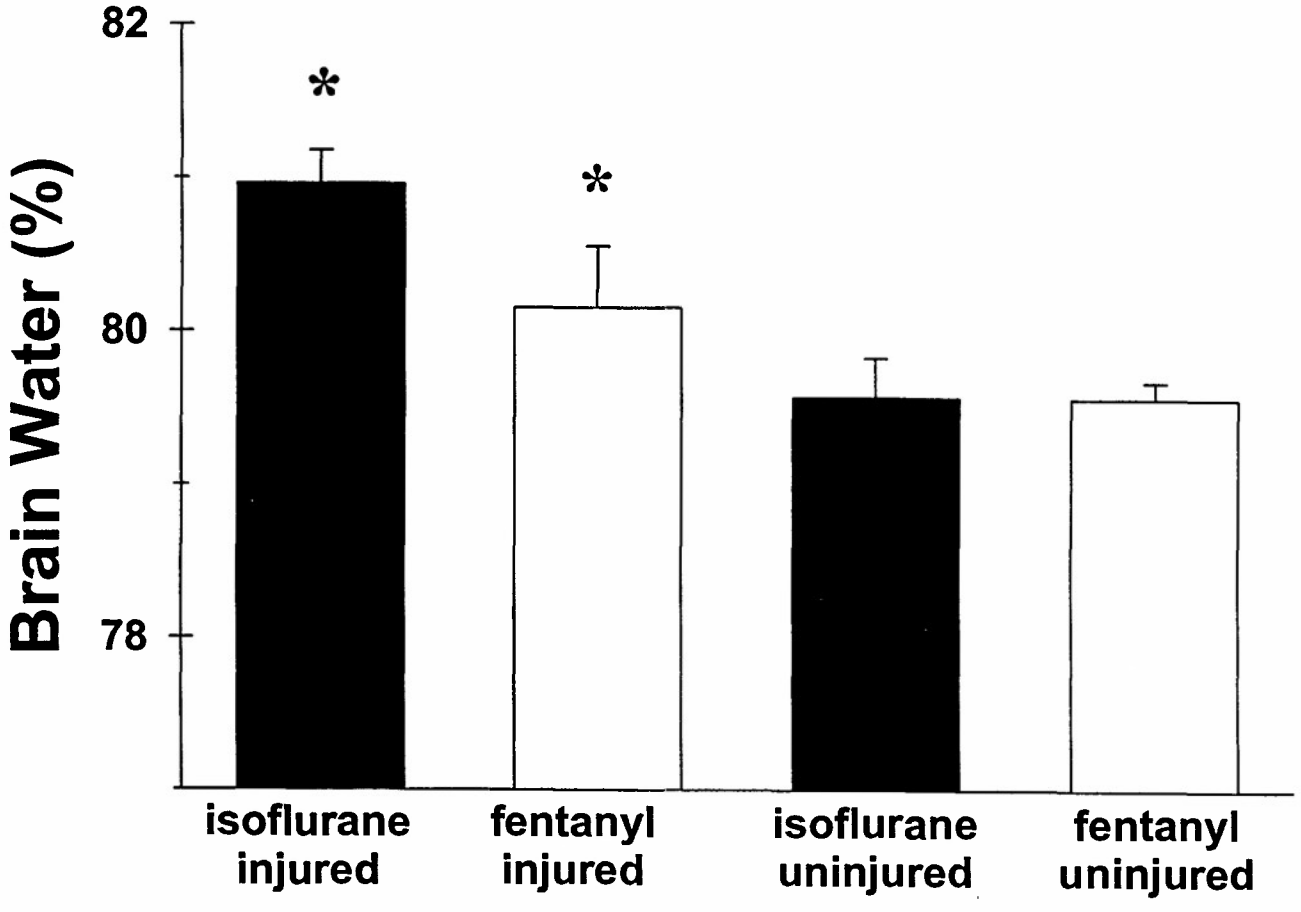

CPP (mm Hg)
180
160
140
120
100
80
60
40
20
0
impact
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Time (hours)
*

Figure 6


Brain Water (%)
82
80
78
*
*
isoflurane injured
fentanyl injured
isoflurane uninjured
fentanyl uninjured

5

CHANGES IN MITOCHONDRIAL MEMBRANE POTENTIAL IN STRETCH-INJURED ASTROCYTES AND NEURONS. S.M. Ahmed*, B.A. Rzigalinski and E.F. Ellis, Dept. of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA.

The dynamics of energy failure in traumatically injured astrocytes and neurons are unclear. In order to better understand mitochondrial function and cell energetics following trauma we utilized the fluorescent dye Rhodamine 123, which is normally sequestered in mitochondria where its fluorescence is quenched. When mitochondrial membrane potential (MMP) decreases, such as with mitochondrial poisons, the dye moves to the cytoplasm and fluorescence is increased. Pure neuronal or astrocytic cultures were subjected to mild (5.7 mm), moderate (6.5 mm) or severe (7.5 mm) stretch-induced injury and the change in MMP measured. There were no significant changes in MMP in mildly to moderately injured neurons at 15 min, 24 or 48 hr post-injury. However severely injured neurons displayed an immediate 33% decrease in MMP that persisted to 48 hr. In contrast, mild and moderate astrocyte injury caused a dramatic, 39-52% drop in MMP at 15 min, with MMP returning to normal by 24 hr. Our results indicate that direct trauma-induced alterations in cell energetics vary greatly in neurons and astrocytes. We suggest that in vivo the deficit induced in astrocytes may alter astrocyte function, which in turn may produce dramatic effects on neuronal function. Supported by NS-27214 and NS-07288.

6

HYPERTHERMIA ADVERSELY AFFECTS OUTCOME AFTER MODERATE HEAD INJURY. Philipp R. Aldana[1*], J. Marquez[1], D. S. Petrin[1], D. Johns[1], W. D. Dietrich[2], P. A. Villanueva[1]. Department of Neurological Surgery[1], Neurotrauma Research Center[2], University of Miami School of Medicine, Miami, Florida, 33101, USA.

Hypothermia has been shown to have beneficial effects after traumatic brain injury (TBI) in both human and animal studies. Conversely, hyperthermia after TBI has been shown to have deleterious effects in animals. No studies have addressed the effects of hyperthermia after moderate head injury in humans.

104 patients admitted with a Glasgow Coma Score 9-12 due to blunt head trauma were studied. Demographics, comorbid factors and characteristics of the hyperthermic episodes (>38.6°C) were examined. The number of patients either dead, in a vegetative state or severely disabled during discharge was significantly larger for the hyperthermic group vs. the normothermic group (42.4% vs. 17.5%, respectively). A significantly larger percentage of the normothermic group had a good outcome compared to the hyperthermic group (50% vs. 20.3%, respectively). Among the hyperthermic patients, those with associated infections had significantly worse outcomes and a higher frequency of hyperthermic episodes than those without infections. We conclude that hyperthermia in the face of an associated infection may adversely affect the outcome of patients with moderate head injury. We advocate maintenance of at least normothermic conditions if moderate hypothermia cannot be achieved and treatment of any underlying infection after TBI.

7

EVIDENCE FOR APOPTOTIC CELL DEATH FOLLOWING SUBDURAL HEMATOMA IN RATS. **B. Alessandri*, X. Di, H. Chen, R. Bullock. Div. of Neurosurgery, Medical College of Virginia, Richmond, VA, 23298, USA**

Subdural hematoma (SDH) is a common and dangerous secondary event following traumatic brain injury. The mechanisms leading to neuronal death, even after SDH removal, are not fully understood. A mechanism which might contribute to cell death is apoptotic cell death (ACD), which has been shown to be involved in the development of traumatic and ischemic brain damage.

The hematoma was produced by subdural injection of 250μL of autologous venous blood in Halothane anesthetized rats. Animals were allowed to survive 1 (n=3), 2 (n=3), 4 (n=3) or 7 days (n=4) after injection of SDH. Brain sections were stained by a commercially available apoptosis detection kit (FragEL™) for apoptotic cells (visualized by dianinobenzidine, DAB; counterstained by hemotoxylin). Brain sections were examined light microscopically and DAB-positive cells were counted in both hemispheres in cortical, subcortical and hippocampal areas.

The DBA-pos. cell counts were 2.3±2, 54.5±8, 13.7±8, and 12.8±3 at 1, 2, 4 and 7 days after SDH, respectively All apoptotic cells were within the cortex, within and in the border zone of the SDH lesion. There were no DAB-pos. cells in the contralateral side. The number of DAB-pos. cells was highly correlated with the lesion area (r^2=0.689, p<0.001).

The results indicate that ACD occurs following SDH, and is maximally seen at 2 days. DAB-pos. cells were only found within or in the border zone of the lesion. The correlation of ACD and lesion area underlines the importance of this type of cell death in SDH. The contribution of ACD to SDH-induced brain damage and its relevance for therapy needs further study.

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VERTICAL VERSUS ANGLED CONTROLLED CORTICAL IMPACT IN RATS. H.L. Alexander*, C.L. Robertson, C.E. Dixon, R.S.B. Clark, S.H. Graham, P.J. Safar, P.M. Kochanek. Safar Center for Resuscitation Research, Univ. of Pittsburgh, PA 15213

Although a variety of modifications of the controlled cortical impact (CCI) model exist, a comparison between the two most common variants, vertical[1] and angled impact[2], has not been performed. Rats were subjected to vertical (n = 8), angled (n = 8) or sham (n = 8) insults (4 m/s, 2.5 mm) to the left parietal cortex, using a CCI model with hypoxemia.[3] Motor (beam balance, d1-5), cognitive (Morris Water Maze, d14-21) and histologic (lesion volume, CA1 and CA3 neuron counts, d21) outcomes were studied. Motor and MWM performance were impaired, but did not differ between injury groups. Lesion volumes also did not differ (vertical = 92.2±7.2 mm^3, angled = 79.4±7.8, p = 0.25). CA1 neuron counts were decreased ipsilateral to injury in both groups vs sham (vertical = 20.4±8.4 cells/hpf, angled = 32.7±15.8, sham = 55.5±3.9, $p < .05$). However, CA3 neuron counts were decreased ipsilateral to injury in the vertical group vs sham (23.2±8.5 vs 52.1±6.6, respectively, $p < .05$), but the angled group (32.7±15.8) was not different from sham. We conclude that the vertical and angled variants of the CCI model produce similar functional deficits; however, the vertical impact appears to produce greater local damage, particularly in CA3 neurons. *[1]J Neurotrauma 12:1015 [2]J Neurosci Methods 39:253; [3]J Neurotrauma 14:179; Support: US Army #DAMD17-97-1-7009*

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CHRONIC OVEREXPRESSION OF AMYLOID PRECURSOR PROTEIN (APP) AFTER TRAUMATIC BRAIN INJURY IN RATS. J. R. Ciallella[1]*, H. Q. Yan[1], X. Ma[1], D. W. Marion[1], S. T. DeKosky[2], and C. E. Dixon[1]. Departments of [1]Neurosurgery and [2]Psychiatry, University of Pittsburgh Medical Center, Pittsburgh, PA USA.

Traumatic brain injury (TBI) and Alzheimer's disease (AD) produce cholinergic and metabolic deficits that may contribute to neurodegeneration. There is increasing evidence linking AD and TBI, including upregulation of APP in head injured patients (McKenzie et al. 1994 *NeuroRep.*6:161). To further investigate this linkage, we tested the hypothesis that controlled cortical impact (CCI) injury would produce chronic upregulation of APP protein levels at 4 weeks following injury. Our previous studies demonstrated significant changes in cholinergic proteins at this time point (Ciallella et al. 1998. *Exp. Neurol.* In Press). APP immunohistochemistry (n=3-5) and western blot (n=4) were performed on cortical and hippocampal regions from injured and sham animals. The same N-terminal antibody was used in all studies. A marked increase in cortical and hippocampal APP protein was demonstrated bilaterally by both immunohistochemistry and western blot in injured rats compared to sham controls. This demonstrates that a single TBI can lead to chronic upregulation of APP, concurrent with chronic alterations in cholinergic markers. Supported by AG05133, NINDS-T32NS07391, CDC-CCR312296, NIH-NS30313, and NIH-NS33150.

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EFFECT OF HYPOTHERMIA AFTER SEVERE TRAUMATIC BRAIN INJURY WITH SECONDARY HYPOXEMIA IN RATS. R.S.B. Clark*, C.L. Robertson, C.E. Dixon, H.L. Alexander, S.H. Graham, P.J. Safar, P.M. Kochanek. Safar Center for Resus Res., U of Pgh, PA 15213.

Many reports have shown benefit from hypothermia (HT) in traumatic brain injury (TBI); but, its effect on TBI with secondary insult remains undefined. We hypothesized that HT would improve outcome after controlled-cortical impact (CCI) with secondary hypoxemia. Rats received severe CCI injury followed by 30 min of hypoxemia,[1] and randomized to normothermia (NT=37°C brain temp, n=19), immediate HT (IHT=32°C, after CCI, n=10), or delayed HT (DHT=32°C, after hypoxemia, n=14) for 4 h. Motor (beam balance/ walking, d1-5), cognitive (Morris Water Maze [MWM], d14-21) and histologic outcomes (lesion volume, hippocampal neuron counts, d21) were evaluated. Motor and MWM performance were impaired but did not differ between groups. Lesion volumes (mm^3) did not differ between groups (NT=65.3±6.9, IHT=50.2±8.2, DHT=53.7±7.9). Neuron counts (CA1, CA3) were decreased 60-70% ipsilateral to CCI, but did not differ between groups. Mortality doubled (43% vs 20-21%) in DHT vs NT or IHT (p = 0.3). HT did not improve outcome after severe CCI with secondary insult. Clinical studies[2] exclude patients with secondary insults, and suggest HT is not effective after severe injury (GCS 3-4). Novel therapies may be needed in this setting. *[1]J Neurotrauma 14:179; [2]NEJM 336:540-6; Support: US Army #DAMD17-97-1-7009*

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THE SUPPRESSION OF HIPPOCAMPAL NGF mRNA AFTER CEREBRAL ISHCEMIA IN RAT TREATED WITH ANTISENSE DNA TO C-*fos*. J-K.Cui[1]*, C. Y. Hsu[2], and P. K. Liu[1]. [1]Department of Neurosurgery, Baylor College of Medicine, Houston, TX 77030; [2]Department of Neurology, Washington University, St Louis, MO 63110.

The biological effects of Fos expression in the brain were examined using phosphorothioated oligodeoxy-nucleotides (s-ODNs) to c-fos, $rncfosr_{115}$. Biotinylated antisense $rncofsr_{115}$ (bio- $rncfosr_{115}$) plus lipofectin were delivered into the brain of male Long-Evans rats (225-250 gm) via intracerebroventricular infusion. The distribution of bio-$rncfosr_{115}$ was detected using antibodies against biotin. Using dot blot analysis on the recovered bio-$rncfosr_{115}$, the bio-$rncfosr_{115}$ uptake in hippocampus peaked at 29-48 hrs, and the internalized bio-$rncfosr_{115}$ was degraded within 72 hr of infusion. The s-ODN uptake in the brain was confirmed by 3'-end-labeling with digoxigenin-dUTP, using terminal transferase and anti-digoxigenin IgG-FITC. The presence of fluorescent aggregates in the brain cells near the vessel wall in animals treated with antisense $rncfosr_{115}$ + lipofectin suggests lipofectin mediated s-ODN transfer across the blood brain barrier. The uptake increased with time and with the dose delivered. The effectiveness of antisense $rncfosr_{115}$ was shown by an inhibition of ischemia-induced Fos expression, and was accomplished by an inhibition of ischemia-induced hippocampal NGF mRNA expression in the brain of animals pretreated with antisense $rncfosr_{115}$. The specificity of Fos suppression was suggested by a lack of antisense $rncfosr_{115}$ effect on the expression of NT-3 and α-actin mRNA.

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LOSS OF GLIAL POTASSIUM CURRENTS AND IMPAIRMENT OF POTASSIUM HOMEOSTASIS FOLLOWING FLUID PERCUSSION INJURY. R. D'Ambrosio*, D.O. Maris, M.S. Grady, and D. Janigro. Dept. of Neurosurgery, Univ. of Washington, Seattle, WA 98104

We compared the early effects of moderate in vivo fluid percussion injury (FPI) on the functional expression of potassium currents expressed in oligodendroglia and astrocytes from acutely isolated rat hippocampal slices. Whole cell recordings were performed from post-FPI and naïve slices of 30 d.o. rats. Cs+ (1 mM) was used to block inward potassium currents. K+-selective electrodes were employed to measure K+ accumulation in radiatum CA3. GFAP immunostaining was enhanced in CA3 24-48 hrs following FPI, while immunostaining for oligodendroglia was reduced. A significant decrease in Cs+-sensitive potassium currents was observed following lesion in both oligodendroglia and astrocytes. Cells characterized by complex electrophysiological profiles as well as those characterized by inward rectification were equally affected (-60% and -55% at -140 mV). Morphologically, complex cells visualized by biocytin staining could be classified as oligodendrocytes. Stimulation (1 Hz) of Schaffer collaterals induced K+ accumulation in radiatum CA3. Slices obtained from naïve rats always showed a recovery of extracellular K+ to basal levels within 10 seconds following stimulation (n=5). Slices obtained from post-FPI rats displayed recovery times ranging from 10 to 40 seconds (n=8). Additionally, 75% of the post-FPI slices generated multiple afterdischarges during stimulation, while only 20% of the control slices did. These results indicate that 1) post-FPI CA3 astrocytes are reactive or injured, 2) loss of Cs+-sensitive potassium current occurs in oligodendrocytes and astrocytes post-FPI, 3) neuronal-activity-induced elevation of $[K^+]_{out}$ is more persistent at early time point post-FPI, 4) hyperexcitability is observed after trauma without detectable neuronal loss. We conclude that FPI may affect extracellular K+-homeostasis by impairing glial potassium currents. Supported by NIH-51614 and RO-1 NS33107.

EFFECT OF CALCIUM CHLORIDE ON REGIONAL CEREBRAL BLOOD FLOW DURING CARDIOPULMONARY RESUSCITATION IN PIGLETS

Melody Palmer Land, John Kuluz, Barry Gelman, Michael Nares, En Xu, and Charles Schleien. Pediatric Critical Care Medicine, University of Miami School of Medicine, Miami, FL 33101.

Introduction: The use of calcium chloride ($CaCl_2$) during CPR remains controversial. $CaCl_2$ may improve the effectiveness of CPR by increasing systemic vascular tone and vital organ perfusion. Alternatively, $CaCl_2$ may cause regional vasoconstriction in the brain and heart, resulting in secondary ischemic injury. We hypothesized that administration of the standard dose of $CaCl_2$ during CPR decreases rCBF.

Methods: Under pentobarbital anesthesia, 2-4 week old piglets underwent 6 min of cardiac arrest by ventricular fibrillation, and 30 min of standard CPR. rCBF was measured with microspheres at baseline and after 5, 15 and 30 min of CPR. $CaCl_2$ 20 mg/kg (n=5) or saline (n=5) was given after 1 and 19 min of CPR. Data (mean±SE) were analyzed by ANOVA and Student's t-test (* $p<.05$).

Results: Ionized (io) Ca decreased from 1.40±.03 at baseline to 1.16±.05* at 15 min and 1.18±.05* at 30 min CPR. After $CaCl_2$, ioCa increased to 2.58±16* at 5 min and 2.04±21* at 30 min, and was not different from baseline at 15 min CPR. Calcium increased aortic pressure (44±2 vs 38±2*) and cerebral perfusion pressure at 5 min CPR. Total CBF was not different between groups at any time point; however, severe regional ischemia (CBF<15 ml/100g/min) was more common after 30 min CPR when $CaCl_2$ was given, particularly in subcortical regions ($p<.03$).

Conclusion: These data show that $CaCl_2$ administration has adverse effects on rCBF during prolonged CPR and may worsen ischemic brain injury. Future studies will determine the effect of $CaCl_2$ on functional and neuropathologic outcome.

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SYSTEMIC AND GLOBAL CEREBRAL OXYGEN EXTRACTION (O_2 Ext) IN ACUTE CARBON MONOXIDE (CO) TOXICITY

Deborah M. Lopez, Jacqueline Weingarten-Arams, Lewis Singer, Edward Conway Jr. Department of Pediatrics, Division of Critical Care Medicine, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, New York 10467

Introduction: Carbon Monoxide is a colorless and odorless gas produced by incomplete combustion of carbon containing compounds. CO affects most notably those organs with high metabolic rates such as the central nervous system. CO causes a left shift of the oxyhemoglobin dissociation curve. Limited adult animal studies have suggested that systemic O_2 Ext is decreased in the presence of CO toxicity. The purpose of this study is to evaluate systemic and cerebral O_2 Ext in a pediatric model of CO toxicity.

Methods: 15 Yorkshire piglets were anesthetized with pentobarbital. Tracheostomy, femoral arterial, pulmooary arterial and retrograde jugular venous bulb pressure catheters were inserted. Following a one hour rest period, baseline data was collected. CO was administered via the endotracheal tube to achieve and maintain a level of 60% carboxyhemoglobin (COHb). Arterial, mixed venous and internal jugular blood samples were drawn within five minutes of each other. Blood samples were measured with the Radiometer OSM 3 Hemoximeter. O_2 Ext was calculated via standard formula. Measurements were stratified by the corresponding COHb level: mild toxicity = 0-10%, moderate = ≥10-40% and severe ≥40%.

Results: 158 sets of blood samples were obtained. Mean values are summarized below. Systemic versus cerebral O_2 Ext were analyzed via two tailed t-test and were significant at all levels of COHb with *$p \leq 0.01$.

COHB%	O_2 delivery (ml O_2/min)	CO (L/min)	Systemic O_2 Ext	Cerebral O_2 Ext
0-10	102.6	1.14	0.40	0.28 *
>10-40	95	1.17	0.43	0.36 *, #
>40	56.8	1.40	0.41	0.33 *, #

In addition, cerebral oxygen extraction significantly increased as the percentage of COHb escalated with no change in systemic oxygen extraction. (ANOVA, # $p = 0.05$)

Conclusion: Cerebral oxygen extraction increased with increasing COHb level. Contrary to adult animal studies, systemic oxygen extraction remained unchanged despite increasing COHb levels. Cardiac output remained the same and as expected oxygen delivery decreased with increasing level of COHb.

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NO LONG-TERM BENEFIT FROM HYPOTHERMIA AFTER SEVERE TRAUMATIC BRAIN INJURY WITH SECONDARY HYPOXEMIA IN RATS

Courtney L. Robertson, Robert Clark, C. Edward Dixon, Steven Graham, Henry Alexander, Stephen Wisniewski, Donald Marion, Peter Safar, Patrick Kochanek. Depts of Anesthesiol/CCM, Pediatrics, Neurology, Epidemiology, and Neurosurgery, Safar Center for Resuscitation Research, University of Pittsburgh, PA 15213.

Introduction: Many reports have shown benefit from hypothermia in traumatic brain injury (TBI); but its effect in the setting of TBI with secondary insult remains undefined. Clinical studies show an increase in morbidity and mortality after severe TBI with secondary brain insult.[1] In experimental rat models, outcomes were worse in brain injury with secondary hypoxia.[2] Recently, we characterized a model of TBI with secondary hypoxemia and reported prominent neuronal apoptosis after injury.[3,4] We hypothesized that hypothermia would improve outcome after controlled-cortical impact (CCI) with secondary hypoxemic insult in rats.

Methods: Rats were subjected to severe CCI injury followed by 30 min of hypoxemia (PaO_2=35-45 mm Hg).[3] Rats were then randomized to normothermia (NT=37°C, n=19), immediate hypothermia (IHT=32°C, after CCI, n=10), or delayed hypothermia (DHT=32°C, after hypoxemia, n=14) for 4 h. Motor (beam balance/ beam walking, d 1-5), cognitive (Morris Water Maze [MWM], d 14-21) and histologic outcome (lesion volume, hippocampal neuron counts, d21) were evaluated.

Results: Motor and MWM performance were impaired, but did not differ between groups. Lesion volumes did not differ significantly between groups (NT=65.3 mm^3 ±6.9, IHT=50.2±8.2, DHT=53.7±7.9). Hippocampal neuron counts (CA1,CA3) were decreased on the injured side, but did not differ between groups (NT-CA1=19.8±4.2 cells/hpf, NT-CA3=19.8±4.6, IHT-CA1=13.2±8.7, IHT-CA3=15.6±7.3, DHT-CA1=13.7±5.8, DHT-CA3=18.5±7.3). Mortality rate did not differ significantly between groups.

Conclusions: Immediate or delayed hypothermia did not improve long-term outcome after severe CCI with secondary hypoxemia in rats. The severity of the combined insult may be outside of the therapeutic window of opportunity. Clinical studies[5] have excluded patients with secondary insult, and have indicated that hypothermia is of limited efficacy in the subset of severely injured (GCS 3-4) patients. Novel therapeutic approaches or combination therapies may by necessary in this setting. [1]*J Trauma* 34:216, [2]*J Cereb Blood Flow Metab* 7:759, [3]*J Neurotrauma* 14:179, [4]*J Neurosci* 17:9172, [5]*NEJM* 336:540; Support: USArmy#DAMD17-97-1-7009

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BRAIN NITRIC OXIDE CHANGES AFTER CONTROLLED CORTICAL IMPACT INJURY IN RATS

Leela Cherian, J. Clay. Goodman, Claudia S.Robertson. Departments of Neurosurgery and Pathology, Baylor College of Medicine, Houston, TX-77030

Introduction: The marked reduction in CBF that occurs after severe controlled cortical impact (CCI) injury in rats can be ameliorated by postinjury infusion of L-arginine. Since L-arginine is a substrate for nitric oxide synthase, these studies suggest that a reduced production of nitric oxide (NO) may play a role in the CBF reduction that occurs after brain trauma. The purpose of this study was to measure brain tissue concentrations of NO after severe CCI.

Methods: 12 Long Evans rats were anesthetized with isoflurane and subjected to severe CCI (5 m/sec, 3 mm deformation) or sham CCI. NO was directly measured using a NO electrode which was inserted in the site of the impact after calibration using S-nitriso N-acetyl –D-L-penicillamine at 37°C. A microdialysis probe was inserted near the NO electrode and perfused with artificial CSF at 2 µl/min. The concentration of nitrate/nitrite was measured using a chemiluminescent method in serial 20 minute collections of dialysate. These measurements were obtained prior to injury, and for 3 hours after injury. Values were expressed as % of the pre-injury baseline values.

Results. Impact injury caused a transient increase in brain tissue NO concentrations to 178 % of the baseline values in the CCI animals, compared to 98% in the sham injured animals ($p=0.002$). After the initial transient increase in NO, the concentrations of NO declined and remained significantly lower than in the sham animals throughout the 3 hr study period. The results are summarized below as median (25th percentile, 75th percentile). A similar reduction in nitrate/nitrite was observed in the microdialysates.

Time after Injury	NO (%baseline) CCI injury (n=6)	NO (% baseline) Sham injury (n=6)	P value
2min	177.5 (158,197)	97.5 (96,102)	0.002
1hr	75.5(70,80)	98.5(96,110)	0.004
2hr	73 (69,79)	101 (95,114)	0.001
3hr	70 (67,78)	95.5 (92,100)	0.002

Conclusions: This study suggests that NO is released immediately after a severe brain injury and subsequently is found in decreased concentrations in the brain for at least 3 hours after injury. The reduction in CBF that occurs after severe CCI may be related to the reduced NO levels.

Supported by NIH grant #PO1-NS27616

101.5

EFFICACY OF CASPASE INHIBITION FOR INTRACEREBRAL HEMORRHAGE IN RATS. K.Matsushita[1], W.Meng[2], M.Yamada[1], M.A.Moskowitz[1], E.H.Lo*[2]. [1]Stroke and Neurovascular Regulation, [2]Neuroprotection Research Laboratory, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA02129, USA.

Compared with ischemia, the mechanisms that underlie neuronal damage following intracerebral hemorrhage remain relatively unexplored. Parenchymal ischemia accompanying hemorrhage is typically mild (CBF 50-75% of baseline); therefore this may favor apoptotic pathways of neuronal cell death. The aim of the present study is to characterize the spatial and temporal profile of apoptosis after hemorrhage and evaluate the therapeutic efficacy of caspase inhibition. In vitro experiments confirmed that collagenase per se was not toxic in cultured neurons. Intrastriatal hemorrhage was then produced in rats by the intracerebral injection of collagenase (0.5u in 1μL). Nissl and TUNEL staining at 24, 48 and 72 hrs post-hemorrhage demonstrated that TUNEL positive apoptotic cells were distributed more in the periphery than in the center between 24 and 48 hrs, and then declined in number at 72 hrs. Pre-treatment with the caspase inhibitor, z-VADfmk (80ng, icv), significantly reduced the number of TUNEL positive cells at 24 hrs.

These findings suggest that apoptosis is an important pathological mechanism following intracerebral hemorrhage and caspase inhibition may have a therapeutic effect.

Supported by Uehara Memorial Foundation (KM), NS10828 (MAM), NS32806 (EHL), and NS37074 (EHL).

101.6

INHIBITION OF INTERLEUKIN 1β CONVERTING ENZYME FAMILY PROTEASES REDUCES COLD INJURY-INDUCED BRAIN TRAUMA AND DNA FRAGMENTATION IN MICE. Y. Morita-Fujimura[2] M. Fujimura[2] M. Kawase[2] K. Murakami[2] L. Litt[1]* and P. H. Chan[2]. [1]Dept. of Anesthesia, Univ. of California, San Francisco; [2]Dept. of Neurosurgery, Neurology and Neurological Sciences, Stanford Univ., School of Medicine Palo Alto, CA 94304.

The interleukin 1β converting enzyme (ICE) family, a protease family implicated in apoptosis, has been reported to be activated after brain injury such as ischemia and trauma, and its inhibitors reduce ischemic brain infarction (Hara *et al.*, 1997, Yakovlev *et al.*, 1997). We examined the effect of z-VAD.FMK, a relatively nonselective inhibitor that blocks both ICE-like and CPP32-like caspases, on cold injury-induced brain trauma in which apoptosis appears to play a role (Tominaga *et al.*, 1992). The vehicle alone or with z-VAD.FMK was intracerebroventricularly administered to mice 15 min before and 24h and 48h after cold injury. At 4h after cold injury, infarction volumes in z-VAD.FMK-treated animals were significantly smaller than infarction volumes in vehicle-treated animals, which were further decreased at 24h and 72h (0.92±1.80 mm^3 ; z-VAD.FMK-treated animals, 7.46±3.53 mm^3 ; vehicle-treated animals, mean ± S.D., n=8). The amount of apoptotic cell death was significantly decreased in z-VAD.FMK-treated animals compared with vehicle-treated animals, as shown by TUNEL staining and DNA gel electrophoresis. Although further investigation is necessary to elucidate mechanisms of ICE inhibitor effects on cold injury-induced brain trauma, these data suggest that ICE inhibitors might be of therapeutic benefit in brain trauma. The ICE family of proteases appears to contribute significantly to cold injury-induced brain trauma. Blocking ICE activity increases neuronal survival by reducing apoptosis. Supported by grants NS14543, NS25372, NS36147 and NOINS82386.

101.7

AXONAL PROTECTION WITH HYPOTHERMIA FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT. H. Koizumi, J.T. Povlishock*. Dept. of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

The protective effects of mild hypothermia following traumatic brain injury (TBI) have been demonstrated in multiple studies within the last decade. However, while this protection has been evaluated in relation to the preservation of neurons and/or the blunting of behavioral abnormalities, little consideration has been given to any potential protection afforded to TBI-induced axonal injury, a known feature of human TBI.

To this end, we evaluated the protective effects of mild hypothermia on axonal injury following TBI in rats. Male Sprague Dawley rats weighing 380-400 grams were subjected to experimental TBI induced by impact acceleration. These rats were also subjected to hypothermia either prior to injury or up to 1 hour postinjury, with their temporalis muscle and rectal temperature maintained at 32°C for an 1 hour period. After this 1 hour period of hypothermia, gradual controlled rewarming to normothermic levels was accomplished over a 90 minute period. Twenty-four hours later, the animals were perfused and semiserial sagittal sections of the brain were reacted for the visualization of the amyloid precursor protein (APP), a known marker of axonal injury. The density of APP/damaged axons within the corticospinal tract at the pontomedullary junction was calculated for each animal.

In all hypothermic animals, a significant reduction in APP/damaged axonal density was found. With pre-iojury, immediate postinjury, and delayed hypothermia, the density of damaged axons was dramatically reduced in comparison to the non-treated controls ($p < 0.05$). These findings indicate that early as well as delayed post-traumatic hypothermia result in considerable protection of those axons injured by the traumatic episode. (supported by NS 20193)

101.8

DNA DAMAGE IS TEMPERATURE DEPENDENT EARLY AFTER TRAUMATIC BRAIN INJURY IN RATS M. Whalen, M. Chen, R. Clark, K. Jin, P. Kochanek, D. Marion, S. Graham. Safar Center for Resuscitation Research and Brain Trauma Research Center, University of Pittsburgh, Pittsburgh, PA 15260

Hypothermia applied before or shortly after traumatic brain injury (TBI) attenuates while hyperthermia exacerbates neurologic damage in experimental TBI (Dietrich et al., 1996). DNA damage occurs in neurons undergoing necrosis and apoptosis after TBI (Clark et al., 1997). One mechanism by which hypothermia might mitigate neurologic injury is suppression of neuronal DNA damage. We hypothesized that neuronal DNA damage after TBI would be temperature-dependent within a clinically relevant range. Anesthetized male adult Sprague-Dawley rats were subjected to controlled cortical impact and maintained at brain temperature 32, 37, or 39°C (± 0.5°C; n=8/group) for 4 h. Coronal (6 μm) cryostat brain sections were then obtained through the center of the contusion. DNA damage was assessed using biotinylated dATP and the Klenow fragment of DNA polymerase I. DNA damage was quantified by light microscopy as the number of positively-labeled cells/100x field in cortex and hippocampal regions. Data are expressed as mean ± SEM. Results were analyzed by ANOVA and Student-Neuman-Keuls test. DNA damage was evident in many cells in the ipsilateral cortex, dentate, and CA3 hippocampus, but was rarely detected in CA1 or the contralateral hemisphere. DNA damage was temperature-dependent in the dentate gyrus (9.8 ± 5.0 vs 31.0 ± 8.3 and 63.6 ± 18.1)(32°C vs 37°C and 39°C, respectively; $p < 0.05$) and CA-3 (4.1 ± 2.1 vs 13.0 ± 2.2)(32°C vs. 39°C; $p < 0.05$), but not in CA-1 or regions of the cortex adjacent to the impact site. DNA damage in regions of hippocampus vulnerable to delayed neuronal death seems to be temperature-dependent early after TBI. One beneficial effect of hypothermia may be inhibition of DNA damage after TBI. Funding: Charles Schertz Fellowship Grant from the Univ. Pitt. Dept. Anesthesiology/CCM, NS30318, KOENS01946

101.9

ALTERED EXPRESSION OF ENDOTHELIN-1 AND THE ENDOTHELIN B RECEPTOR SUBTYPE (ETB) AFTER SPINAL CORD INJURY. J. A. Ellison, A. E. M. Mautes, H. Minehart, R. Willette, and L. J. Noble*. Depts. of Neurosurgery, University of California at San Francisco and Saarland University Medical School, Homburg/Saar, Germany, and Dept. of Cardiovascular Pharmacology, SmithKline Beecham Pharmaceuticals.

Glial activation is a prominent feature of the injured spinal cord. There is increasing evidence that ET-1 may participate in glial activation via the ETB receptor. In this study, we begin to address this putative role of ET-1 in the contused spinal cord of the rat.

At 3 hours to 3 weeks after a moderate spinal cord injury or after sham surgery, a 3 cm length of cord, centered over the impact or sham surgery, was removed and divided into proximal, injury, and distal segments. Sections were prepared for immunolocalization of ET-1 and in situ hybridization analysis of ETB mRNA expression. Levels of mRNA expression were quantitated by optical density analysis of the xray film exposed to slides hybridized with the ETB probe. The data were analyzed using Kruskal-Wallis, followed by Mann-Whitney U.

There is enhanced immunoexpression of ET-1 at all time points and a significant increase in ETB mRNA signal along the axis of the injured cord at 1 to 3 weeks post injury as compared to sham surgery. ET-1 is localized in reactive glia, bordering central and dorsal column cavities, and macrophage-like cells. There is pronounced ETB mRNA in similar phenotypes in the lesion and bordering the penumbra of the injury from 1 to 3 weeks post injury.

The enhanced expression of ET-1 and ETB mRNA in glial and macrophage phenotypes suggest that local ET-1 may influence both glial reactivity and macrophages. Supported by NS23324.

101.10

THE ROLE OF CALPAIN-MEDIATED SPECTRIN PROTEOLYSIS (CMSP) IN TRAUMATICALLY INDUCED AXONAL INJURY (AI). A. Büki[1], J.T. Povlishock[1], R. Siman[2] and C.W. Christman[1]* [1]Dept. of Anatomy, Medical College of Virginia; Virginia Commonwealth University, Richmond, VA 23298-0709, [2]Cephalon, Inc., West Chester, PA 19380

Traumatic brain injury (TBI) has long been associated with generation of AI. Such axons are not mechanically severed at impact, instead showing progressive changes that lead to axonal disconnection. In severely injured axons, it has been shown that the axolemma is perturbed, suggesting the influx of Ca^{2+} and the unleashing of Ca^{2+}-mediated overt proteolytic degradation. Experimental studies, however, have failed to confirm this assumption, suggesting that alterations in axonal permeability trigger more discrete and evolving cytoskeletal changes.

To explore the role of Ca^{2+}-induced proteolysis in AI, this study was undertaken in an animal model of TBI coupled with antibodies targeting both CMSP and focal neurofilament compaction (NFC). Rats were subjected to impact acceleration TBI and allowed to survive for 15 min to 2 h, when the brains were prepared for the visualization of double label reaction products related to the presence of CMSP and NFC. Using LM and EM, these strategies revealed that TBI consistently evoked focal CMSP immunoreactivity (IR). This focal IR was also correlated with concomitant change in the underlying cytoskeleton reflected in NFC. These changes were seen at 15 min postinjury and continued over the entire 2 h observation period. We confirmed these changes at the EM level. At 15 min post injury, IR associated with CMSP was confined primarily to the subaxolemmal network. With increasing survival, its distribution became more widespread moving from the subaxolemmal compartment to fill the axoplasm.

These findings suggest that, in moderate to severe TBI, CMSP occurs and impacts upon concomitant cytoskeletal change. While these studies further implicate Ca^{2+} in the demise of severely injured axons, they do not imply an all or none effect, rather they show evidence for progressive change that may be amenable to rapid therapeutic intervention. This work is supported by grants NS 20193 and The Martin Rodbell Fellowship.

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DNA DAMAGE IS TEMPERATURE DEPENDENT EARLY AFTER TRAUMATIC BRAIN INJURY IN RATS

Miehael Whalen, Minzhi Chen, Robert Clark, Kunlin Jin, Patrick Kochanek, Donald Marion, and Steven Graham. University of Pittsburgh Dept. Anesthesiology/CCM, the Safar Center for Resuscitation Research and Brain Trauma Research Center, University of Pittsburgh, Pittsburgh, PA 15260

Introduction: Hypothermia applied before or shortly after traumatic brain injury (TBI) attenuates while hyperthermia exacerbates neurologic damage in experimental TBI (Dietrich et al., 1996). DNA damage occurs in neurons undergoing necrosis and apoptosis after TBI (Clark et al., 1997). One mechanism by which hypothermia might mitigate neurologic injury is suppression of neuronal DNA damage. We hypothesized that neuronal DNA damage after TBI would be temperature-dependent within a clinically relevant range.

Methods: Anesthetized male adult Sprague-Dawley rats were subjected to controlled cortical impact and maintained at brain temperature 32, 37, or 39°C (± 0.5°C; n=8/group) for 4 h. Rats were saline perfused and coronal (6 µm) brain sections were obtained through the center of the contusion. DNA damage was assessed using biotinylated dATP and the Klenow fragment of DNA polymerase I. DNA damage was quantified by light microscopy as the number of positively-labeled cells/100x field in cortex and hippocampal regions. Data are expressed as mean ± SEM. Results were analyzed by ANOVA and Student-Neuman-Keuls test.

Results: DNA damage in cells was evident in ipsilateral cortex, dentate, and CA3 hippocampus but was rarely detected in CA1 or in the contralateral hemisphere. DNA damage was temperature-dependent in the dentate gyrus (9.8 ± 5.0 vs 31.0 ± 8.3 and 63.6 ± 18.1 cells/100x field)(32°C vs 37°C and 39°C, respectively; $p < 0.05$) and CA-3 (4.1 ± 2.1 vs 13.0 ± 2.2 cells/100x field)(32°C vs. 39°C; $p < 0.05$), but not in CA-1 or peritrauma regions of the cortex.

Conclusions: DNA damage in regions of hippocampus vulnerable to delayed neuronal death appears to be temperature-dependent early after TBI. One beneficial effect of hypothermia may be inhibition of DNA damage after TBI.

Funding: Charles Schertz Fellowship Grant from the Univ. of Pittsburgh Dept. Anesthesiology/CCM, Laerdal Fnd, NS30318, and KO8NS01946.

45

INDUCIBLE 72kd HEAT SHOCK PROTEIN IS INCREASED AFTER TRAUMATIC BRAIN INJURY IN HUMANS: EVIDENCE FOR THE STRESS RESPONSE

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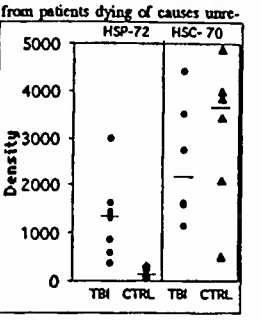
Introduction: Induction of the 72-kDa heat shock protein (hsp-72) is a key event in the stress response. We have shown that hsp72 is increased in neurons after traumatic brain injury (TBI) in rats[1]. We hypothesized that the inducible hsp-72, but not the constitutive heat shocks protein (hsc-70), would be increased in human brain after TBI.

Methods[2]: Brain tissue samples were obtained from adult patients (n=8) undergoing emergent surgical decompression for management of increased intracranial pressure after TBI. All patients had clinical or radiographic evidence of cerebral herniation. Control samples (n=5) were obtained postmortem from patients dying of causes unrelated to CNS trauma. Total protein was extracted and examined with Western blot gel densitometry using monoclonal antibodies to hsp-72 and hsc-70. Immunohistochemistry was also done using the hsp-72 antibody to provide cellular localization of the protein.



Results: The TBI patients had a mean age of 36y, mean GCS of 7 and mean GOS of 3.4. Western blot analysis showed that hsp72 was increased in patients after TBI vs controls (median[range], 1359[372-2986] vs. 131[71-308], P=0.002, Mann-Whitney). In contrast, hsc-70 was not different in TBI vs. controls. (median[range], 2191[1143-4409] vs. 3657[538-4879], P=0.85, Mann-Whitney) Immunohistochemistry showed that cells with increased immunoreactivity included endothelium, glia, and neurons.

Conclusions: Hsp-72, but not hsc-70, is increased after TBI in humans. These data suggest that the stress response is induced locally in injured brain after TBI Further study is needed to completely characterize the stress response after TBI in humans. [1]J Neurotrauma 1998, 15:171-181. [2]Approved by the UPMC Institutional Review Board.

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46

PENTOXIFYLLINE EFFECT ON FUNCTIONAL, BIOCHEMICAL AND HISTOLOGICAL RATINGS AFTER EXPERIMENTAL SPINAL CORD IMPACT

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Introduction: Pentoxifylline (PTX) may increase blood flow, cellular deformability, and decrease platelet aggregation, inflammation and secondary damage after acute spinal cord injury. This study examines the effects of two PTX treatment protocols on functional, biochemical, and histological recovery after acute spinal cord impact injury in an anesthetized rat model.

Methods: 60 Hooded Long-Evans pentobarbital anesthetized rats were injured by standardized 10gX25mm weigbt-drop method onto exposed spinal cord (T10). Treatment was randomized, blinded and given in equal diluent volumes at 5, 30, and 60 min after impact: Group I: Saline; Group II: PTX 10 mg/kg, 5 mg/kg, 5 mg/kg; and Group III: PTX 25 mg/kg, 15 mg/kg, 15 mg/kg. Blinded to group, 3 scales (BBB[1], Tarlov[2], Rivlin-Tator Angleboard[3]) were serially applied to assess motor function for 28 days. At autopsy, spinal cord segments above, at and below impact site were analyzed for biochemical markers of injury (serotonin to metabolite ratio) and by 3 histologic scales (qualitative, lesion size, and myelin density). Analysis was by ANOVA and RM-ANOVA, with appropriate after testing.

Results: At baseline, the 3 groups were equivalent. At 30 and 60 min after impact injury and blinded treatment, group I had significantly higher mean HR and BP than both PTX groups ($p<.02$). All groups showed improvement in motor function over 28 days ($p<.01$), with no significant differences among groups ($p>.05$). Biochemical analysis showed the highest serotonin and metabolite levels at the impact site, with no significant serotonin/metabolite ratio difference among groups ($p>.05$). Histological evaluation confirmed comparable injury at impact sites, with proximal sections least affected for all groups ($p<.05$). Further qualitative assessment blinded to group showed significant changes in histology above vs below impact aite in Group I control rats, but not in Group II or III PTX treated rats.

Conclusions: Subtle but significant patterns of medication treatment effect can be reliably tracked by these functional, biochemical, and histological outcome measures in this rat model of acute spinal cord impact injury. Lack of dramatic functional or biochemical correlates of histologic improvement suggest that alternative PTX dose or treatment duration may be required if PTX post-injury treatment is to be effective.

Refs: [1]Basso et al. J Neurotrauma 1995. [2]Tarlov. Arch Neurol Psych 1954. [3]Rivlin et al. J Neurosurgery 1977.

47

PRELIMINARY RESULTS ON THE IMPACT OF APOE GENOTYPES ON CEREBROSPINAL FLUID (CSF) EXCITATORY AMINO ACIDS (EAA) AND METABOLITES IN TRAUMATIC BRAIN INJURED (TBI) ADULTS

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Introduction: Apolipoprotein E (APOE) genotype has been linked to beta amyloid deposits and a microtubule-associated protein, tau, in an isoform-specific manner. APOE4 may impair neuronal growth potentially impairing recovery following injury.[1] Wilson et al[2] reported that patients with APOE4 alleles had poorer verbal memory and psychomotor slowing following injury when compared to patients without APOE4 alleles. The purpose of this project was to determine whether the trajectory of EAA and lactate/pyruvate ratio was altered based on APOE-genotype following a TBI.

Methods: The APOE genotypes were identified in 24 adults by genomic DNA amplification followed by digestion with HhaI restriction enzyme. Serial CSF samples from a ventriclostomy catheter placed within the right ventricle were removed by gravity drainage every 12 hours for the first 72 hours after injury. Samples were immediately placed into a freezer at -80 degrees C for storage. Aspartate and glutamate were measured using high-pressure liquid chromatography (HPLC) with fluorescence detection. Lactate and pyruvate were measured using ultraviolet detection.

Results: Of the 24 subjects, 6 had APOE 2/3 genotype, 12 had APOE 3/3 genotype and 6 had APOE 3/4 genotype. Aspartate ranged from .06 to 3.34 (M=.6083; SD=.59); glutamate ranged from 71.1 to 722.8 (M=158.36; SD=119.6) and the L/P ratio ranged from 8.22 to 75.62 (M=28.19; SD=12.8). Patients were grouped based on the presence or absence of APOE4 allele. Using a repeated measure analysis of variance, significant differences existed across time in aspartate, glutamate and L/P ratio. Aspartate and glutamate levels were elevated in patients carrying the APOE4 allele.

Conclusions: The results of this study suggest that the presence of the APOE4 allele is related to enhanced levels of EAA (glutamate and asparate) which contribute to secondary damage following TBI. The mechanism of this relationship is not known and warrants further study.

1. Mahley RW et al. (1996). Annals of the New York Academy of Scienees. 777:139-45.
2. Wilson J et al., (1998). Journal of Neurotrauma 15(1):80.

E48

MK-801 improves functional outcome in rats after controlled cortical impact. RA Ruppel, PM Kochanek, CE Dixon, HL Alexander, SH Graham, RSB Clark, SR Wisniewski, DW Marion, PJ Safar. Safar Center for Resuscitation Research, Univ of Pitt, Pgh, PA

Excitotoxicity is implicated as a key mechanism of secondary neuronal damage after traumatic brain injury (TBI). The NMDA receptor antagonist MK-801 has been shown to attenuate cerebral injury in focal ischemia and some models of TBI, but it has not been tested in controlled cortical impact (CCI). We hypothesized that MK-801 would improve functional and histopathologic outcomes in rats following CCI. Anesthetized Sprague-Dawley rats (n=8/grp) were subjected to CCI (4 m/s, 2.5 mm depth), then randomized to immediate treatment with either MK-801 (1 mg/kg IP) or vehicle. Rats treated with MK-801 recovered motor function significantly earlier than vehicle controls, as shown by beam balance/walking performance (d 1-5). MK-801 treated rats also showed improvement in the probe trial of the Morris water maze (d 14-20) vs vehicle (p<0.05), but no differences were seen in latencies to target. Contusion volume and hippocampal cell counts (d 21) did not differ between the groups. These data demonstrate an important role for excitotoxicity early after cerebral contusion and support continued evaluation of anti-excitotoxic therapies for use in TBI.

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E49

PROTECTION WITH MK-801 AGAINST SUSCEPTIBILITY OF MICE EXPRESSING HUMAN APOLIPOPROTEIN E4 TO CA1 NEURONAL INJURY FROM TRAUMA AND OXIDATIVE STRESS. R.A. Wallis[1,3*], K.L.Panizzon,[1,3] B. Teter,[2,3] G.M. Cole,[1,2,3] S.A. Frautschy,[1,2,3] J.R. Gilbert[4]. [1]Dept. of Neuro., UCLA; [2]Dept. of Geri., UCLA, LA, CA 90024; [3] Sepulveda VAMC, Sepulveda, CA 91343; [4]Dept. of Neuro., Duke University, NC 27710.

Susceptibility of CA1 neurons to trauma and oxidative stress was assessed in transgenic mice expressing the human apolipoprotein E4 gene and promoter in the ApoE knockout background. Mild trauma to hippocampal slices from ApoE knockout mice produced virtually full recovery of mean orthodromic (ortho.) and antidromic (anti.) population spike (PS) amplitude recovery of 94% ± 2 and 95% ± 1 one hr after trauma, while slices from ApoE4 mice showed ortho. and anti. PS recoveries of CA1 of 16% ± 5 and 14% ± 3 *(p<0.05)*. MK-801 32 μM treatment reversed ApoE4 susceptibility with CA1 ortho. and anti. PS recoveries of 66% ± 5 and 64% ± 3 *(p<0.05)*. Oxidative stress (H_2O_2 5 μM, 6 min), to ApoE knockout mouse slices showed ortho. and anti. PS recovery after 1 hr of 89% ± 2 and 91% ± 2, while slices from ApoE4 mice showed ortho. and anti. PS recoveries of 23% ± 2 and 19% ± 2 *(p<0.05)*. MK-801 reversed the susceptibility of ApoE4 mice to oxidative stress with ortho. and anti. PS recoveries of 83% ± 2 and 79% ± 2 *(p<0.05)*. These findings suggest that the ApoE4 gene increases susceptibility of CA1 neurons to trauma and oxidative stress through excitotoxic mechanisms. Supported by the VA Research Service.

E50

TREATMENT OF SPINAL CORD INJURY WITH GK-11. Gaetano Menna, Wencheng Huang and Wise Young. Neuroscience Center, Rutgers, The State University of New Jersey, 604 Allison Rd, Piscataway, New Jersey 08854.

GK-11 is a long-lasting blocker of NMDA receptors. Several studies have reported that NMDA receptor blockers reduce tissue damage. We assessed the effects of GK-11 on a well-standardized rat spinal cord contusion model, comparing 2.7 mg/kg, 0.9 mg/kg, and 0.3 mg/kg doses and vehicle started at 15 minutes or 60 minutes after 12.5 mm or 25.0 mm contusions with the NYU weight drop impactor. A total of 134 adult Long-Evan's hooded rats were studied in this study. The rats were anesthetized with intraperitoneal pentobarbital (male 60 mg/kg and female 45 mg/kg) and injured at T9-10 cord exposed with laminectomy. At 24 hours after injury, the spinal cords were rapidly removed and frozen. Six cord samples were removed from each rat and each sample was approximately 5 mm in length. To quantify tissue damage, we measured spinal cord lesion volumes, cell volume fractions (CVF), tissue Na , K concentrations and edema at and around the impact site. The results show that GK11 did not have any beneficial effects in tissue Na, K, water concentrations, cell volume fractions and lesion volumes compared to vehicle groups (p>0.05). The analyses also rule out a possible effect of GK-11 only on the surrounding cord because repeated measures analyses did not reveal any consistent treatment-related Na, K, CVF or lesion volume differences at specific sample sites. We therefore conclude that GK-11 in the dose ranges of 0.3-2.7 mg/kg given at 15 and 60 minutes after injury does not alter the cell loss in spinal cords contusion injury in the presence of pentobarbital anesthesia.

E51

GROUP I METABOTROPIC GLUTAMATE ANTAGONIST REDUCES NEURONAL DEGENERATION AND BEHAVIORAL DEFICITS AFTER RAT FLUID PERCUSSION INJURY. Q-Z. Gong*, S.D. Shields, S. Murphy, R.F. Berman, J.P. Muizelaar, B.G. Lyeth. Dept. Neurological Surgery, U.C. Davis, Davis, CA 95616, USA.

Acute activation of Group I metabotropic glutamate receptors (mGluRs) contributes to traumatic brain injury (TBI) pathophysiology (J Neurosci 16:6012, 1996). We infused over 1 hr, the selective mGluR Group I antagonist, (RS)-1-aminoindan-1,5- dicarboxylic acid (AIDA) (0, .4, 2, 10 nmol) (n=6/group) into the hippocampus beginning at 5 min after parasagittal fluid percussion TBI. At 24 hrs after TBI, coronal sections were stained with Fluoro-Jade, a fluorescent marker for neuronal degeneration. Positive staining cells were counted in 4 sections/rat. Significantly fewer (p<0.05) CA2-3 Fluoro-Jade positive neurons were detected in the 10 nmol AIDA group (184 ±32) compared to the vehicle group (310 ±47). In a second experiment, rats were administered 10 nmol AIDA or vehicle (n=10/group) after TBI as above and tested in the Morris water maze for acquisition of a spatial learning/memory task on days 11-15 post-injury. The mean swim distance for the 10 nmol AIDA-treated group (1973 ±146cm) was significantly (p<0.04) shorter than vehicle controls (1493 ±142cm). Post-injury blockade of Group I mGluRs appears to reduce neuronal degeneration and improve functional outcome after TBI. Supported by NIH NS29995

216.1

DELAYED 45-CALCIUM ACCUMULATION FOLLOWING TRAUMATIC BRAIN INJURY IS AGE-DEPENDENT AND REFLECTS SECONDARY CELL DEATH IN THE THALAMUS

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Calcium flux is considered an important factor in the pathophysiology of traumatic brain injury. Our previous work has shown diffuse ^{45}calcium ($^{45}Ca^{++}$) accumulation in the cortex immediately after lateral fluid percussion (LFP) injury lasting at least 2 days in P17, P28, and adult rats. In addition to this first, acute period of isotope accumulation these studies suggested a second, delayed period of $^{45}Ca^{++}$ accumulation in the thalamus. To determine the developmental profile of this delayed $^{45}Ca^{++}$ accumulation 36 P17, 34 P28, and 17 adult rats were anesthetized and subjected to a moderate-severe (~2.75 atm) LFP. Immediately, 6 hours, 1 day, 2 days, 4 days, 7 days, and 14 days after injury the rats were injected with $^{45}Ca^{++}$ (1µg/g, i.v.) and processed for autoradiography. Optical densities were measured in 16 regions of interest, including 6 thalamic nuclei. $^{45}Ca^{++}$ accumulation was evident within the ipsilateral cerebral cortex immediately after injury (~40% increase), returning to sham levels within 4 days in all three age groups ($p<0.03$). In contrast, differences existed between the age groups for delayed $^{45}Ca^{++}$ accumulation in the thalamus. While P17s showed no delayed $^{45}Ca^{++}$ accumulation, P28 and adult rats showed significant accumulation in the thalamus starting at 2 days and increasing out to 14 days (~50% increase, $p<0.03$). Since histological analysis indicated cell death at the time of the delayed $^{45}Ca^{++}$ accumulation, this delayed accumulation could represent retrograde degeneration due to diffuse axonal injury and/or Ca^{++}-induced apoptosis. The age-dependency of delayed $^{45}Ca^{++}$ accumulation in the thalamus may be attributed to differential biomechanical consequences of the LFP and/or greater calcium buffering capacities of younger animals. The results suggest that two temporal patterns of $^{45}Ca^{++}$ accumulation exist following LFP: acute calcium flux associated with the injury-induced ionic cascade and delayed calcium accumulation associated with secondary cell death. Supported by NS30308, NS27544.

216.2

NMDA antagonists enhance delayed neurodegeneration in the hippocampus following traumatic brain injury.

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Traumatic cortical injury in adult rats causes two types of lesions, an acute local lesion within the cortex and a distant lesion in the ipsilateral CA3 hippocampus which evolves in a delayed fashion. The effect of pre- and posttreatment with the NMDA antagonist CPP on delayed neurodegeneration in the hippocampus was analysed by means of stereological morphometry. Male Fisher 344 rats were anesthetized with tribromoethanol, placed in a stereotaxic apparatus and a craniotomy was performed over the right sensorimotor cortex. A force of 380gxcm produced by a 20g falling weight was selected to produce brain contusion. Rats were sacrificed by perfusion fixation at 3 days after trauma. Pretreatment with CPP (30 mg/kg i.p. at 2,1 and 0 hrs prior to trauma) resulted in amelioration of hippocampal neuronal loss within the ipsilateral CA3 subfield. When treatment with the same dose-regimen was initiated at 1 hr after trauma no effect on numbers of hippocampal CA3 pyramids was evident, whereas treatment with CPP that was initiated at 4 or 7 hrs after trauma resulted in significantly greater neuronal cell loss compared to vehicle treated rats. CPP demonstrated no effect on neuronal densities and total cell numbers within the ipsilateral CA3 subfield when treatment was initiated at 10 hrs after trauma. These observations reveal that during a critical period following traumatic brain injury, NMDA antagonists enhance slow neurodegeneration and can therefore have neurodestructive properties. Supported by BMBF grant 01KO9515ITPA3.

216.3

IS GUANOSINE BEING MISTAKEN FOR THE PEROXYNITRITE MARKER 3-NITROTYROSINE IN MODELS OF CNS INJURY?

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A marker used to identify peroxynitrite activity following CNS injury is the 3-nitrotyrosyl residue of proteins. Recently, a number of studies have purported measurement of 3-nitrotyrosine (3-NT) in brain protein digest by HPLC. These assays vary substantially in processing, chromatographic and detection methodologies. Halliwell and collaborators (J. Neurochem. 70:2220-2223, 1998) reported measurement of an artifactual substance in brain tissue which exhibited chromatographic, electrochemical and chemical properties nearly identical to 3-NT. It was suggested that this artifact might confound the detection of 3-NT in brain tissue. We have developed an HPLC assay for the measurement of 3-NT that circumvents the problem of artifact detection. This was accomplished by using gradient elution, ion pairing and multi-channel electrochemical detection. Using this technology, we were able to measure, in injured brain protein digests, 3-NT as a percentage of tyrosine (3-NT/TYR) at levels much lower (0.004%) than purported (J. Cereb. Blood Flow Metab. 18:123-129, 1998). In fact, at 24 hrs, after impact-acceleration head injury in rat, hippocampal 3-NT/TYR was small did not differ from sham animals. However, in the same model, another peak that eluted very close to 3-NT increased significantly after injury. This same peak was found to increase in microdialysate with rat head injury as well, with no apparent change in 3-NT. The former response was blocked by L-NAME, the non-selective inhibitor of nitric oxide synthase. We suggest that this may be the same artifact reported by Halliwell and collaborators. Isolation of this peak material followed by LC-MS, LC-NMR, LC-EC and LC-UV confirms the identity as guanosine. We recommend including guanosine as an HPLC standard to avoid misidentification with 3-NT. (Supported by Warner-Lambert/Parke-Davis)

216.4

CHANGES IN CORTICAL ENERGY METABOLISM AFTER CLOSED HEAD INJURY IN THE MOUSE.

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The induction of cytokines had been demonstrated after acute stroke and trauma. Temporal pattern of changes in energy balance has been closely linked to cytokine expression. To investigate a possible correlation between cytokine production and energy metabolism after closed head injury (CHI), the present study was designed in a mouse model of CHI. Injury was performed using a weight drop device (Chen et al., 1996) and brains were frozen 4-24h after sham surgery and at 5 min, 4, 12 and 24h (n=4/group) following CHI. ATP, glucose and lactate contents were determined by computer-assisted bioluminescence imaging in serial tissue sections. Results: i) ATP content was significantly decreased at 4, 12 and 24h on the injured hemisphere as compared to sham. On the contralateral side ATP did not change in comparison to sham. ii) glucose content ipsilaterally significantly decreased at 5min, and remained lower up to 24h. Contralateral glucose content did not change significantly as compared to sham. iii) no changes in lactate could be discerned. Conclusions: CHI in the mouse lead to ipsilateral cortical energy depression. This may be related to the early production of harmful mediators such as cytokines, reactive oxygen species or vasoactive neurotransmitters that locally impair the blood supply.

216.5

ADENOVIRUS-MEDIATED TRANSFER AND EXPRESSION OF β-GAL IN INJURED HIPPOCAMPUS IS NOT INHIBITED AFTER TRAUMATIC BRAIN INJURY IN MICE.

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In models of focal cerebral ischemia, adenoviral (Ad) gene transfer is attenuated or markedly delayed vs naïve[1]. After controlled cortical impact (CCI)-induced traumatic brain injury (TBI) in mice, CA1 and CA3 hippocampus both exhibit delayed neuronal death, with DNA damage at 24h, morphological loss of CA3 by 72h, and complete loss of hippocampal parenchyma by 21d.[2] We hypothesized that Ad-mediated expression of β-Gal in hippocampus would be attenuated after CCI in mice.

Isoflurane anesthetized C57BL6 mice (n=16) were subjected to either CCI to left parietal cortex or sham injury (burr hole). Ad-CMV-β-Gal (10^9 PFU/ml) was then immediately injected into left dorsal hippocampus. At 24 or 72h, mice were saline perfused and β-Gal expression was quantified (mU/mg protein). Separate mice (n=8) were used to examine the distribution of β-Gal staining in vibratome sections.

Robust β-Gal expression in left hippocampus was detected in sham and was similar at 24h (48.4±4.1) vs 72h (68.8±8.8, NS). CCI did not reduce β-Gal expression in ipsilateral hippocampus (68.8±8.8 vs 88.1±7.0 at 72h, sham vs CCI, NS); but, CCI reduced β-Gal expression in contralateral hippocampus (14.2±3.9 vs 2.5±0.2 at 72h $p < 0.05$ sham vs CCI). β-Gal was seen in many cell types in ipsilateral hippocampus. Contralateral expression was restricted to periventricular cells and CA3 neurons.

Despite the eventual nearly complete loss of ipsilateral hippocampus by 21 d in this model, Ad-mediated gene transfer is robust in this structure early after TBI. This supports the use of this approach to test novel genes targeting hippocampal neuronal death in this model. Inhibition of gene expression in contralateral hippocampus by injury may reflect reduced CSF circulation or failure of axonal transfer of Ad after CCI. [1]*Abe et al, 1997.* [2]*Whalen et al, 1999.* Support: NS30318 and GM44100

216.6

FENTANYL VERSUS ISOFLURANE ANESTHESIA: EFFECT ON OUTCOME AFTER TRAUMATIC BRAIN INJURY IN RATS.

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Despite the routine use of fentanyl for initial sedation of patients after severe traumatic brain injury (TBI), it remains to be determined if it is the optimal sedative agent. Isoflurane is the most commonly used anesthetic in experimental models of TBI. Recent studies in experimental cerebral ischemia suggest that isoflurane is neuroprotective (vs fentanyl) in part by increasing cerebral blood flow (CBF) and reducing metabolic demands. To our knowledge, fentanyl has not been directly compared to isoflurane in experimental TBI. We hypothesized that isoflurane would be neuroprotective vs fentanyl when administered early after TBI in rats.

Male Sprague-Dawley rats (n=9/group) were subjected to controlled cortical impact to the left parietal cortex and randomized to receive either fentanyl (10 mcg/kg bolus followed by a 25 mcg/kg/h iv infusion) or isoflurane (1% by inhalation) for 4 h. Motor (beam balance, beam walking, d 1-5) and cognitive (Morris water maze performance, d 14-20) function were used to assess functional outcome and rats were perfused for the assessment of lesion and hippocampal volumes on d 21.

Rats treated with isoflurane had markedly better motor and cognitive function vs those treated with fentanyl (both $p < 0.05$ on multiple days); although, there were no differences in either contusion or hippocampal volumes between treatment groups. We speculate that the increase in CBF in concert with metabolic suppression produced by isoflurane may be neuroprotective after TBI. Therefore the use of isoflurane may mask the beneficial effects of novel treatments tested in experimental models of TBI. In addition, fentanyl may not represent the optimal sedative agent, and may even be detrimental in the acute phase after severe TBI. Support: USArmy#DAMD17-97-1-7009

B6

FENTANYL VERSUS ISOFLURANE ANESTHESIA: EFFECT ON OUTCOME AFTER TRAUMATIC BRAIN INJURY IN RATS. K.D. Statler, P.M. Kochanek, C.E. Dixon, H. Alexander, D.S. Warner, R.S.B. Clark, S. Wisniewski, S.H. Graham, L.W. Jenkins, X. Ma, D.W. Marion, P. Safar. Safar Center for Resuscitation Research, Univ. of Pittsburgh, Pgh, PA, 15260 and Duke Univ., Raleigh Durham, NC, 27710.

Despite routine use of fentanyl in patients after traumatic brain injury (TBI), it is unclear if it is the optimal analgesic. Isoflurane is routinely used in TBI models. Studies in cold lesion and ischemia suggest isoflurane is neuroprotective vs. fentanyl. To our knowledge, fentanyl and isoflurane have not been compared in TBI. We hypothesize that isoflurane is neuroprotective vs. fentanyl early after TBI. Male Sprague-Dawley rats (n=18) underwent controlled cortical impact and received 4 h of fentanyl (10 mcg/kg bolus, 50 mcg/kg/h infusion) or isoflurane (1% inhalation). Functional outcome (beam balance, beam walking and Morris water maze [MWM] tasks) and lesion volumes were assessed. Motor and MWM performances were better in rats treated with isoflurane vs. fentanyl ($p<0.05$). Lesion volumes were not different between groups. We speculate that isoflurane may be neuroprotective after TBI by increasing CBF, suppressing metabolism, and/or modulating excitotoxicity. Isoflurane may mask beneficial effects of novel treatments in experimental TBI. Finally, fentanyl may not be the optimal analgesic agent early after TBI in humans. Support: USArmy#DAMD17-97-1-7009

B7

INFLUENCE OF POST-TRAUMATIC HYPOXIA ON BEHAVIORAL AND HISTOPATHOLOGICAL OUTCOME FOLLOWING MODERATE SPINAL CORD INJURY IN RATS. Y. Yanagawa*, A. Marcillo, R. Garcia, K. Loor, W. D. Dietrich, Department of Neurological Surgery and The Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL

Pulmonary dysfunction leading to secondary hypoxia is a common complication of spinal cord injury (SCI). The purpose of this study was to investigate the consequences of an induced post-traumatic hypoxic event following SCI. Forty-five female Sprague-Dawley rats were randomly assigned to 1 of 4 groups, including 1) laminectomy and normoxia, 2) laminectomy and hypoxia, 3) NYU weight-drop and normoxia, and 4) NYU weight-drop and hypoxia. For these studies, a moderate injury was induced by adjusting the height of the weight-drop (10 gm) to 12.5 mm above the exposed spinal cord (T10). Immediately after injury, PaO_2 in hypoxic rats was kept between 30-35 mmHg for 30 min, with 56% nitrous oxide, 31% nitrogen, and 13% oxygen. PaO_2 in the normoxic group was maintained over 100 mmHg, while $PaCO_2$ in all rats was maintained at 35-40 mmHg. The behavior of the rats was checked every 7 days using the BBB locomotor rating scale. Rats were sacrificed at 8 wks after behavioral testing and perfusion fixed for quantitative histopathological analysis of lesion areas. Although post-traumatic hypoxia tended to improve BBB scores, no significant difference in locomotor performance was demonstrated between the traumatized groups. In contrast, the percent of gray matter sparing at the impact epicenter was significantly reduced in hypoxic vs. normoxic SCI rats ($p<0.05$). These studies demonstrate that although moderate hypoxia following SCI does not significantly affect locomotive recovery, this secondary insult worsens gray matter pathology.

B8

SECONDARY HYPOXIA 24 HOURS AFTER CONTROLLED CORTICAL IMPACT INCREASES INJURY IN CEREBRAL CORTEX BUT NOT HIPPOCAMPUS IN THE MOUSE. E. A. Welsh*, J. Keller, R. Raghupathi, G.P. Sinson, T.K. McIntosh. Dept. of Neurosurg., Univ. Penn. Sch. Med., Philadelphia, PA 19104.

The objective of this study was to determine whether an episode of hypoxia 24 hr after brain trauma augments histologic injury. Male C57BL/6 mice (n=10) were subjected to controlled impact injury using a deformation depth of 1 mm and impact velocity of 5 m/sec. After recovery for 24 hr, hypoxia was produced by lowering the percentage O_2 to 9% for 5 min and 7% for an additional 30 min. After an additional recovery period of 5 days, the animals were perfusion-fixed with FAM, and the brains were embedded in paraffin, sectioned, and stained with acid fuchsin/thionin. The stained sections were examined for histologic alteration and the volume of cortical infarction was measured.

Histopathologic alteration was not detected in any region of the contralateral hemisphere. Hypoxia significantly increased the size of the cortical lesion: Sham-hypoxia = 1.95 ± 0.42 mm^3 (mean $\pm$ SD, n=5) vs. Hypoxia = 3.15 ± 0.48 ($p<0.01$). The only other histologic alteration detected was in the dentate granule cell layer of the ipsilateral hippocampus. There was both loss of neurons and acidophilic transformation of neurons in this layer. However, the number of acidophilic dentate granule cells was not altered by hypoxia (Sham-hypoxia = 74 ± 4 vs. Hypoxia = 70 ± 19). These results indicate that the traumatized cortex remains vulnerable for 24 hr to a level of hypoxia which does not cause histologic injury in the contralateral hemisphere.

Supported by NIH Grant NS-08803.

B9

HYPOXIA EXACERBATES CA3 HIPPOCAMPAL NEURONAL DAMAGE AFTER FLUID PERCUSSION BRAIN INJURY IN RATS. Namiko Nomura*, Kojiro Wada, Yoshitaro Matsushita, Hiroshi Nawashiro, Katsuji Shima Dept. of Neurosurgery, National Defense Medical College, Tokorozawa, Saitama, Japan

We have reported that increased vulnerability of hippocampal CA3 neurons to hypoxia after mild concussion. The present study was designed to determine if a model of moderate fluid-percussion (F-P) brain injury with hypoxia exacerbates hippocampal CA3 lesions, if those lesions are associated with apoptosis using the terminal deoxynucleotidyl transferase-mediated biotin-dUTP nick-end labeling method (TUNEL). Anesthetized Sprague-Dawley rats were injured with a moderate severity fluid percussion pulse (3.5-4.0 atmospheres) administered over the right parietal cortex. The experimental animals were divided into 2 groups, traumatic brain injury (TBI) group (n=6), which was subjected to TBI alone, and TBI + hypoxia group (n=6), which was subjected to TBI followed by 20 min of moderate hypoxia (F_iO_2: 10%). Three days following TBI, % neuronal density per 1-mm length of CA3 neurons in the ipsilateral hippocampus was significantly decreased in the TBI + hypoxia group (45.2 $\pm$ 29.6 %; $p < 0.05$) compared to the TBI alone group (90.8 $\pm$ 24.1 %). No significant difference in the number of TUNEL positive cells was observed at 6-h, 24-h and 3-day (n=2) in both groups. These results suggest that TBI with moderate hypoxia induced more hippocampal damage due to not only apoptosis but also necrosis.

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SOLUBLE FAS IS INCREASED IN CSF FROM INFANTS AND CHILDREN AFTER HEAD INJURY

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Introduction: Fas, a member of the TNF-receptor family, and its ligand FasL, provide a system for regulating intercellular programmed-cell death (PCD), where binding of FasL to Fas receptor triggers apoptosis. Fas has been identified on neurons and astrocytes, and FasL is present on microglia and inflammatory cells, thus, PCD in brain after injury may be regulated in part by Fas-FasL interactions. Accordingly, we examined CSF from infants and children after traumatic brain injury (TBI) for alterations in Fas and Fas-L. ***Methods:*** CSF was obtained from 20 patients with severe TBI who required neurointensive care including intraventricular catheter placement. Samples (n = 68) were collected on d 1 - 10 and were immediately centrifuged to remove cells. Control CSF was obtained from 14 children without TBI or meningitis. Fas and FasL were measured by ELISA. ***Results:*** TBI patients ranged in age from 1 mo - 11 y, 18 survived and 2 died. CSF Fas was increased 3-fold in TBI patients vs control (see table). Post hoc analysis also revealed an association between Fas and age ($p = 0.05$), and suspected child abuse. ***Conclusions:*** These data suggest that TBI induces alterations in the Fas/FasL system. Additional patients and multivariate analysis are required to further define associations between Fas and age and suspected child abuse. Therapies targeting cell death receptors, such as Fas, may represent effective strategies aimed at reducing PCD after TBI. **Support:** RO1 NS38620, KO8 NS01946, & P50 NS30318

Group	Fas (mU/ml)	FasL (pg/ml)
Control (n = 14)	269 ± 59	88 ± 14
TBI (n = 20)	759 ± 90*	163 ± 42
Accidental TBI (n = 16)	660 ± 164	168 ± 48
Suspected Child Abuse (n = 4)	1230 ± 94†	146 ± 93

*mean ± SEM, $p < 0.01$ vs control. † $p = 0.05$ vs accidental.

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INCREASED ADENOSINE CONCENTRATION IN CEREBROSPINAL FLUID AFTER SEVERE TRAUMATIC BRAIN INJURY IN INFANTS AND CHILDREN: ASSOCIATION WITH SEVERITY OF INJURY

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Introduction: Traumatic brain injury (TBI) in children results in a myriad of pathophysiologic derangements that contribute to secondary injury, including hypoperfusion, energy failure and excitotoxicity. In addition, a number of endogenous neuroprotectants are produced after TBI, including adenosine, which increases cerebral blood flow and reduces metobolic demands[1,2]. In a prior evaluation of cerebrospinal fluid (CSF) of children following severe TBI[3], we demonstrated increased peak levels of adenosine after TBI. In the current study, we evaluate the CSF of an expanded sample of infants and children following severe TBI, and examine the contribution of age, GCS, mechanism of injury and time after injury to CSF adenosine levels. **Methods:** Samples (n=304) of ventricular CSF were collected from 27 infants and children (2 mo to 14 y) during the first 7 d after severe TBI (GCS <8). Control CSF samples (n=21) were obtained from infants and children without TBI or meningitis. Adenosine was measured using HPLC. **Reaults:** Mean adenosine level was markedly increased in CSF of children following TBI vs control (peak 33.5 ± 9.5 and mean 24.3 ± 9.5 vs control mean 3.8 ± 0.5 nmol/L, $p<0.001$). Using a multiple regression model, the increase in CSF adenosine was independently associated with GCS ≤4 vs >4 and time after injury (both $p<0.05$). However, increased adenosine was not independently associated with mechanism of injury (abuse vs other) or age (≤4 vs > 4y). **Conclusions:** We conclude CSF adenosine concentration is increased in infants and children after severe TBI. This increase was especially pronounced in children with the most severe injuries. Unlike mediators of secondary damage, such as glutamate[4], adenosine was not associated with child abuse or age ≤4 y. We speculate that adenosine may play an important role in endogenous attempts at neuroprotection after TBI. [1]Cerebrovasc Brain Metab Rev 1:26, 1989; [2]J Cereb Blood Flow Metab 14:853, 1994; [3]Crit Care Med 24:A136, 1996; [4]Pedatrics 102:704, 1998 **Acknowledgment:** Univ of Pittsburgh Center for Injury Research and Control/CDC, Laerdal Foundation, and NS 38087

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SYSTEMIC TREATMENT WITH A PAN-CASPASE INHIBITOR IMPROVES HIPPOCAMPAL NEURON SURVIVAL AFTER TRAUMATIC BRAIN INJURY IN MICE

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Introduction: Traumatic brain injury (TBI) produces cell death both immediately after injury as a result of direct mechanical disruption, and in a delayed fashion as a result of secondary injury. Programmed cell death (PCD), or apoptosis, contributes to secondary cell death. The caspase family of cysteine proteases serve as effectors and executioners of PCD, and caspase inhibitors reduce cell death *in vitro* and *in vivo*. We hypothesized that systemic administration of the pan-caspase inhibitor boc-aspartyl(OMe)-fluoro-methylketone (BAF) would reduce hippocampal cell death after controlled cortical impact (CCI) in mice. ***Methods:*** Anesthetized mice were subjected to severe CCI to the left parietal cortex. Immediately after CCI mice were given 100 nmol BAF or vehicle (DMSO) i.p. in a randomized fashion. In one squadron of mice Caspase-3 activity was measured in injured brain at 24 h (n = 3-4/group). Separate mice underwent motor function tests (beam and round tube balance) at baseline and 24 h after CCI, then were killed for assessment of hippocampal neuron survival and DNA fragmentation using TUNEL (n = 5/group). ***Results:*** BAF treatment prevented the increase in relative caspase activity typically produced by CCI vs vehicle (85 vs 174% of uninjured hemisphere, $p = 0.04$). The number of surviving CA1 hippocampal neurons, cells vulnerable to PCD in this model, were increased in BAF treated mice vs vehicle (247 ± 28 vs 149 ± 13, $p = 0.02$). TUNEL-positive cells in hippocampus were similar between groups. Motor function was worse in BAF treated mice vs vehicle ($p < 0.05$). ***Conclusions:*** Pan-caspase inhibition using systemic treatment with BAF completely inhibits caspase-3 activity and enhances survival of vulnerable neurons 24 h after TBI in mice. Surpringly, motor function at 24 h after TBI is worsened. This may be due to preservation of dysfunctional neurons, or nonspecific effects of BAF. Additional studies evaluating the long-term effects of pan-caspase inhibition after TBI are ongoing. Further investigation to determine the optimal treatment paradigm targeting caspase inhibition after TBI is warranted. **Support:** RO1 NS38620 and P50 NS30318.

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ISOFLURANE IMPROVES LONG-TERM NEUROLOGIC OUTCOME COMPARED TO FENTANYL AFTER TRAUMATIC BRAIN INJURY IN RATS

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Introduction: Despite routine use of fentanyl in patients after traumatic brain injury (TBI), it is unclear if it is the optimal sedative/analgesic agent. Isoflurane is commonly used in models of TBI. Recent studies in cerebral ischemia and focal cryogenic lesion suggest that isoflurane may be neuroprotective vs fentanyl.[1,2] To our knowledge, fentanyl and isoflurane have not been directly compared in TBI. We hypothesized that isoflurane would be neuroprotective vs fentanyl when given early after TBI in rats. **Methods:** Adult rats (n=18) underwent controlled cortical impact (CCI) with physiologic monitoring and then received 4h of N_2O:O_2 (2:1) and either fentanyl (10 mcg/kg bolus, 50 mcg/kg/h infusion) or isoflurane (1% inhalation). Shams (n=8) underwent identical preparation and anesthesia but no CCI. Functional outcome (beam balance, beam walking, Morris water maze [MWM] tasks) was assessed over 20d in injured and sham rats. Lesion volume was quantified on d21. Additional rats (n=14) underwent CCI and anesthesia as described above with intracranial pressure (ICP) monitoring (Codman intraparenchymal transducer) for 4h. Brain water (wet-dry weight method) was assessed at the end of the anesthetic period. **Results:** After injury, motor and MWM performances were better in isoflurane vs fentanyl treated rats ($p<0.05$, ANOVA) but did not differ between shams. Lesion volumes were similar between groups. There was increased frequency of ICP>20 mm Hg and higher brain water in rats treated with isoflurane vs fentanyl ($p<0.05$, ANOVA). **Conclusions:** Rats treated with isoflurane had improved long-term functional outcome after CCI compared to those treated with fentanyl, despite increases in ICP and brain water. We speculate that isoflurane may mediate improved long-term functional outcome after CCI in rats through promotion of cerebral blood flow, suppression of metabolism, and/or modulation of excitotoxicity. Fentanyl may not be the optimal sedative/analgesic agent early after TBI in humans. **Support:** USArmy#DAMD17-97-1-7009. 1. Mira, *et al.* Anesthesiology 1998; 391-400. 2. Murr, *et al.* Anesth Analg 1995; 80: 1108-15.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

1 JUN 2001

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports. Request the limited distribution statement for reports on the enclosed list be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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