NEURAL RESPONSES TO INJURY: PREVENTION, PROTECTION, AND REPAIR
Annual Technical Report
1994

Submitted by

Nicolas G. Bazan, M.D., Ph.D.
Project Director

Period Covered: 20 September, 1993, through 19 September, 1994

Cooperative Agreement DAMD17-93-V-3013

between

United States Army Research and Development Command
(Walter Reed Army Institute of Research)

and

Louisiana State University Medical Center
Neuroscience Center of Excellence

Protecting the Auditory System and Prevention of Hearing Problems

Project Directors:
Richard Bobbin, Ph.D.
Charles Berlin, Ph.D.

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00ZYIZ00S18064

6. AUTHOR (S)
Nicolas G. Bazan, M.D., Ph.D., Program Director
Director, LSU Neuroscience Center
Professor of Ophthalmology, Biochemistry and Molecular Biology and Neurology

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Louisiana State University Medical Center
LSU Neuroscience Center
2020 Gravier Street, Suite B
New Orleans, LA 70112

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P. O. Box 12211
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13. ABSTRACT *(Maximum 200 words)*

The LSU Neuroscience Center is a comprehensive, multidisciplinary, and transdepartmental entity that unites fundamental neurobiology and the clinical neurosciences in the common goal of elucidating the workings of the brain and contributing to the treatment of currently incurable diseases of the nervous system. The objective of the present program is to find solutions to neuroscience-related problems of interest to the U.S. Army Medical Research and Development Command. The program is focused on exploiting novel neuroprotective strategies that lead to prevention of and repair after neural injury. Converging approaches using state-of-the-art tools of cell biology, neurochemistry, neuroimmunology, neurophysiology, neuropharmacology, molecular biology and virology are proposed. Over the next four years, this program aims to: 1) carry out seven research projects in the basic and clinical neurosciences; 2) expand central, shared facilities with the addition of highly specialized instrumentation not currently available to our scientists; 3) develop laboratory space to permit the physical consolidation and coordination of this research effort; and 4) institute a coordination unit to monitor, facilitate, and administrate the cooperative research programs, as well as to meet the associated budgetary, human resources, facilities, and communications needs for the attainment of the proposed program goals.

14. SUBJECT TERMS

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17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
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This Technical Report covers the progress made in the first year of this Cooperative Agreement in one project of the original proposal. We hope that this format of the report will facilitate its handling. The table of contents for all the projects has been included in each volume as well as letters from members of the External Advisory Committee of the LSU Neuroscience Center who have conducted an initial review of the work done supported by this Cooperative Agreement.

Nicolas G. Bazan, M.D., Ph.D.
Director, LSU Neuroscience Center
Program Director, USAMRDC Cooperative Agreement

Table of Contents

Abstracts: Psychoneuroimmunology Research Society

1. HSV-1 latently-infected mice display an altered response to stress: Implications for antiviral immunity.
2. Mouse lymphocytes express an orphan opioid receptor
3. Morphine suppresses peritoneal and splenic CTL activity in a dose-dependent fasion in alloimmunized mice
4. The frequency of exposure to morphine differentially affects CTL activity in alloimmunized mice.

Manuscripts:

1. Carr DJJ, Carpenter GW, Garza HH, Baker ML, Gebhart BM (in press) Cellular mechanisms involved in morphine-mediated suppression of CTL activity. In: *The Brain Immune Axis in Substance Abuse* (Sharp, Friedman, Maddin and Eisenstein, eds), Plenum Press.
2. Carpenter GW and Car DJJ (submitted) Pretreatment with β-funaltrexamine blocks morphine-mediated suppression of CTL activity in alloimmunized mice.
3. Carr DJJ and Carpenter GW (submitted) Morphine-induced suppression of spenic CTL activity in alloimmunized mice is not mediated through a δ-opioid receptor.
4. Carpenter GW, Garza HH, Gebhardt BM, Car DJJ (in press) Chronic morphine treatment suppresses CTL-mediated cytolysis, granulation and cAMP responses to alloantigen

Project Director: Nicolas G. Bazan, M.D., Ph.D.

Participating Scientists: Geoffrey Allen, Ph.D.
Gary D. Clark, M.D.
Victor Marcheselli, M.S.
John Hurst, Ph.D.
Leo Happel, M.D.
Walter Lukiw, Ph.D.

Participating Scientists: Claude A. Burgoyne, M.D.
Emily Varnell
Mandi Conway, M.D.

Manuscripts

1. Chew SJ, Beuerman RW, Kaufman HE (in press) Real-time confocal microscopy of keratocyte activity in wound-healing after cryoablation in rabbit corneas. *Scanning* 16.

"Role of Growth Factors and Cell Signaling in the Response of Brain and Retina to Injury" . . . .

Project Directors: Prescott Deininger, Ph.D.
Nicolas G. Bazan, M.D., Ph.D.

Participating Scientists: Julia Cook, Ph.D.
Haydee E. P. Bazan, Ph.D.
William C. Gordon, Ph.D.
Elena Rodriguez De Turco, Ph.D.
Victor Marcheselli, M.S.

"Effect of Ischemia-reperfusion Damage on Neurochemical and Neuropathological Responses in Transgenic Mice with Reduced or Enhanced Expression of Growth Factors" . . . .

"Neuropathological responses in transgenic mice having growth factor receptors either depleted or overexpressed." . . . .

Figure 1. A neuron-specific expression vector for the PDGF dominant negative mutant.
Letter to Rick Huntress, Transgenic Services Coordinator, DNX Corporation
Manuscript

1. Thompson HW, Cook JL, Nguyen D, Rosenbohm T, Beuerman RW, Kaufman HE (submitted) In vivo gene transfer to corneal epithelium by retroviral vector administration in eyedrops.

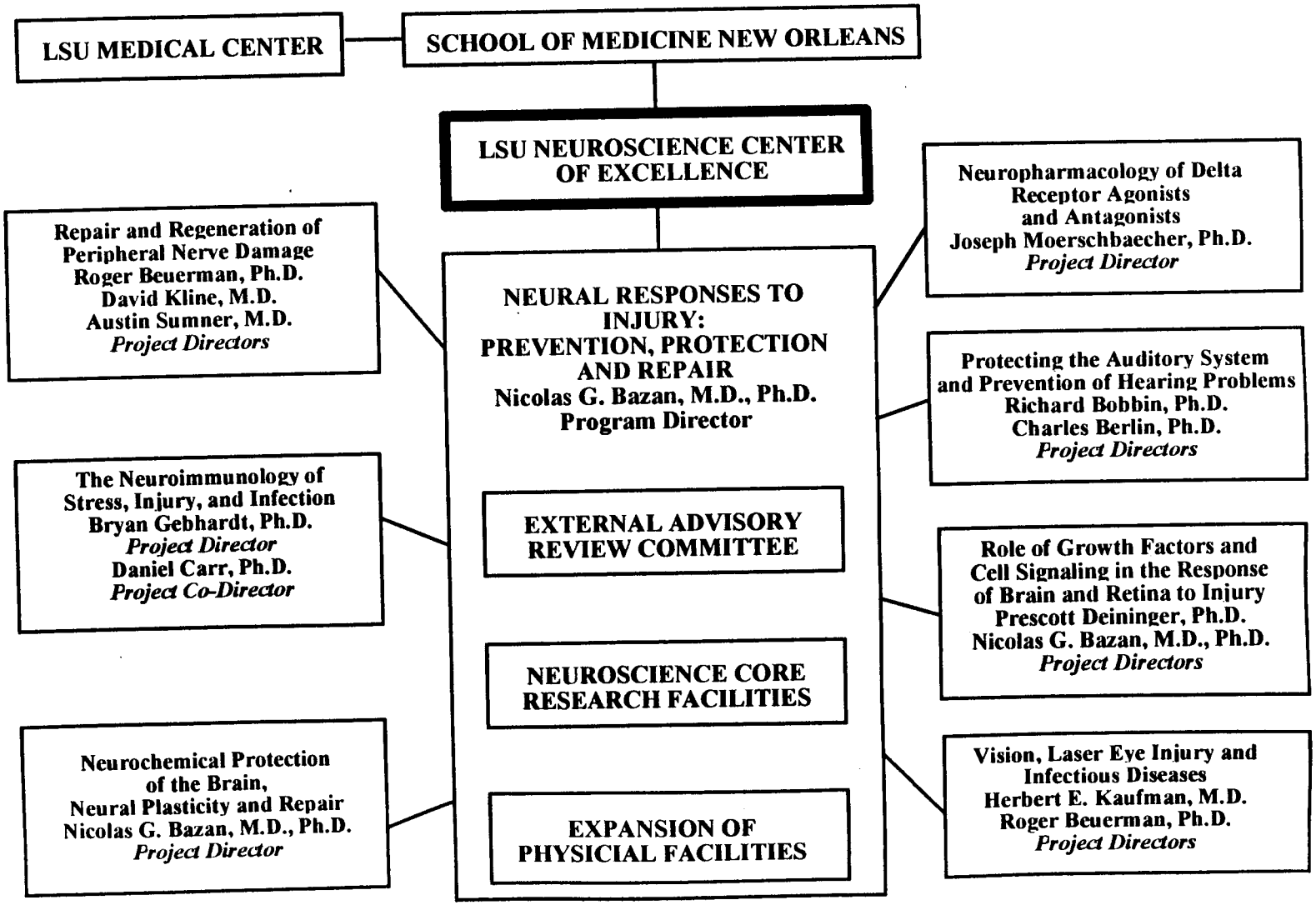
TABLE OF CONTENTS FOR THIS VOLUME

(continued on next page)

"Protecting the Auditory System and Prevention of Hearing Problems" (continued)

Cooperative Agreement Between the US Army Medical Research and Development Command and The LSU Neuroscience Center of Excellence

DAMD17-93-V-3013 20 September, 1993 - 19 October, 1997 $13,860,000



SCHOOL OF
MEDICINE IN NEW ORLEANS
Louisiana State University
Medical Center
2020 Gravier Street, Suite "B"
New Orleans, LA 70112-2234
Telephone: (504) 568-6700
Telefax: (504) 568-5801

Neuroscience Center
Office of the Director

19 October, 1994

Commander
U.S. Army Medical Research and Development Command (USAMRDC)
ATTN: SGRD-RMI-S
Fort Detrick
Frederick, MD 21702-5012

Re: Annual report, Cooperative Agreement No. DAMD17-93-V-3013
Neural Responses to Injury: Prevention, Protection, and Repair

Dear Sir,

Please find enclosed the original and five copies of the first annual report for the Cooperative Agreement, referenced above, between the USAMRDC and the Louisiana State University Medical Center School of Medicine, Neuroscience Center of Excellence. This report represents the research carried out during the first year of this agreement (20 September, 1993, to date). It is organized per project, each corresponding to a chapter of the original application.

In addition to the research conducted in the first year of this agreement, the planning for the two additional floors of research space which are to be added to the Lions/LSU Clinics Building, 2020 Gravier Street, New Orleans, LA, has been completed, including all specifications necessary for the start of bidding. Enclosed is one copy each of the program manual (1 vol.) and the project manual (3 vols.) which has been generated by Cimini, Meric and Duplantier, Architects and Planners, for bidding purposes. It should be noted that there will actually be three floors constructed in this one project, two as funded by this Cooperative Agreement and one which is funded by LSU to be used by the School of Medicine for other purposes.

As planned, I arranged to have three meetings between the LSU investigators and their counterparts in the Army to provide program briefings for the work that they were planning to conduct under this agreement as well as to exchange ideas and information of mutual interest. The agendas for each of these meetings are enclosed. These provided both the LSU scientists and those of the Army the opportunity to discuss the work being done, the direction, and the significance to problems of interest to the Department of Defense.

On 2 December, 1993, several of our investigators, excluding the Auditory and Laser/Vision groups, met at the Walter Reed Army Institute of Research, Washington, D.C., with Drs. Frank Tortella, Joseph Long, Mark DeCoster and Jit Dave. These discussions revolved around the neurochemical and neuropharmacological aspects of the program project and provided a forum for the Army scientists tobegin interactions and exchange of information with our investigators.

On 31 January, 1994, the LSU auditory physiology group, represented by Drs. Charles Berlin and Richard Bobbin, and I met at Fort Rucker, AL, with Dr. Kent Kimball and Dr. Ben T. Mozo. These meetings involved presentations and discussions about the protection of the auditory system and prevention of hearing problems in humans.

The LSU investigators involved with the vision research, composed of Dr. Herbert Kaufman, Dr. Roger Beuerman and myself, met on 7 February, 1994, at Brooks Air Force Base, San Antonio, TX. These scientists and those of the Ocular Hazards Research Unit of the US Army Medical Research Detachment made presentations and conducted discussions focused on protection from, repair of, and prevention of laser injuries, specifically to the eye. Each of these information exchanges provided very useful direction and advice for the LSU investigators. These workshops will be conducted annually for the term of this agreement.

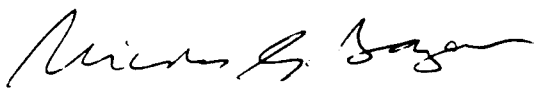
At the end of the first year of this program, as planned, I requested that two of the members of the External Advisory Committee of the LSU Neuroscience Center, Dr. Dennis W. Choi, Jones Professor and Head of the Department of Neurology, Washington University School of Medicine, and Dr. Fred Plum, Anne Parrish Titzell Professor and Chairman of the Department of Neurology, Cornell University Medical College, provide a critical review and a written report of the progress of the research accomplished under this Cooperative Agreement. Dr. Choi was given a copy of this annual report and subsequently made a site visit on 15 September, 1994, to the LSU Neuroscience Center. (The agenda for his meeting is attached.) At that time he met with a number of the investigators and administrators involved with whom he discussed many facets of the research being performed under this Agreement. His opinion of the work being done is attached.

Dr. Fred Plum made a site visit on 26 September, 1994, having also been provided previously with a copy of this annual report. He was also given the opportunity to examine the research and other progress made under this agreement and his written critique is also attached. Please note that, near the end of his letter (bottom of page two, first four paragraphs of page 5), Dr. Plum also included a description of projects not directly supported by the Cooperative Agreement but which are very positively impacted by any support of Neuroscience projects. The

reviewers were very complimentary of the positive consequences resulting from this support.

We are very pleased with the progress that has been made. We would like to thank you for the assistance you have given us. Please let me know if there is any further information that I can provide you.

Sincerely,



Nicolas G. Bazan, M.D., Ph.D.
Villere Professor of Ophthalmology,
Biochemistry and Molecular Biology,
and Neurology
Director, LSU Neuroscience Center

NGB/eht
enclosures

JOINT WORKSHOP ON "NEURAL RESPONSES TO INJURY: PREVENTION, PROTECTION AND REPAIR"

Sponsored by the LSU Neuroscience Center and Walter Reed Army Institute of Research, Department of Medical Neurosciences

December 2, 1993
Building 40, Room 2133

"Overview of LSU Program" **N. Bazan**	9:00
"Repair and Regeneration of Peripheral Nerve Damage" **R. Beuerman, D. Kline, J. England**	9:20
"The Neuroimmunology of Stress, Injury and Infection" **D. Carr**	10:10
Break	10:20
"Neurochemical Protection of the Brain, Neural Plasticity and Repair" **N. Bazan**	10:40
"Neuropharmacology of Delta Receptor Agonists and Antagonists" **J. Moerschbaecher**	11:15
"Stress and the Dopamine System" **J. Rao**	11:45
Box Lunch Served ($2.00 each)	12:00
"Role of Growth Factors and Cell Signaling in the Response of Brain and Retina to Injury" **N. Bazan and J. Cook**	12:10
"An Overview of Neuropharmacology Research at WRAIR on Nervous System Injury and Protection" **Frank Tortella**	13:00
"Animal Models of Spinal Cord Injury and Mechanisms of Blood Flow Changes" **Joseph Long**	13:30
"Evaluation of Excitatory Amino Acids in Neuronhal Cell Culture" **CPT DeCoster**	13:50
"Molecular Biology of Nervous System" **Jit Dave**	14:10
Overall Discussion	14:30
Adjourn	15:00

Joint Workshop on Neural Responses to Injury:
Prevention, Protection and Repair
Walter Reed Army Institute of Research, Dept. of Medical Neuroscience
U.S. Army Aeromedical Research Laboratory, Fort Rucker, AL
SCHEDULE FOR JANUARY 31, 1994

January 30
12:00 PM - depart New Orleans by car
Hotel: **Comfort Inn, 615 Boll Weevil Circle, Enterprise, AL 36330**
Tel. 205-393-2304, Fax. 205-347-5954

January 31
Visiting - Dr. Kent Kimball, Director, Plans and Programs, USAARL
Dr. Ben T. Mozo, Research Physicist, USAARL
Fort Rucker, AL 36362-5292
Tel. (205) 255-6917, Fax. (205) 255-6937

9:00 AM - Welcome
9:20 AM - Overview of LSU Program - **Nicolas G. Bazan**
9:45 AM - Protection the Auditory System and Prevention of Hearing Problem via Efferent Activation in Humans - **Charles Berlin**
10:30 AM - Break
11:00 AM - Prevention of Hearing Problems in Animals - **Richard Bobbin**
12:00 PM - General Discussion and Lunch
13:00 PM - Adjourn

OCULAR HAZARDS RESEARCH
U.S. ARMY MEDICAL RESEARCH DETACHMENT
7914 A DRIVE (Bldg 176)
BROOKS AIR FORCE BASE, TEXAS 78235-5138

February 7, 1994

Leave New Orleans on Continental flight #1445 at 6:00 PM, arrive San Antonio on Continental flight #1120 at 8:53 PM.

Hyatt Regency San Antonio
123 Losoya St., San Antonio, TX 78205
Confirmation #HY0000605552

February 8, 1994

8:30 *Overview of USAMRD program*
Bruce Stuck, Director, USAMRD

8:45 *Review of Accidental Laser Exposures and Human Tissue Response*
Donald Gagliano, Commander, USAMRD

9:00 *Overview of LSU Program*
Nicolas G. Bazan, Director, LSU Neuroscience Center

9:10 *The Program: Vision, Laser Eye Injury, and Infectious Diseases*
Herbert Kaufman, Chairman, Ophthalmology Dept. LSU

10:00 *Confocal Approach to Cellular Reactions in Wound Healing and of the Lamina Cibrosa.*
Roger Beuerman of the LSU Neuroscience Center

10:30 **BREAK AND LAB TOUR**

10:50 *Neurochemical Protection of the Brain, Neural Plasticity, and Repair*
Nicolas Bazan, Director, LSU Neuroscience Center

11:40 *Basic Fibroblast Growth Factor (bFGF) Treatment of Laser-Injured Retina*
Steven T. Schuschereba, Chief, Biology Section, USAMRD

12:10 *Role of Growth Factors and Cell Signaling in the Response of Brain and Retina to Injury: Focus on the Retina*
Nicolas Bazan, Director, LSU Neuroscience Center

12:50 **LUNCH**

2:50 Depart San Antonio on Southwest flight #803

5:55 Arrive New Orleans on Southwest flight #1055

LETTERS FROM MEMBERS OF THE EXTERNAL ADVISORY COMMITTEE

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE
AT WASHINGTON UNIVERSITY MEDICAL CENTER

NEUROLOGY

Dennis W. Choi, M.D., Ph.D.

Andrew B. and Gretchen P. Jones Professor and Head
Neurologist-in-Chief, Barnes Hospital

October 17, 1994

Nicholas G. Bazan, MD, PhD
Director, LSU Neuroscience Center
School of Medicine in New Orleans
Louisiana State University Medical Center
2020 Gravier Street, Suite "B"
New Orleans, LA 70112-2234

Dear Nick:

Thank you for the invitation to visit LSU on September 15 and review early progress made under the LSU Neuroscience Center of Excellence Cooperative Agreement with the U.S. Army Medical Research and Development Command.

You have assembled an impressive array of faculty researchers to study diverse aspects of nervous system injury. Overall, I find the individual projects to be thoughtful and well chosen. With you as director, I am sure that they will be most ably integrated. Your project 3 "Neurochemical Protection of the Brain, Neuroplasticity and Repair" is in my view the clear focal point of the overall program. The identification of new PAF antagonist drugs capable of regulating excitatory synaptic transmission and excitotoxic central nervous system injury, is an attractive and attainable goal. The novel pharmacology theme is also well developed in Dr. Moerschbaecher's Section 4 "Neuropharmacology of Delta Receptor Agonist and Antagonist". Involvement of clinician-investigators in clinical departments, such as Dr. Sumner in Project 1 or Dr. Kaufman in Project 5 are strengths of the program that will enhance its ability to identify human therapeutic interventions.

Progress in the first months of operation appears to be on target. Substantial synergy can be expected between the research programs specifically outlined in this collaborative agreement, and the larger intellectual framework formed the LSU Neuroscience Center of Excellence. Your role as director of both efforts is a vital feature that will ensure maximization of this synergy. In summary, I am most enthusiastic about this LSU-U.S. Army Cooperative Agreement, both for its specific merit and as a prototype mechanism for facilitating effective collaboration between academic and military institutions.

Best regards.

Sincerely,

Dennis Choi

Box 8111
660 South Euclid Avenue
St. Louis, Missouri 63110
(314) 362-7175 • FAX (314) 362-2826

525 EAST 68th STREET NEW YORK N Y 10021

THE NEW YORK HOSPITAL-CORNELL MEDICAL CENTER

FRED PLUM, M.D., CHAIRMAN
ANNE PARRISH TITZELL PROFESSOR OF NEUROLOGY
CORNELL UNIVERSITY MEDICAL COLLEGE
NEUROLOGIST-IN-CHIEF
THE NEW YORK HOSPITAL- CORNELL MEDICAL CENTER
(212) 746-6141
FAX (212) 746-8532

September 28, 1994

Nicholas G. Bazan, M.D., Ph.D.
LSU Neuroscience Center
2020 Gravier Street
Suite B
New Orleans, LA 70112-2234

Dear Dr. Bazan:

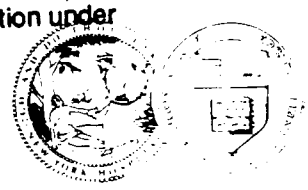
I am pleased to submit this reviewer's report of a Cooperative Agreement between the LSU Neuroscience Center and the US Department of the Army entitled, "Neural Response to Injury: Prevention, Protection and Repair" (henceforth designated as "Injury Study"). The agreement will span four years of effort by the LSU Center; this report describes progress obtained during its first year, extending from September 1, 1993 to August 31, 1994.

Nicholas G. Bazan, M.D., Ph.D. both directs the LSU Neuroscience Center of Excellence and serves as the Program Director of the Injury Study. In addition to Dr. Bazan's personal investigative efforts, seven additional study groups are engaged in research directly related to the Injury Study, as indicated in the administrative diagram attached to this report.

Dr. Bazan's outstanding personal and scientific qualities are the two most important factors in assuring the future success of the LSU-U.S. Army Cooperative Agreement. His leadership and intellectual "taste", as well as his joy in and dedication to brain science penetrate every aspect of the LSU Neuroscience Institute. His enthusiasm has spread to infect his colleagues and many other departments of the Medical School with his high scientific standards and integrity. His knowledge suffuses every dimension of basic neuroscience. His diplomacy and gentle handling of his staff creates their huge loyalty. His energy is contagious. Furthermore, he has the wonderful quality of scientific generosity: always ready to help and encourage others, he is entirely responsible for the continuously improving quality of young persons who are coming to LSU to learn and do important neuroscience.

In addition to the above, Dr. Bazan's specific research is internationally recognized as being of the highest caliber. His personal research contributions to the Injury Study during the past year reflects these high qualities in several ways. They have been published in the most competitively prestigious biomedical research journals. They also add new understandings to both the normal and potentially abnormal effects of the platelet-activating factor (PAF). PAF already is known to be a potent mediator of inflammatory and immune responses. What Bazan and his team now have found is that in low concentrations, PAF transmission may enhance memory and repair mechanisms in brain. Alternately, if released in excessively large concentrations or in combination with certain other molecules, PAF appears capable of causing immune-related tissue damage such as occurs with intense inflammation and/or the induction of genetic prostaglandin synthesis, a step that also may injure brain tissue. This fundamental research emphasizes the complexity and often bidirectional responses that may occur when injury strikes the brain. The results are important and illustrate the difficulties which must be overcome in establishing prevention, protection and repair of brain injuries.

Drs. Bazan and Prescott Deininger have succeeded in developing a series of transgenic mice expressing a dominant mutant of platelet derived growth factor (PDGF). Remarkably enough, the animals thus far have shown no major behavioral alteration under



normal developmental conditions. Their reaction to ischemia, seizures and other circumstances has not yet been tested.

Let me turn now to some of the other, supporting projects: **Drs. R. Benerman, D. Kline and A. Sumner** have made good progress in their studies of neurotrophic factors and other mechanisms in human and experimental neuromas resulting from blunt and crush nerve injuries. Basic fibroblast growth factor (bFGF) was the most prominent factor found in human post-nerve injury neuromas with other specific factors either absent or reaching only very low levels of concentration. More precisely analytic experiments await the analyses of fresh neuronal material from the experimental preparations.

Drs. Herbert Kaufman and Roger Benerman have made brilliant advances using confocal microscopy to examine the cellular details of the human retina. To a degree never before possible they have safely demonstrated in awake human subjects the acute pathophysiology of laser injuries to cornea and their early transformation into fibroblasts. Detailed identification of anterior chamber cells has been possible and current efforts are underway to examine at great magnification the optic disc itself. Ocular fungus and herpes infections can be identified immediately and without introducing foreign substances against the cornea or into the eye. Application of the tool should have an important place in clinically applied military medicine.

During the past year, the investigators also have pursued their earlier discovery that ambient chilling of monkeys latently infected with H. Simplex induces an acute recurrence of cutaneous herpes. Furthermore, chronic ingestion of the beta blocker, propanelol, has been found to ameliorate or prevent the active recurrence. Clinical trials of this important discovery must be pursued as it has important practical aspects.

During the year, the necessary work to establish and equip the glaucoma research laboratory was undertaken. Next year's report can be expected to provide research results from that laboratory.

Dr. Joseph Moerschbaecher and his colleagues in pharmacology have initiated preliminary studies on the influence of delta opoid agonists-antagonists on learning and antinociception. Somewhat surprisingly, the agent damps the CO_2 response of breathing but has no antinociceptive effect. The same investigator is analyzing how anxiogenic drugs affect dopamine neurons in the ventral tegmental area of the rodent brain.

In another preliminary approach, **Drs. H.W. Thompson et al** have initiated experiments passing retroviral gene carriers into the eye with externally applied eye drops, thereby developing a new approach to deliver protection against certain ophthalmologic infections or enhancing the potential success of corneal transplant.

Drs. Richard Bobbin and Charles Berlin, thanks to the DOD grant, have added an excellent postdoctoral student as well as important new equipment to their laboratory. The laboratory's principal subject of interest is to find mechanisms for preventing the audiologic damage produced by intense sound. In guinea pigs, this has been achieved by stimulating calcium-dependent mechanisms in cochlear neurons. In another study, the laboratory has found in human studies that during the delivery of loud, binaural sounds, men and women suppress the noise in opposite sided ears from each other.

The above individual achievements provide only a part of the considerable effort, enthusiasm and success that the U.S. Army grant has brought to the LSU Neuroscience Center of Excellence (NCE). The following steps forward can also be emphasized:

1) Morale in the LSU-NCE rides at high pitch, encouraging scientific collaboration and the generation of new ideas.

2) Funds have been granted to subsidize the necessary equipment and technical personnel to establish a brain bank. Presently, approximately 50 specimens are available in storage with the Center holding good clinical records of the preterminal illness.

3) A program of "starter" grants designed to assist young investigators in conducting merit-deserving, self designed research projects has been initiated.

4) A highly popular state-wide Graduate School outreach summer program has been successfully concluded, attracting a strong interest in neuroscience among gifted college students.

5) An interdisciplinary graduate program in neuroscience was initiated and strongly encouraged by the faculty during 1993-94. As a result, nearly all of the graduate students (including the new entering class) are of very good quality. Indeed, other participating departments say that the Neuroscience graduate students are the best among the LSU biological sciences programs.

Summary. Under the generous auspices of a U.S. Army Cooperative Agreement, the LSU Neuroscience Center of Excellence is not only thriving but headed for far greater future productivity than at any time in the past. The admirable success of the program depends heavily on the foresight, intelligence, creativity and energy of two outstanding scientists, Herbert Kaufman and, especially, Nicholas G. Bazan. Their achievements and those of their colleagues totally warrant continuation of support. Indeed, every indication is that their extramural, non-Army support will continue to grow, making the program stronger and stronger as the years elapse.

One serious problem remains - that of sufficient space in which to do the studies that Dr. Bazan and his colleagues already have conceived so well. Prompt attention to and effective application of must be given to the DOD funds already awarded to construct new research space which will greatly increase the LSU Neuroscience team's opportunities for creative discovery.

I and my colleagues on the External Advisory Board of the LSU Neuroscience Center of Excellence strongly endorse the quality and number of achievements that have come from the U.S. Army-LSU-NCE collaboration. Thanks to strong leadership for the Center and a high degree of internally high morale and interdependence within the Center, it can be anticipated that the Cooperative Agreement will have a major impact on national neuroscience research as well as the specific medical needs of the U.S. Army.

Sincerely,

Fred Plum

Fred Plum, M.D.

FP/moc

PROTECTING THE AUDITORY SYSTEM AND PREVENTION OF HEARING PROBLEMS

Project Directors:

Richard Bobbin, PhD
Charles Berlin, PhD

Participating Scientists:

Sharon Kujawa, PhD
Carlos Erostegui, MD
Douglas Webster, PhD

FOREWARD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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(X) 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 Commission on Life Science, National Research Council (NIH Publication no. 86-23, Revised 1985).

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

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

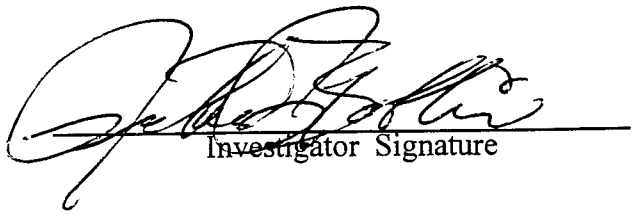
Investigator Signature

ANIMAL USE
20 SEPTEMBER, 1993, THROUGH JULY, 1994

DAMD17-93-V-3013

The experimental animals used during this period for the project, Neural Responses to Injury: Prevention, Protection, and Repair, **Subproject: Protecting the Auditory System and Prevention of Hearing Problems**, are as follows:

Species	Number Allowed	Number Used	LSU IACUC #
guinea PIG	99	88	1061



Investigator Signature

4. TABLE OF CONTENTS:

page

5. ABSTRACT: (Maximum of 200 words)

ANIMAL PROJECT: This study aims to demonstrate and explore mechanisms for preventing the effects of intense sound. We discovered that continuous, moderate level ipsilateral primary stimulation (CM-LIPS) will produce complex changes in the mechanics of the cochlear partition. Calcium ions, but not efferent nerves, are involved in these changes. We discovered that "toughening" guinea pigs results in changes in efferent activity, in cochlear partition mechanics, and, most dramatically, the OHC response to ATP is greatly attenuated.

HUMAN PROJECT: Major findings include: (1) binaural stimulation generates more than twice the emission suppression of ipsilateral or contralateral stimulation in a forward masking paradigm; (2) Females show larger emissions than males; **(3) Binaural noise causes more emission suppression when the clicks are delivered to the right ears of females than when the clicks are delivered to left ears; (4)In males, exactly the opposite is seen, that is binaural noise suppresses left ears more than right ears;** (5) Using tone bursts we can show more controllable and measurable emission than using broad band clicks.

6. INTRODUCTION: The nature of the problem that this proposal addresses is that soldiers may be exposed to intense noise hazards which will affect their hearing. The hypothesis to be tested is that noise induced hearing loss can be prevented or attenuated.

The literature suggests that the efferent nerve fibers synapsing on the outer hair cells (OHCs) in the cochlea may attenuate the effects of intense sound. One example of this may be the demonstration that chronic low level sound will "toughen" ears so subsequent intense noise will induce less damage (e.g., Canlon et al., 1988; Campo et al., 1991; Franklin et al., 1991). We demonstrated that sound will activate cholinergic efferent fibers leading to the cochlea (Kujawa et al., 1994a). The released acetylcholine (Ach) hyperpolarizes the OHCs (Erostegui et al., 1994) which in turn attenuates the mechanical response of the cochlear partition to sound as measured by distortion product otoacoustic emissions (DPOAE; Kujawa et al., 1992, 1993, 1994a). These results predict that low level sound will activate the efferents and consequently will reduce the effects of intense sound by attenuating cochlear partition motion. Whether this occurs in "toughening" remains to be determined. Also, excitatory amino acid drugs may impact the effects of sound since glutamate, the transmitter between the inner hair cells (IHCs) and the afferent nerve endings, damages the afferents (Eybalin, 1993; Puel et al., 1994). Whether the efferents prevent glutamate-induced damage remains to be explored.

The human experimentation program was proposed to develop

emission-based tests to detect abnormal cochlear function rapidly and accurately. The procedure offers the equivalent of a non-invasive acoustic microscope to analyze the integrity of the OHCs; this is an important tool since OHC damage is always seen in humans who have suffered noise damage. The hypothesis is that "noise tender" ears that are particularly susceptible to noise damage will show different emission suppression patterns from ears that are "tough".

Our intention remains to study 100 musicians and industrial workers who have had matched exposures to noise in an efferent suppression paradigm. Before completing the final design of this major study we need to lay basic parametric groundwork relative to the nature of the stimuli, their delivery (whether binaural, contralateral only, or ipsilateral), their analysis, and whether there are any pertinent gender and ear differences which have to be entered into the final subject selection.

In summary, in animals we will test the hypothesis that: (1) the impact of noise on hearing can be lessened; (2) certain classes of drugs may prevent (or exacerbate) noise-induced hearing loss. In human subjects, we will explore the concept that some individuals are more or less susceptible to noise damaging effects. We will describe those populations to determine the basis of this "toughness" or susceptibility to damage from noise. We will examine whether the techniques which we may discover aid in preventing noise-induced hearing loss in soldiers.

7. BODY:

ANIMAL PROJECT: In year #1 we initiated an acute and a chronic experiment. The acute experiment focused on the effects of ipsilateral sound (experiment #2 in the original application with modifications based on the new data of Kirk and Johnstone, 1993). The chronic experiment examined "toughening" together with whole cell voltage clamp experiments (experiment #1, #3 & #4 in the original application with modifications based on data of Subramaniam et al., 1994). These experiments follow directly our stated specific aims for year #1 and the first three TECHNICAL OBJECTIVES: to extend studies which demonstrate that contralateral, ipsilateral, or "toughening" sound will prevent the effects of intense noise, to test the role of the efferents, and to explore cellular mechanisms.

The methods were described (Kujawa et al., 1992; 1993; 1994a). Briefly, guinea pigs are anesthetized (urethane: 1.5 gm/kg) and tracheotomized. ECG is monitored and temperature maintained at 38° ± 1°C. The right auditory bulla is exposed, opened and tendons of the middle ear muscles are sectioned. For drug application to the cochlea, holes are placed in the cochlear basal turn: one in scala tympani for the introduction of perfusates and one in scala vestibuli to allow fluid escape. Perfusates are introduced into scala tympani at approximately 2.5 μl/min for 10 min through a pipette coupled to a syringe pump. DPOAEs ($2f_1-f_2$ & f_2-f_1) are recorded in response to primary stimuli ($f_2/f_1=1.2$) delivered to the right ear of each animal by an acoustic

probe/hollow ear bar assembly. Acoustic signals present within the canal are detected by a microphone system (Etymotic Research, ER-10) contained within the probe. Microphone output is directed via a preamplifier (Etymotic Research, ER-1072) to a signal analyzer (Hewlett Packard 3561A). To extract the DPOAE from the canal spectrum, the signal is sampled, digitized and submitted to Fast Fourier Transform (FFT) analysis. The resulting spectrum is averaged (over 25 samples) and displayed at the spectrum analyzer (1 kHz window, 3.75 Hz BW). DPOAE amplitude is defined as the spectral peak corresponding to the DPOAE frequency. The contralateral stimulus is a wideband noise with an overall level of 70 dB SPL and flat from 0.9 to 15.8 kHz.

The acute experiment examined the influence of continuous, moderate-level (60 dB SPL) ipsilateral primary stimulation (CM-LIPS) on the f_2-f_1 DPOAE at 1.25 kHz. The stimulation and response monitoring protocols consisted of quiet for 15 min, then turning the primaries on for 9 min, off for 1 min and then on for an additional 3 min. CM-LIPS resulted in a complex change in the magnitude of f_2-f_1 (see Appendix 1, Figure #4a): there was an initial increase, followed by a slow decrease. When the sound was turned off for 1 min of rest and then turned back on there was an immediate lower value, an "off-effect" which then rapidly grew bigger. The "on-effect" and the slow decrease have been described separately (Brown, 1988; Kirk and Johnstone, 1993; Whitehead et al, 1991). We believe the "off-effect" to be a new discovery. Such changes did not occur in $2f_1$-f_2 (Appendix 1, Figure

#4b). This indicates to that f_2-f_1 is a sensitive indicator of low-level sound-induced alterations in cochlear partition mechanics, possibly involving the OHCs and possibly a form of "toughening". We explored mechanisms for the CM-LIPS-induced alteration in mechanics. We first ruled out whether efferent nerves were inducing these changes by applying drugs to the nerve fibers and hair cells by cochlear perfusion: curare which blocks Ach and tetrodotoxin (TTX) which blocks nerve action potentials. In addition, the efferent nerve fibers were sectioned acutely. None of these treatments induced a statistically significantly alteration in the CM-LIPS-induced alteration in the mechanics (Appendix 1, Figures #7, #8 & #9). The treatments did disable the efferents as indicated by the blockade of the effect of contralateral sound (Appendix 1, Figure #9). Therefore, we concluded that the efferents play no role in the CM-LIPS-induced alteration in the mechanics. Second, we examined the role of calcium. Lowering the calcium with EGTA or BAPTA and utilizing the calcium channel antagonist, magnesium, all reversed the polarity of the "off-effect" (Appendix 1, Figures #10 & #11) indicating that calcium plays an important role in part of the CM-LIPS-induced alteration in mechanics. These results were presented at the Acoustical Society Meeting in June of 1994 (Appendix 1) and a manuscript is in preparation.

In the chronic experiment, the "toughening" sound used is a continuous low-level band of noise (85 dB SPL, band centered at about 1.5 kHz with 3 dB cut off at 1.025 and 2.125 kHz)

which is similar to that used by others (Boettcher et al., 1992; Canlon et al., 1988, 1992; Campo et al., 1991; Franklin et al., 1991; Henderson, 1994; Henselman et al., 1994; Mensh et al., 1993a, 1993b; Subramaniam et al., 1991a, 1991b, 1994). Others show a sound to be a "toughening" sound by demonstrating that it induces less change (i.e., less temporary threshold shift) in the cochlea during its continuous presentation over several days (Franklin et al., 1991; Subramaniam et al., 1994). To demonstrate this we are exposing animals to the "toughening" sound and monitoring at 1, 3, 5, 7, 9, 10 and 11 days. When taken out of the exposing chamber, the animals are immediately anesthetized and tested. The DPOAEs tested are: $2f_1-f_2$ at 8, 6, 5, 4, 3, 2, 1.25, & 1 kHz; and f2-f1 at 0.312, 0.5, 0.75, 1, 1.25, 1.5, & 2 kHz, maintaining $f_2/f_1=1.2$. The effects of efferents activated by contralateral sound on these DPOAEs is being documented as is the effects of CM-LIPS. Preliminary results indicate that the toughening sound induces a TTS by suppressing DPOAEs, especially the f_2-f_1, a new finding. At 3 days the suppression of DPOAEs is maximum, and then the DPOAEs move towards recovery over the next few days (Appendix 2, Figure 1). The trend towards recovery indicates that we are observing a "toughening" of the ear (Franklin et al., 1991; Subramaniam et al., 1994). At day 7 the suppressive effects of the contralateral efferents are enhanced at 1 kHz, a new and unexpected finding (Appendix 2, Figure 2). In the CM-LIPS-induced alterations "toughening" appears to result in eradication of the "on-effect" and the "off-effect" and an enhancement of the slow decrease

(Appendix 2, Figure 3). A manuscript describing these effects of "toughening" is being prepared.

To examine the cellular basis for these "toughening"-induced changes in the cochlea, we carried out whole cell voltage clamp recordings from the OHCs of these animals after the DPOAE recordings were made. To date only OHCs from days 10 and 11 animals have been examined. The experiments utilized standard whole cell variant of the patch clamp technique (Erostegui et al., 1994). Results indicate the current change in response to ATP in a barium-containing external solution was statistically reduced (Appendix 2, Figure 4) and the ATP response in normal solutions was abolished (no figure shown). The results indicate ATP receptors have down-regulated (i.e., become less in number) during "toughening". ATP (which depolarizes OHCs, Kujawa et al., 1994b) may be released together with Ach (which hyperpolarizes OHCs, Erostegui et al., 1994) from the efferent nerve endings. With ATP receptors possibly down-regulating, then the Ach receptor response may become larger. This may be the molecular mechanism for "toughening".

HUMAN PROJECT:

Experiment in 2 dB steps outlines critical zones of the effect: We have completed two studies on efferent suppression which show the nature of, and optimal levels for demonstrating, the effect (Berlin et al., 1993). In one study (Hood et al. 1994) we systematically manipulated the intensity of the clicks in one ear and the contralateral noise. The linear clicks were presented at

50, 55, 60, 70 dB peak Sound Pressure to the left ear. Then noise was introduced from 10 dB below the click to 10dB above the click. The noise was increased systematically in 2 dB steps. In this lengthy and time consuming study we clarified that the most powerful contralateral suppression is seen when the click is only 55-60 SPL (17 to 22 dB HL) but the noise is 5 to 10 dB more intense than the click (See Appendix 3, Figure 1).

Binaural stimulation is more powerful than ipsilateral and contralateral: In a second experiment we reasoned that, because of the bilateral nature of efferent Medial Olivocochlear innervation (Warr et al., 1986), a bilateral stimulus would be more powerful at demonstrating suppression than a contralateral stimulus. However, the paradigm would have to be changed to allow binaural noise stimulation without interfering with click echoes. Therefore we designed an experiment to follow the paradigm displayed in Appendix 3, Figure 2. We systematically varied the interval following the termination of the noise. The spacings were 1, 2, 5, 10, 20, 50, 100 and 200 msecs and the noise was only 408 msecs in duration. The clicks were presented only to the left ear (we had not yet realized the importance of comparing ears) and the noises were presented either binaurally, ipsilaterally or contralaterally (Berlin, et al. 1994). Results shown (Appendix 3, Figures 3 - 6) indicate that binaural stimulation is more powerful than either ipsilateral or contralateral suppression. Furthermore, as might be predicted, the closer to the end of the noise the clicks were presented, the more

their resultant emissions were suppressed.

The noise becomes an effective suppressor when it is between 80 and 160 msecs in duration: A third experiment (Wakefield et al. 1995) systematically varied the **duration of the noise**, keeping the onset of the click within one msecs of the end of the noise. That study showed that more than 80 msecs and less than 160 msecs outlines the noise duration in which a robust suppressive effect might be expected in humans. This is critical information because it suggests that noise durations to which humans are exposed must exceed 80-100 msecs before any expected "toughening" or efferent pre-stimulation effects can be expected. This is consistent with reports offered by Liberman and colleagues about the minimum durations needed for efferent effects to be seen in cats (1992; See Appendix 3, Figure 7).

There are unexpected gender and ear differences: It has been suggested recently (McFadden, 1993) that well-known dichotic perceptual asymmetries studied, and ascribed to cortical and cognitive asymmetries, may in fact be influenced by peripheral and efferent asymmetries. In our original presentation we had not anticipated this possibility and therefore had to investigate the validity of McFadden's suggestions before we embarked on our major study of 100 noise-exposed subjects. In his paper, which was published after we had submitted this project for approval, McFadden (1993) speculated that, based on some asymmetries seen in the literature, efferent suppression would be relatively less in right ears and females than in left ears and males.

We therefore designed and completed a fourth experiment with 12 males and 12 females based on our forward masking studies described above. These data (Appendix 3, Figures 8 - 9) show McFadden's predictions appear to be accurate in absolute values if not in proportions. We found that in males, left ears were much more suppressible than right ears, but in females it was the right ear that was more suppressible, but not by nearly as much as the left ears in males (Barham et al. 1994). Since left ears show much more damage than right ears in noise exposure surveys of males, this observation has great scientific relevance.

Subjects studied: We have studied one noise-traumatized subject (FRC) in great detail. This is a subject who claims noise damage and tinnitus in one ear only after sudden close-proximity exposure to a fire siren which apparently damaged one ear more than the other. His confreres in the Fire Department have had similar exposures but none have reported his complaint. None has yet been studied because we are looking for ways to match noise exposure in his particular milieu. He does have emissions in both ears, but the traumatized ear has far less emission than the other ear. His suppression performance is also asymmetrical, but the findings are difficult to interpret until we resolve the gender and ear differences discussed above.

New Equipment Needed: In all the experiments we have completed so far on suppression and forward masking we have had to contend with an idiosyncracy in the design and structure of the ILO88 system. It seems that, when a time value is selected for the

duration of the noise, or the duration of the interval between the end of the noise and the onset of the click, a number of defaults come into play. First, all noise durations, regardless of how we might describe them in the nominal programs, are multiples of 81 msecs. Thus our selection of 460 msecs on the menu actually generated a noise burst of 406 msecs. And our selection of a 1 msec interval between the end of the noise and the onset of the clicks had its own inherent flaw; the first click indeed is presented 1 msec after the end of the noise. But this first click **must occur** along with three additional clicks in an indivisible group of four, each of which occurs 20 msecs after the preceding click. Thus in an experiment designed to assess the effect of time interval on suppression, only the **first click** is at the "Correct" interval from the end of the noise. Three additional clicks contaminate the average and each of them is a successive 20 msecs away from the end of the noise. If we were to present only a **single click** in that interval, three additional blank 20 msec noise segments are averaged into the final echo display, an artifact which reduces all our effects by a factor of three. This was unpredicted at the beginning of our experiments, and assuming the developer cannot make the major changes necessary, we are embarking on the formidable task of making our own system which will be capable of performing this experiment exactly to our requirements and specifications.

8. CONCLUSIONS:

ANIMAL PROJECTS: The completed research indicates that we are able to "toughen" guinea pig cochlea to the effects of intense sound by the use of moderate level continuous sound. Future work will confirm this by an experiment where we "toughen" the animals for 11 days, wait 5 days, and then expose the animals to an intense damaging sound. The mechanisms for the "toughening" appear to involve the efferent nerve fibers, the mechanics of the cochlear partition and OHCs. We discovered that the changes in the mechanics can be detected with the f_2-f_1 DPOAE. In future experiments we will explore the changes in f_2-f_1 elicited by the CM-LIPS more closely. This is a new and exciting finding and indicates that short term (min) changes in cochlear mechanics occur. The molecular mechanisms appear to involve changes in calcium. How these short term changes (min) in calcium and in f_2-f_1 impact "toughening" or noise exposure will be a new question to be explored. Regarding the long term (days) "toughening", the changes in the ATP response will be replicated to insure that the effects are real. In addition, the Ach-evoked response will be vigorously explored: This is more difficult since fewer numbers of isolated OHCs respond to Ach (20%) normally compared to the number (50%) that respond to ATP. Finally, the changes in efferent activity at only certain frequencies was unexpected and must be explored.

HUMAN PROJECT: We have found gender and ear differences in otoacoustic emission suppression which make our project much more complicated but also more interesting than before.

Resultant Actions: These findings have prompted a mid-course correction. We must now account for and design into our studies a set of gender and ear differences we had not anticipated in the original proposal. In order to avoid losing precious time we are instituting three parallel actions: 1. Completion of tone burst studies to minimize effects of pre-existing peripheral hearing loss on emissions. 2. Construction of a larger patient data base which will facilitate experiments which include both gender and ear variables. 3. Execution of program changes which present only a single click or tone burst in one 20 msec window for analysis. We are also developing our own proprietary system which can produce and analyze exactly the type and time sequence of stimuli we would like without the intrusive three-memory dead space alluded to above.

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10. Appendix:

Appendix 1. Kujawa, S.G., Fallon, M., and Bobbin, R.P., A suppressive "off-effect" in the f_2-f_1 DPOAE response to continuous moderate-level primary stimulation. Poster presented at **Acoustical Society of America**, Boston, June 1994.

Appendix 2. Additional figures for the animal studies.

Appendix 3. Figures for the human studies.

Appendix 4. Berlin, C.I., Hood, L.J., Hurley, A.H., Wen, H., and Kemp, D.T., Binaural noise suppresses linear click-evoked otoacoustic emissions more than ipsilateral or contralateral noise. Manuscript in preparation.

LIST OF APPENDICES

CHAPTER: PROTECTING THE AUDITORY SYSTEM AND PREVENTION OF HEARING PROBLEMS

PROJECT DIRECTORS: RICHARD P. BOBBIN, PH.D.
CHARLES I. BERLIN, PH.D.

APPENDIX 1

Kujawa, S.G., Fallon, M., and Bobbin, R.P., A suppressive "off-effect" in the f_2-f_1 DPOAE response to continuous moderate-level primary stimulation. **Acoust. Soc. Am.**, Boston, June 1994.

CHAPTER: PROTECTING THE AUDITORY SYSTEM AND PREVENTION OF HEARING PROBLEMS

PROJECT DIRECTORS: RICHARD P. BOBBIN, PH.D.
CHARLES I. BERLIN, PH.D.

A Suppressive "Off-Effect" in the $f_2 - f_1$ DPOAE Response to Continuous Moderate-Level Primary Stimulation

Sharon G. Kujawa, Maureen Fallon and Richard P. Bobbin

Kresge Hearing Research Laboratory,

Dept. of Otolaryngology,

LSU Medical Center, New Orleans, LA

INTRODUCTION

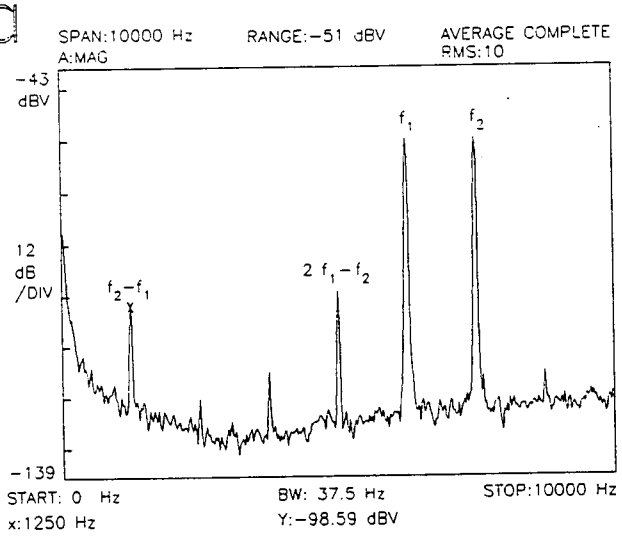
Following a short period of amplitude enhancement, the f_2-f_1 distortion product otoacoustic emission (DPOAE) can be observed to undergo a gradual decline during continuous ipsilateral stimulation with primary tones[1-2,4]. In the present experiments, we studied these time-varying changes in response amplitude along with an exacerbation of f_2-f_1 amplitude suppression that we observed following a short rest from continuous stimulation. We tested the dependence of these amplitude alterations on several stimulus variables (intensity, duration) and on DPOAE type (quadratic vs cubic).

Our ultimate goal in these experiments is to identify the mechanism(s) underlying the effect of continuous, moderate-level ipsilateral stimulation on the f_2-f_1 DPOAE. First, we tested the hypothesis that the amplitude alterations are mediated by the olivocochlear (OC) efferents. For these experiments, we employed pharmacologic antagonists of OC efferent activity (curare, bicuculline), an antagonist of all action potential-mediated activity (tetrodotoxin) and OC bundle section. We employed the known efferent-mediated contralateral suppression of DPOAEs[3] as an internal control. Next, we tested the hypothesis that the f_2-f_1 amplitude alterations are related to local calcium-dependent changes occurring at the level of the outer hair cells (OHCs). For these experiments, we reduced available perilymph calcium or replaced it with another divalent cation, magnesium.

METHODS

1 Baseline Measures: DPOAEs (f_2-f_1 at 1.25 kHz; $2f_1-f_2$ at 5 kHz) were recorded in anesthetized (urethane, 1.5 g/kg) guinea pigs. The right auditory bulla was opened widely to expose the cochlea and tendons of middle ear muscles were sectioned in all animals. Responses were elicited by primary stimuli at 6.25 kHz (f_1) and 7.5 kHz (f_2) (Fig. 1a) presented via a closed, calibrated probe system. Probe microphone output was led to a signal analyzer for averaging (10 spectra) and display. Fig.1 displays typical DPOAE responses to equilevel primaries at 60 dB SPL (span = 10 kHz; BW = 37.5 Hz). DPOAE amplitude alterations during continuous primary stimulation were monitored using a narrower window (1 kHz; CF = DP frequency; BW = 3.75 Hz) to improve the noise floor (−15 to −20 dB SPL; see for example, Fig. 1b). Amplitude growth functions (25 – 70 dB SPL in 5 dB steps) were consistent with laboratory norms.

a



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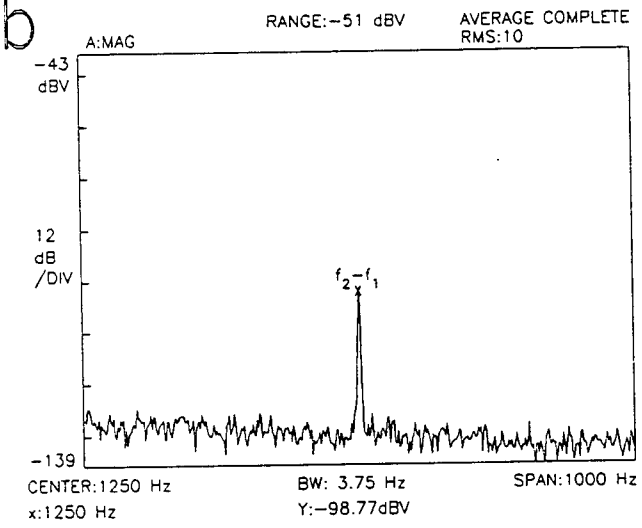
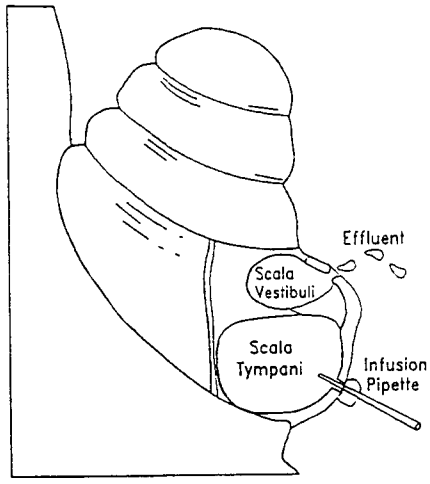


INTRODUCTION

Following a short period of amplitude enhancement, the f_2-f_1 distortion product otoacoustic emission (DPOAE) can be observed to undergo a gradual decline during continuous ipsilateral stimulation with primary tones[1-2,4]. In the present experiments, we studied these time-varying changes in response amplitude along with an exacerbation of f_2-f_1 amplitude suppression that we observed following a short rest from continuous stimulation. We tested the dependence of these amplitude alterations on several stimulus variables (intensity, duration) and on DPOAE type (quadratic vs cubic).

Our ultimate goal in these experiments is to identify the mechanism(s) underlying the effect of continuous, moderate-level ipsilateral stimulation on the f_2-f_1 DPOAE. First, we tested the hypothesis that the amplitude alterations are mediated by the olivocochlear (OC) efferents. For these experiments, we employed pharmacologic antagonists of OC efferent activity (curare, bicuculline), an antagonist of all action potential-mediated activity (tetrodotoxin) and OC bundle section. We employed the known efferent-mediated contralateral suppression of DPOAEs[3] as an internal control. Next, we tested the hypothesis that the f_2-f_1 amplitude alterations are related to local calcium-dependent changes occurring at the level of the outer hair cells (OHCs). For these experiments, we reduced available perilymph calcium or replaced it with another divalent cation, magnesium.

3 Cochlear Perfusion: Perfusion and effluent holes were placed as shown in Fig. 3. Perfusion solutions were introduced at a rate of 2.5 μl/min and all perfusions were 10 min in duration. Two perfusions of artificial perilymph (AP) were followed by perfusions of experimental drugs separated by artificial perilymph washes (W). After each perfusion, the DPOAE was monitored during continuous ipsilateral primary stimulation and before, during and after WBN stimulation of the contralateral ear. To insure that drugs were not being diluted with replacement CSF during the long response monitoring periods post-perfusion, a subgroup of animals (N=4) received drugs after first undergoing blockade of the cochlear aqueduct and results were compared with animals in which the aqueduct was patent.



OC Nerve Section: In a subgroup of animals (N=4), OC fibers were sectioned where they cross the brainstem midline. The cut was made spanning the anterior-posterior extent of the exposed IVth ventricle floor at brainstem midline at a depth of 2-3 mm.

BASELINE MEASURES

4 Continuous ipsilateral stimulation alters f_2-f_1 but not $2f_1-f_2$

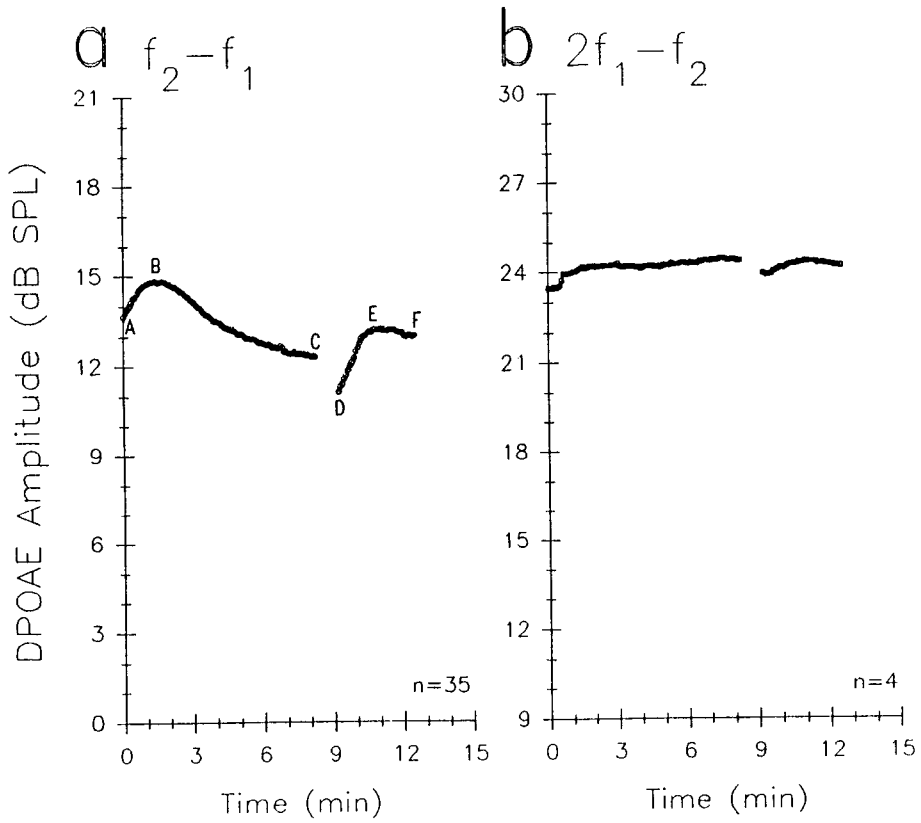


Table 1

DPOAE Amplitude Alterations (dB)

Condition	"On- Effect" (Δ A–B)	"On- Effect" (Δ D–E)	Pre-Rest Suppression (Δ B–C)	"Off-Effect" (Δ C–D)
Baseline: f_2-f_1 (n=35)	+1.18	+2.09	−2.53	−1.17
Baseline: $2f_1-f_2$ (n=4)	–	–	–	−0.43

5 f_2-f_1 amplitude alterations are dependent on continuous stimulation and on primary level

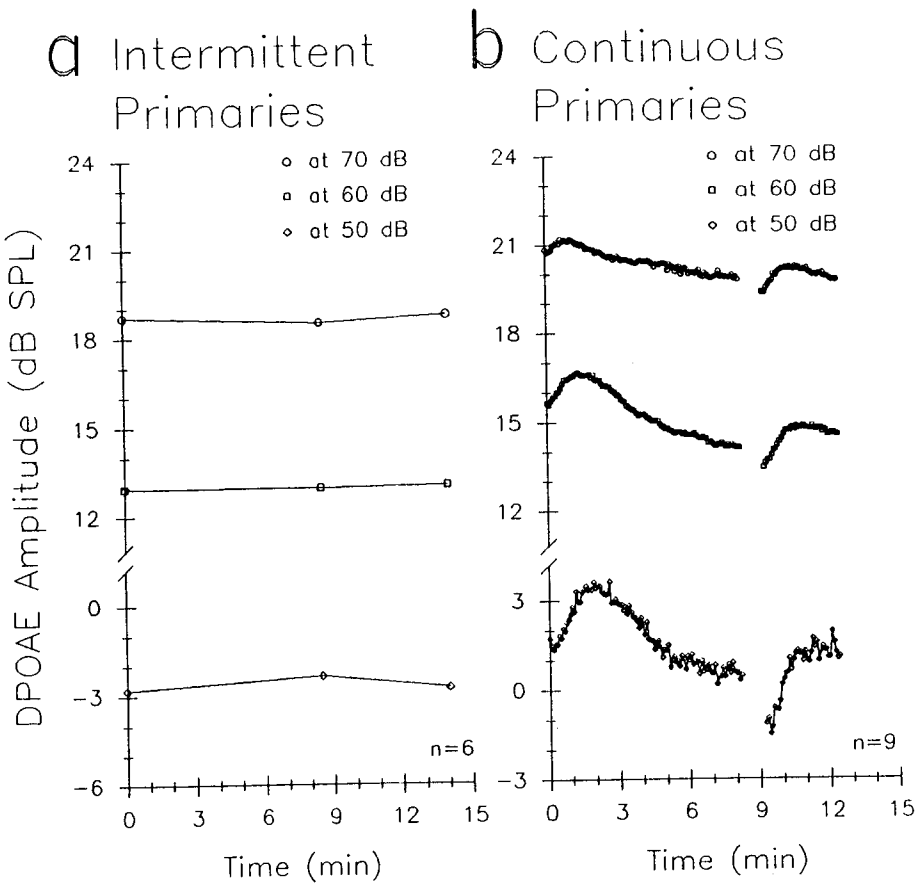
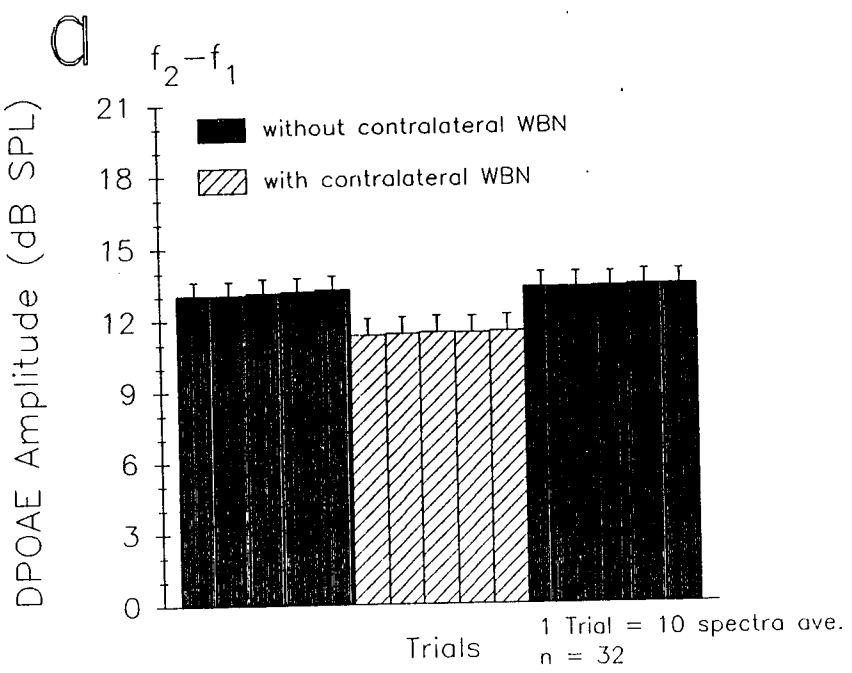


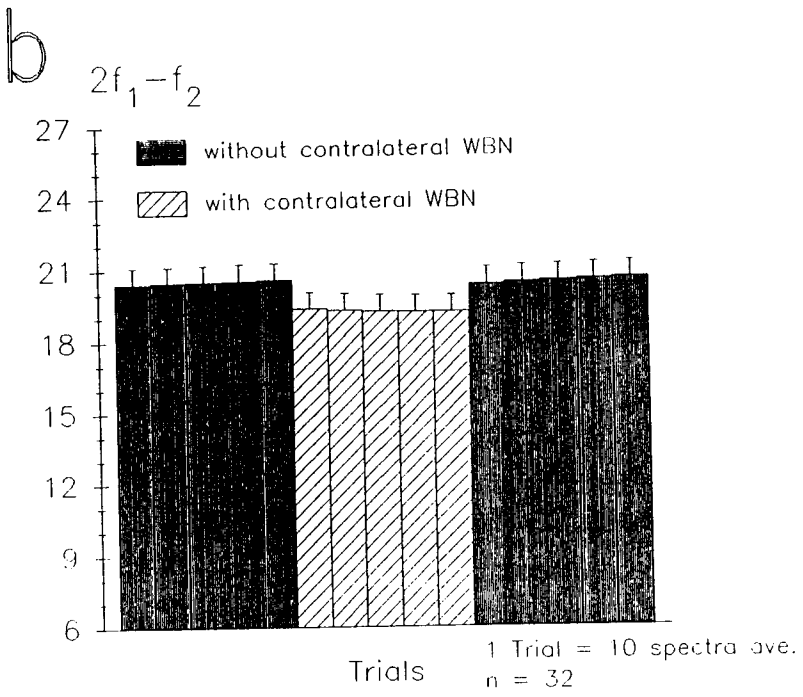
Table 2

DPOAE Amplitude Alterations (dB)

Condition (continuous primaries)	"On- Effect" (Δ A-B)	(Δ D-E)	Pre-Rest Suppression (Δ B-C)	"Off-Effect" (Δ C-D)
70 dB SPL (n=9)	+0.36	+0.95	-1.40	-0.51
60 dB SPL (n=9)	+1.02	+1.48	-2.55	-0.73
50 dB SPL (n=9)	+1.90	+3.24	-3.17	-2.01

6 Contralateral WBN suppresses BOTH f_2-f_1 and $2f_1-f_2$





OC MANIPULATIONS

7 Intracochlear bicuculline and curare reduce but do not block f_2-f_1 amplitude alterations

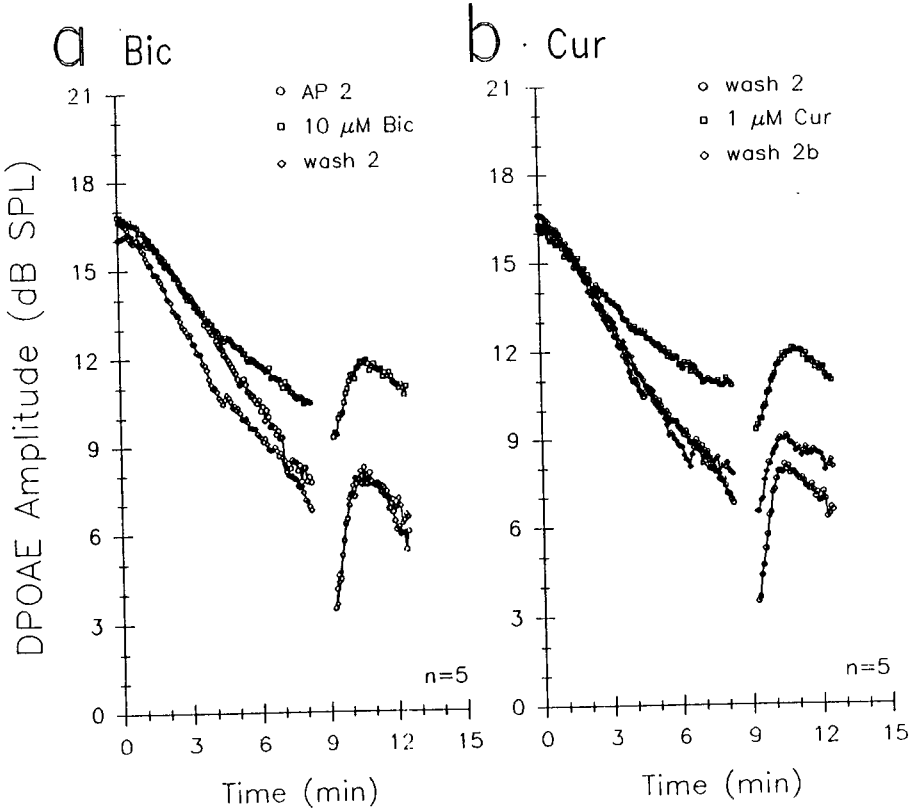


Table 3

DPOAE Amplitude Alterations (dB)

Condition	"On- Effect" (Δ A-B)	"On- Effect" (Δ D-E)	Pre-Rest Suppression (Δ B-C)	"Off-Effect" (Δ C-D)
AP2 (n=5)	-*	+4.75	-8.29	-4.26
Bic (n=5)	-	+2.62	-6.32	-1.19
W2 (n=5)	-	+4.62	-9.82	-3.31
Cur (n=5)	-	+2.77	-5.33	-1.53
W2b (n=5)	-	+2.62	-8.52	-1.30

* Point B unavailable for measurement due to stimulations immediately preceding these measures.

8 f_2-f_1 amplitude alterations are unaffected by intracochlear TTX and OC bundle section

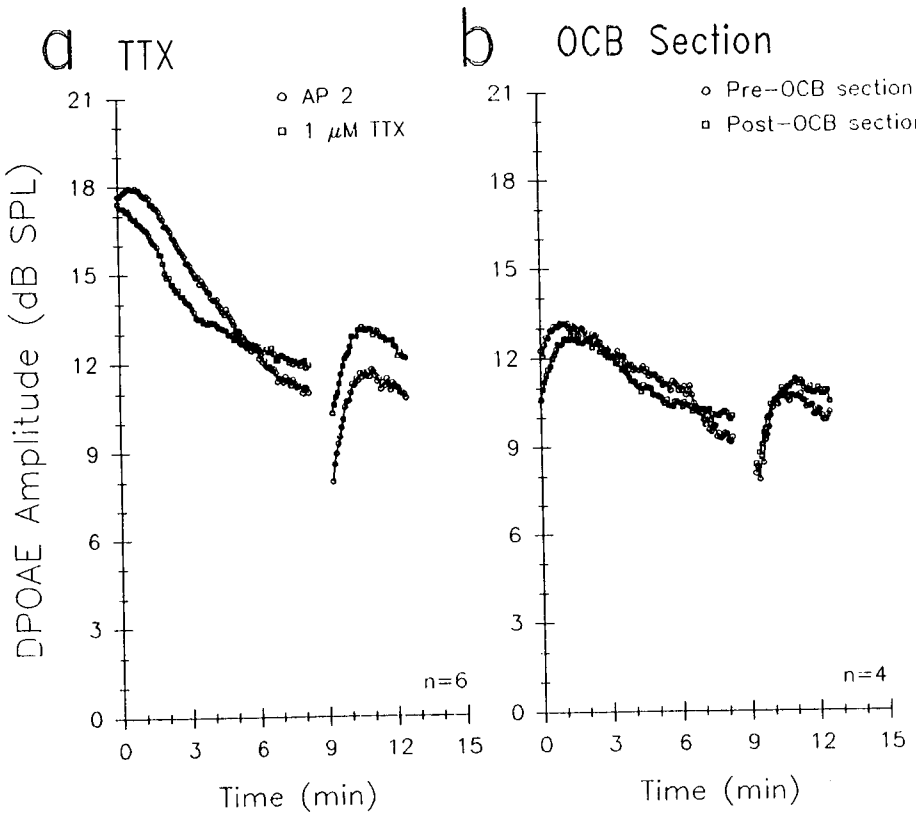


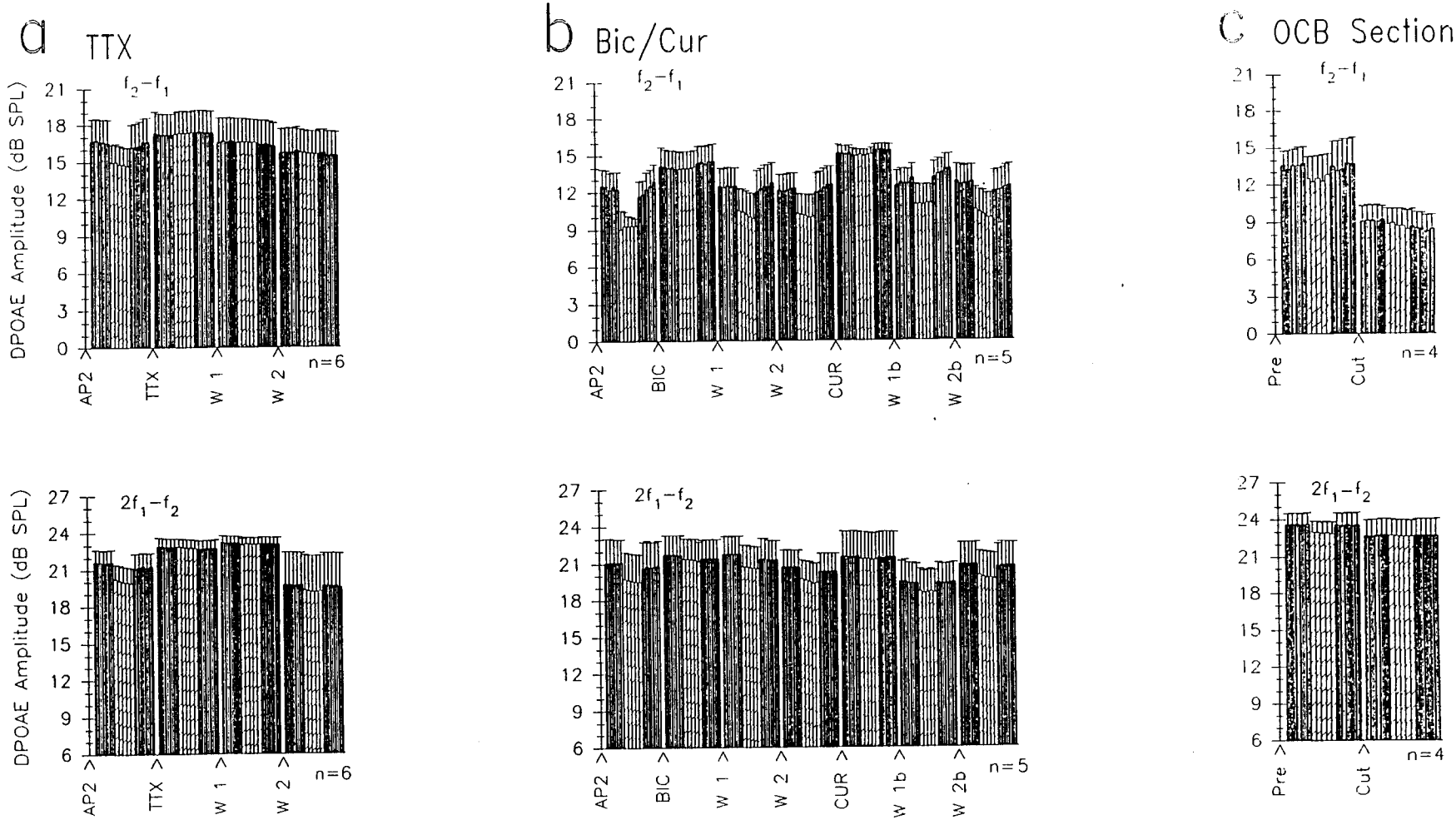
Table 4

DPOAE Amplitude Alterations (dB)

Condition	"On- Effect" (Δ A-B)	"On- Effect" (Δ D-E)	Pre-Rest Suppression (Δ B-C)	"Off-Effect" (Δ C-D)
AP2 (n=6)	-*	+3.77	-*	-3.02
TTX (n=6)	-	+2.81	-	-1.62
W2 (n=6)	-	+4.93	-	-2.72
Pre-OCB Section (n=4)	+1.09	+2.12	-2.63	-1.08
Post-OCB Section (n=4)	+2.15	+2.88	-2.74	-1.63

* Point B unavailable for measurement due to stimulations immediately preceding these measures.

9 All OC manipulations block contralateral suppression of BOTH f_2-f_1 and $2f_1-f_2$



Ca^{2+} MANIPULATIONS

10 Reducing perilymph Ca^{2+} suppresses/ reverses f_2-f_1 amplitude alterations

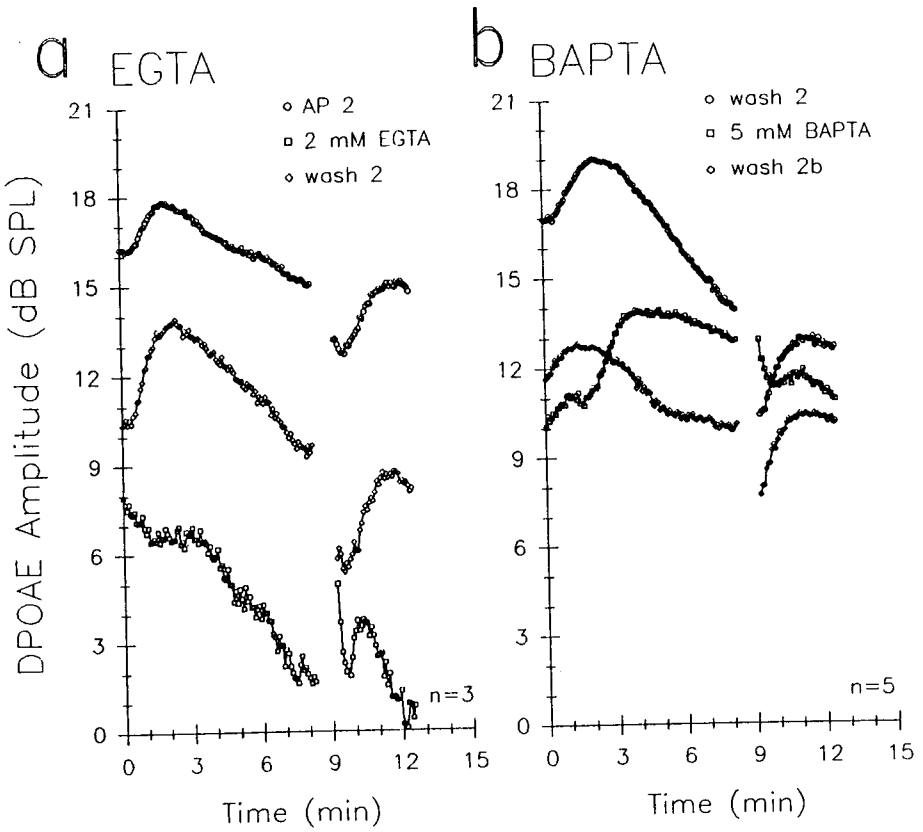


Table 5

DPOAE Amplitude Alterations (dB)

Condition	"On- Effect" (Δ A-B)	"On- Effect" (Δ D-E)	Pre-Rest Suppression (Δ B-C)	"Off-Effect" (Δ C-D)
AP2 (n=3)	+1.59	+1.85	-2.84	-1.82
EGTA (n=3)	0.00	0.00	-6.28	+3.29
W2 (n=3)	+3.53	+2.93	-4.25	-3.84
AP2 (n=5)	+2.06	+2.54	-5.12	-3.52
BAPTA (n=5)	-	-	+2.87	-0.01
W2 (n=5)	+1.18	+2.72	-2.72	-2.41

11 Mg^{2+} suppresses the "on-effect" and reverses the "off-effect"

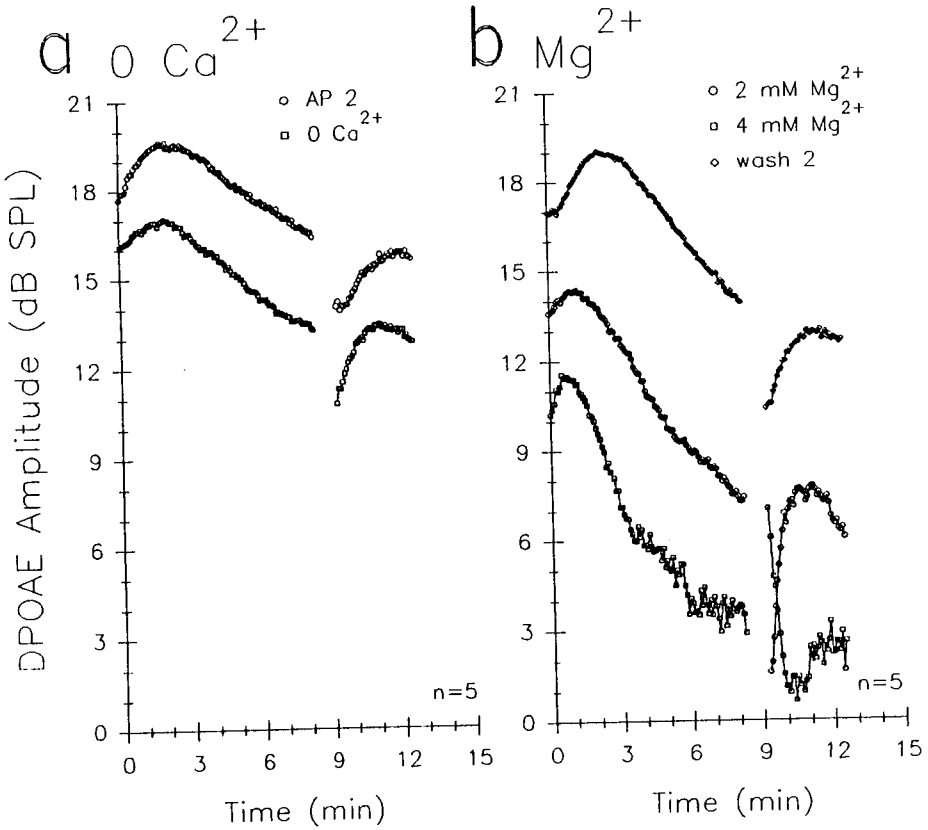
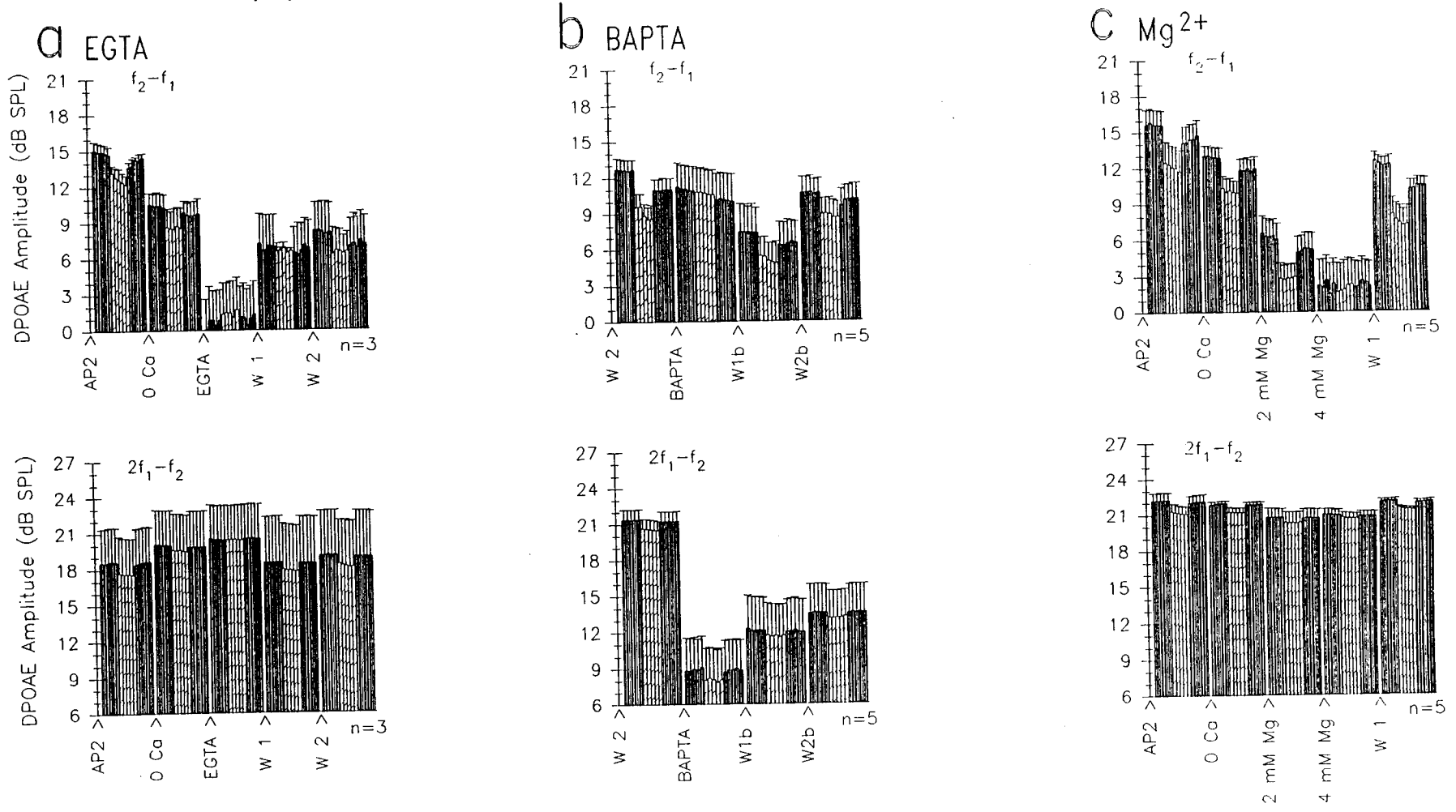


Table 6

DPOAE Amplitude Alterations (dB)

Condition	"On- Effect" (Δ A-B)	(Δ D-E)	Pre-Rest Suppression (Δ B-C)	"Off-Effect" (Δ C-D)
AP2 (n=5)	+1.93	+1.71	-3.26	-2.33
0 Ca^{2+}(n=5)	+0.94	+2.63	-3.76	-2.47
2 mM Mg^{2+}(n=5)	+0.76	+6.08	-6.94	-5.82
4 mM Mg^{2+}(n=5)	+1.33	-	-8.66	+4.15
W2 (n=5)	+2.03	+2.54	-5.09	-3.52

12 Ca^{2+}-reducing manipulations block contralateral suppression of BOTH f_2-f_1 and $2f_1-f_2$



SUMMARY OF RESULTS

1. Results confirmed previous reports of an initial f_2-f_1 DPOAE amplitude enhancement (Δ A–B; "on–effect") followed by a gradual decline in response amplitude during continuous primary stimulation (Δ B–C). Following a 1 min rest from such stimulation, however, we observed the f_2-f_1 DPOAE to be further suppressed from its pre–rest level (Δ C–D; "off–effect"). To our knowledge, this is the first report of post–rest amplitude suppression in this DPOAE. Response amplitude increased rapidly upon return to continuous stimulation (Δ D–E). These amplitude alterations (Fig. 4a) were observed in all animals tested and were stereotyped in magnitude and time course both across animals and over repeated trials in the same animal. "On–effects" (Δ A–B; (Δ D–E) reached maximum values within the first 2 min of stimulation.

2. Corresponding alterations in the amplitude of the $2f_1-f_2$ distortion product were very small and were variable in direction (Fig. 4b), consistent with previous reports [1–2,4].

3. On average, f_2-f_1 DPOAE responses to intermittent primaries (60 dB SPL) varied less than 0.1 dB over the same (approx. 13 min) period of monitoring (Fig. 5a).

4. Response alterations were intensity–dependent over the ranges tested (Fig. 5b). Specifically, larger f_2-f_1 DPOAE amplitude changes were observed at lower primary levels.

5. Introduction of a WBN to the contralateral ear suppressed ipsilaterally–recorded f_2-f_1 (1.73 ± 0.02 dB) and $2f_1-f_2$ (1.23 ± 0.05 dB) DPOAEs in all animals tested (Figs. 6a–b).

6. Amplitude alterations in the f_2-f_1 DPOAE observed during continuous primary stimulation were reduced but not blocked by the OC efferent antagonists bicuculline (10 μM) and curare (1 μM) (Figs. 7a–b). Similar findings were obtained for animals in which the cochlear aqueduct had been blocked to prevent entry of CSF.

7. Amplitude alterations in the f_2-f_1 DPOAE were largely unaffected by TTX (1 μM) which should block all action potential–mediated activity (Fig. 8a). In these same animals, TTX reduced the CAP to the level of the noise floor.

8. Amplitude alterations in the f_2-f_1 DPOAE were largely unaffected by midline section of OC neurons (Fig. 8b). These cuts should effectively remove most of the MOC efferent innervation to each cochlea but should leave the LOC efferents essentially intact.

9. Amplitude alterations in the f_2-f_1 DPOAE were suppressed, prevented or reversed in AP solutions which reduced available Ca^{2+} (zero Ca^{2+} with 2 mM EGTA, zero Ca^{2+} with 5 mM BAPTA; Figs. 10a–b) or replaced it with another divalent cation, Mg^{2+} (zero Ca^{2+} with 2 or 4 mM Mg^{2+}; Figs. 11a–b).

10. All of these manipulations prevented the known efferent–mediated contralateral suppression of DPOAEs (f_2-f_1 and $2f_1-f_2$; Figs. 9a–c, 12a–c). Moreover, several of the manipulations were associated with increases in the absolute amplitudes of the DPOAEs (Figs. 9a–b) suggesting that the manipulations interfered with a tonic suppressive efferent influence on the cochlear mechanical response to sound.

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2 Kirk, D. L. and Johnstone, B. M. (1993) Modulation of f_2-f_1: Evidence for a GABA–ergic efferent system in apical cochlea of the guinea pig. Hear. Res. 67, 20–34.

3 Kujawa, S. G., Glattke, T. J., Fallon, M. and Bobbin, R. P. (1994) A nicotinic–like receptor mediates suppression of distortion product otoacoustic emissions by contralateral sound. Hear. Res. 74, 122–134.

4. Whitehead, M. L., Lonsbury–Martin, B. L. and Martin, G. K. (1991) Slow variation of the amplitude of acoustic distortion at f_2-f_1 in awake rabbits. Hear. Res. 51, 293–300.

ACKNOWLEDGMENTS

The authors are grateful to Ted Glattke for discussions and Han Wen for programming assistance. This work was supported by NIH DC00007, DC00722 and DAMD 17–93–V–3013.

APPENDIX 2

Additional figures for the animal studies.

CHAPTER: PROTECTING THE AUDITORY SYSTEM AND PREVENTION OF HEARING PROBLEMS

PROJECT DIRECTORS: RICHARD P. BOBBIN, PH.D.
CHARLES I. BERLIN, PH.D.

OC MANIPULATIONS

7 Intracochlear bicuculline and curare reduce but do not block f_2-f_1 amplitude alterations

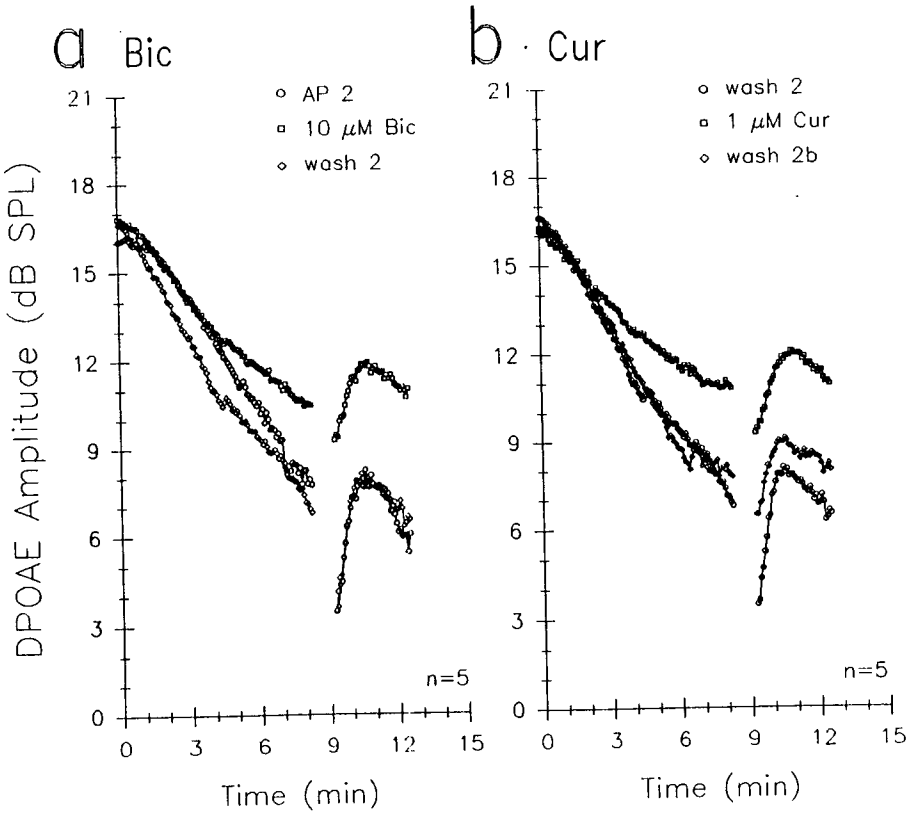

Table 3

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* Point B unavailable for measurement due to stimulations immediately preceding these measures.

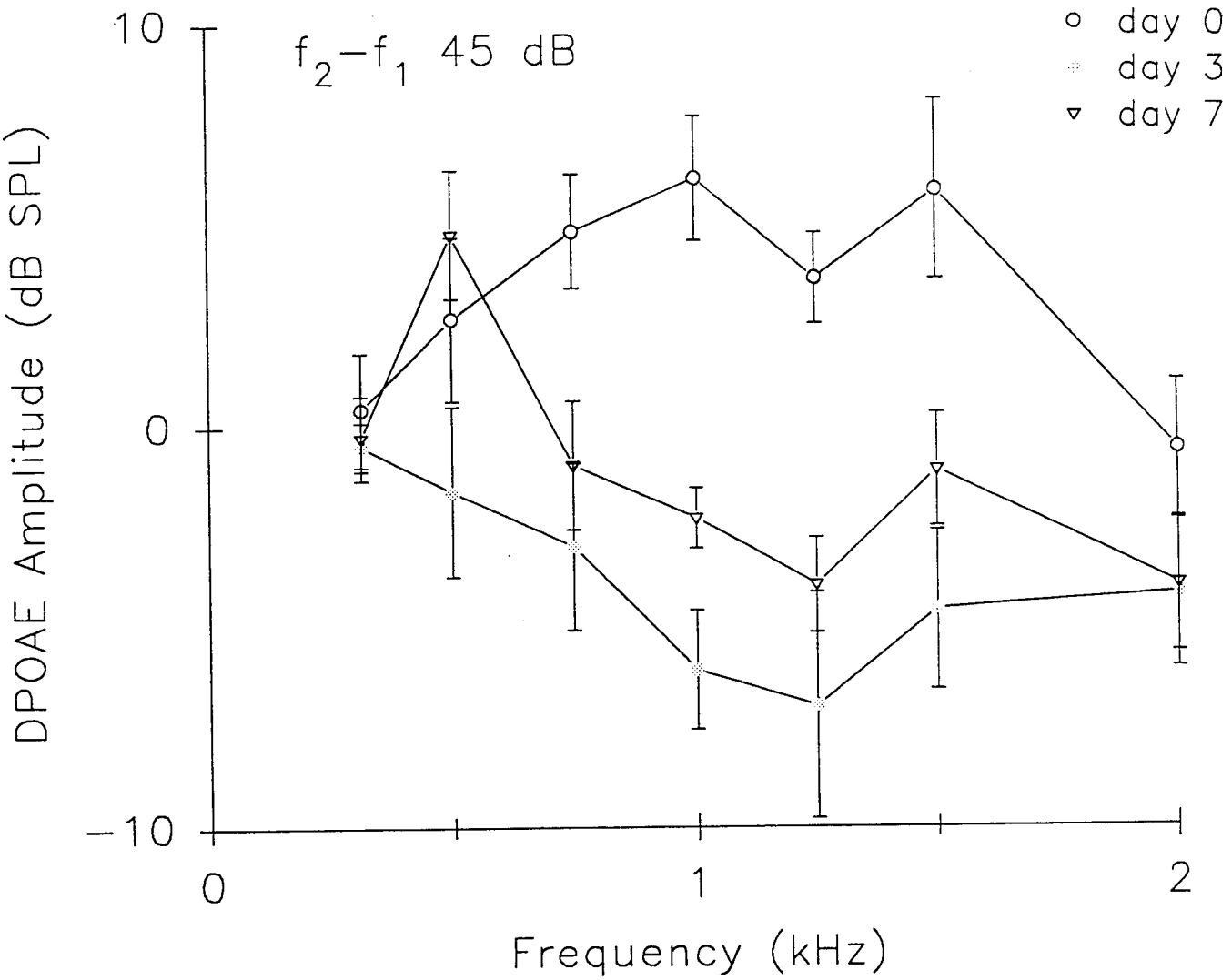
Figure Legends

Figure 1. Audiogram of the effects of toughening sound at 0 days, 3 days and 7 days on f_2-f_1 at 45 dB. The figure illustrates the suppressive effects at day 3 and the trend towards recovery at day 7. Data points indicate means plus or minus standard error for five animals for each day.

Figure 2. The contralateral wide band noise decreases the amplitude of the f_2-f_1 DPOAE to a greater extent at day 7 than at day 0 of toughening sound. Data points indicate means plus or minus standard error for five animals for each day.

Figure 3. Eleven days of toughening sound exposure (noise exposure) abolishes the "on-effect" and "off-effect" and increases the rate of the slow decrease. Data points indicate means for six animals.

Figure 4. Eleven days of toughening sound exposure (noise) significantly decreases the current response of outer hair cells to ATP.



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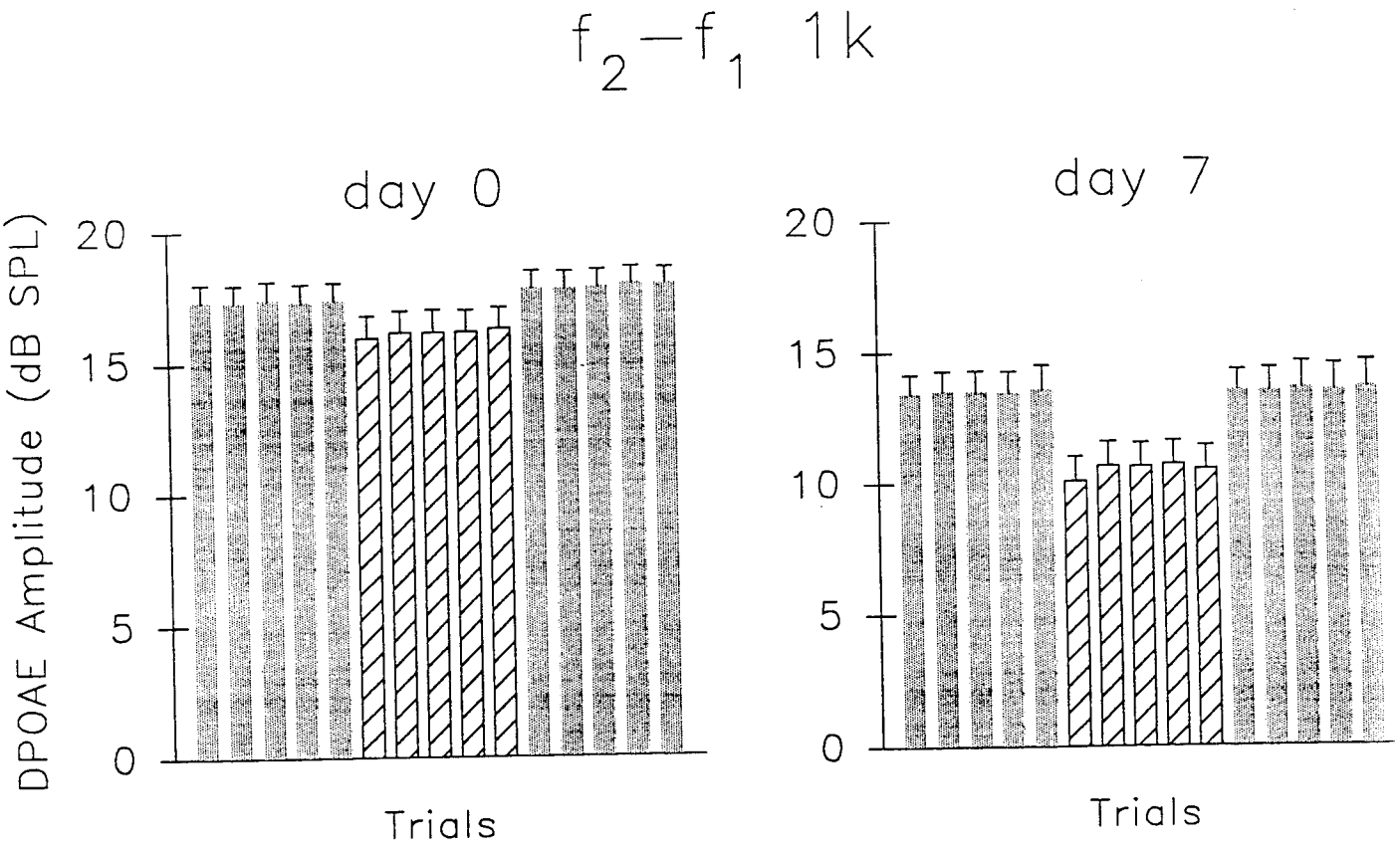

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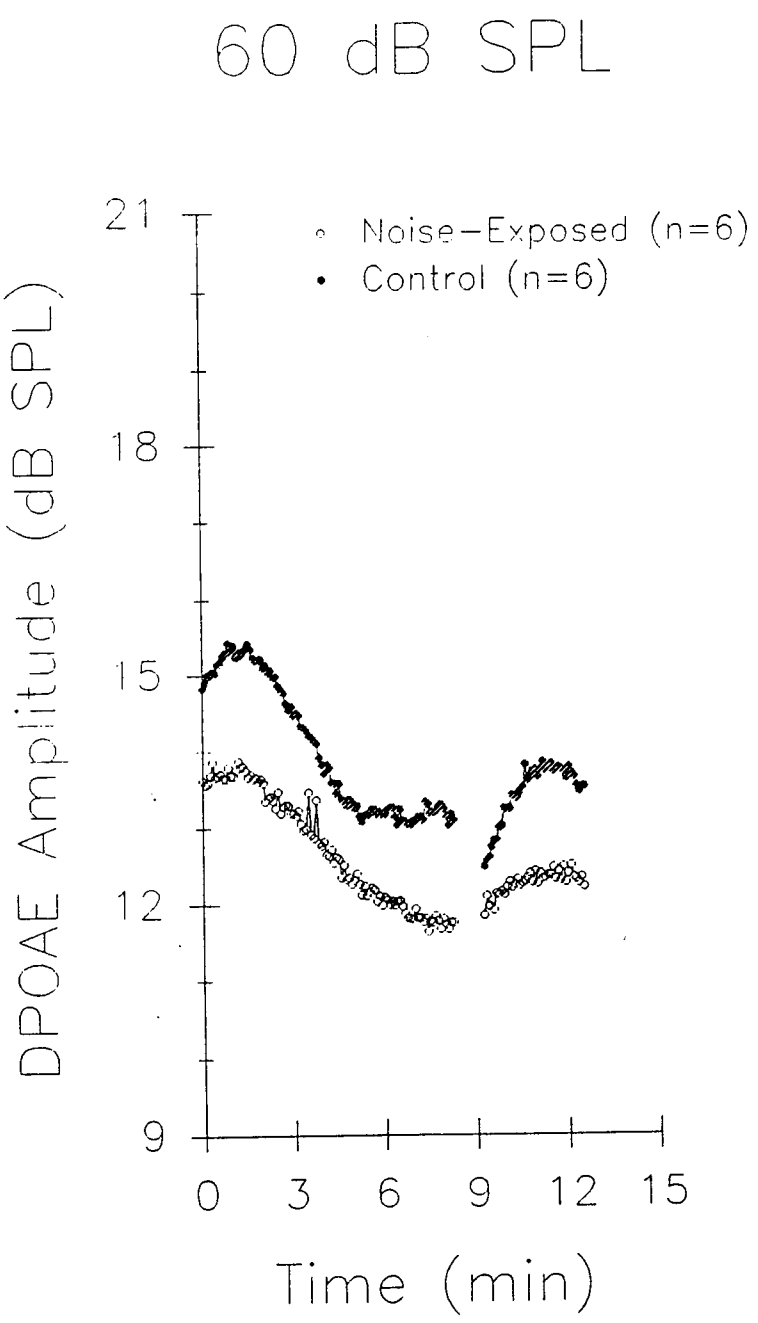

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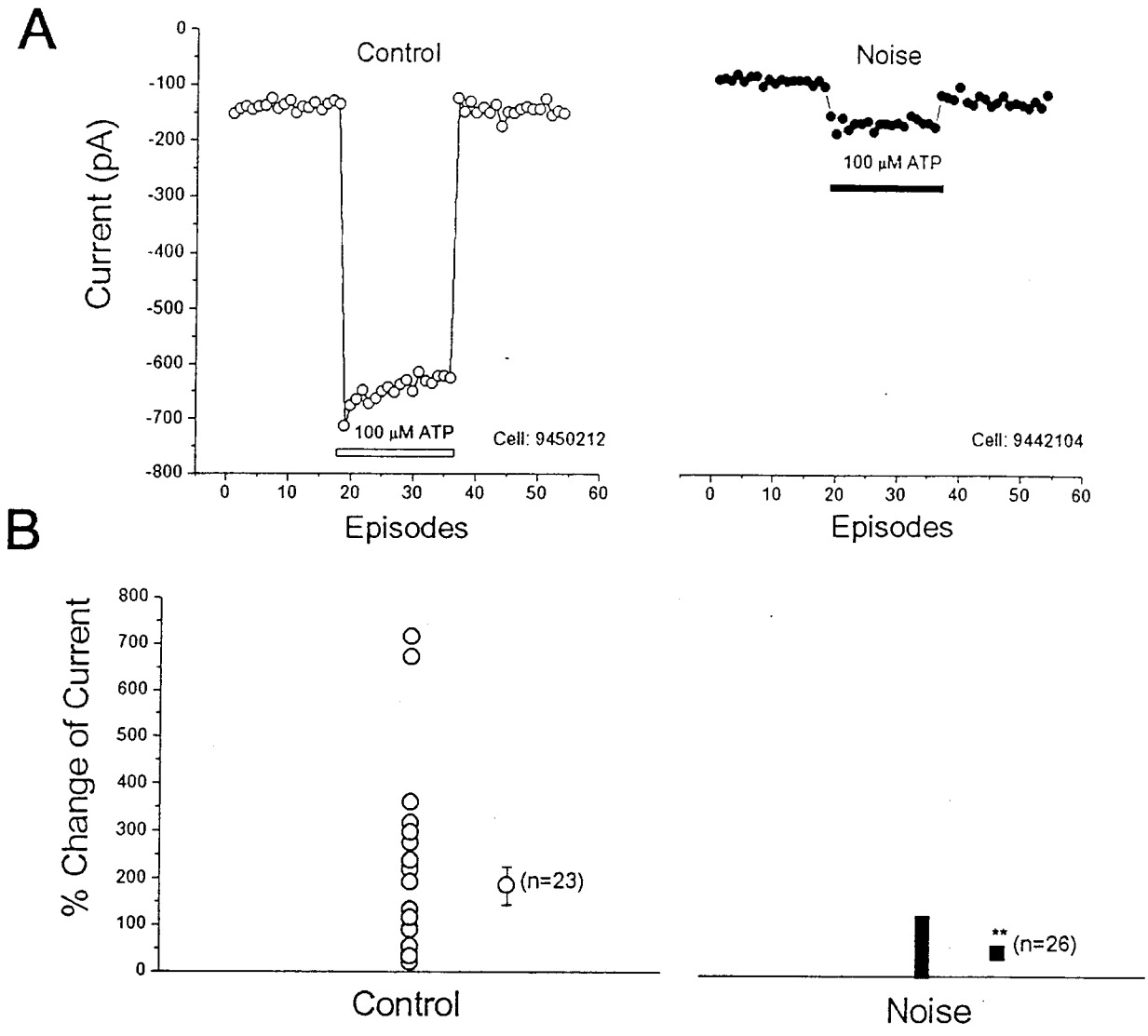

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APPENDIX 3

Figures for the human studies.

CHAPTER: PROTECTING THE AUDITORY SYSTEM AND PREVENTION OF HEARING PROBLEMS

PROJECT DIRECTORS: RICHARD P. BOBBIN, PH.D.
CHARLES I. BERLIN, PH.D.

Figure Legend

Figure 1. Contralateral suppression of linear clicks (50 to 70 dB peak Sound Pressure) by white noise. The white noise was presented in two dB steps from 10 dB below the click to 10 dB above the click. The largest shifts are seen when the clicks were at 55 dB peak Sound Pressure, approximately 17 dB HLn.

Figure 2. Setting up the efferent forward suppression paradigm for binaural, ipsilateral and contralateral noise.

Figure 3. Binaural noise suppresses emissions more than ipsilateral or contralateral noise in the forward masking paradigm.

Figure 4. The effects of 1,10,50, and 200 msecs time separation on emission suppression.

Figure 5. Further differentiation of separation time as a factor in binaural noise suppression.

Figure 6. Test-re-test reliability in the binaural experiment.

Figure 7. Duration of the noise becomes more effective in suppression above 80 msecs.

Figure 8. Female subjects show more suppression to binaural noise when the target click is in the right ear than the left.

Figure 9. Male subjects show more suppression to binaural noise when the target click is in the left ear than the right.

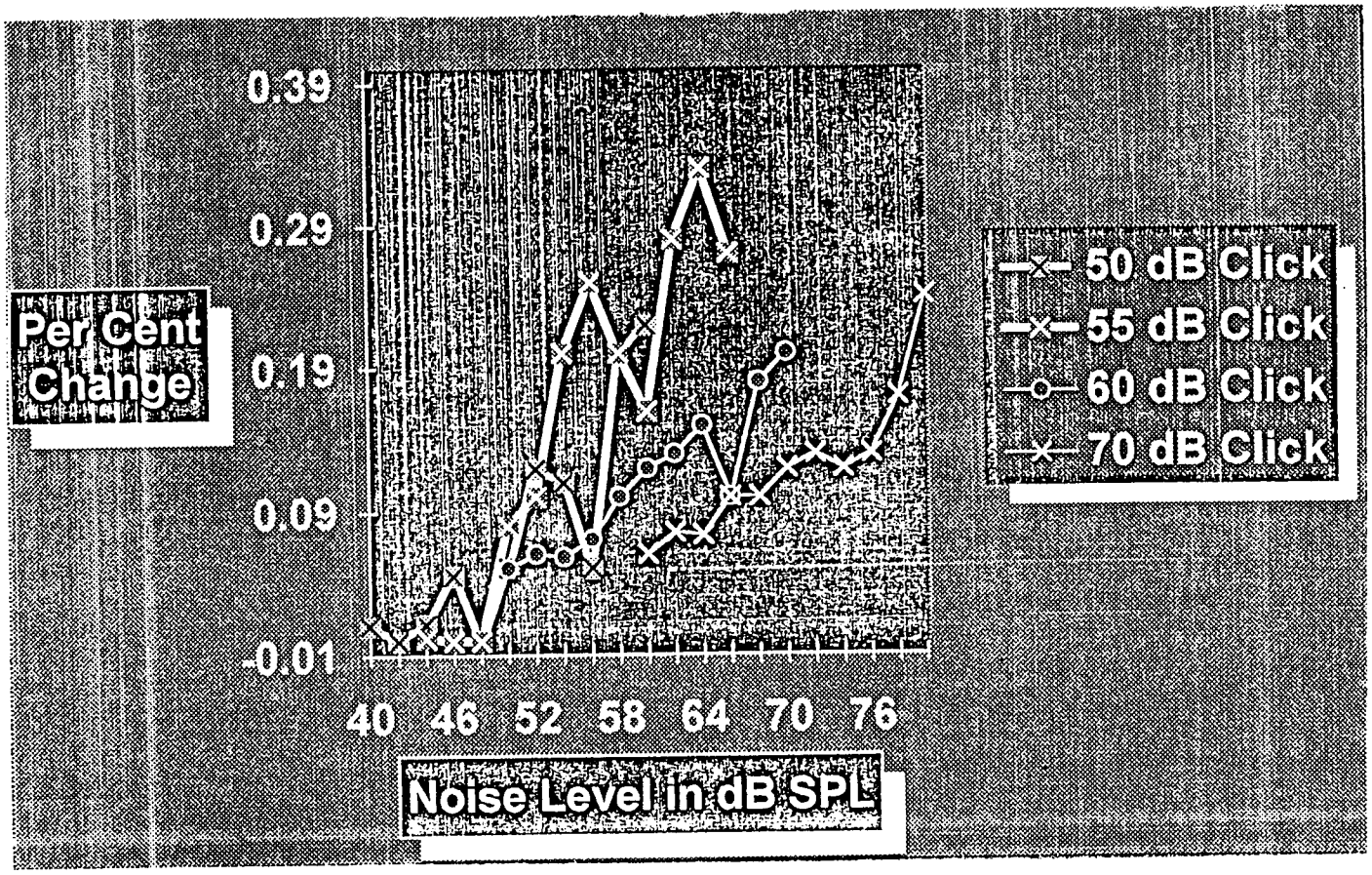

0.39
0.29
0.19
0.09
-0.01
Per Cent Change
40 46 52 58 64 70 76
Noise Level in dB SPL
50 dB Click
55 dB Click
60 dB Click
70 dB Click

Figure 1.

Setting up the efferent suppression paradigm

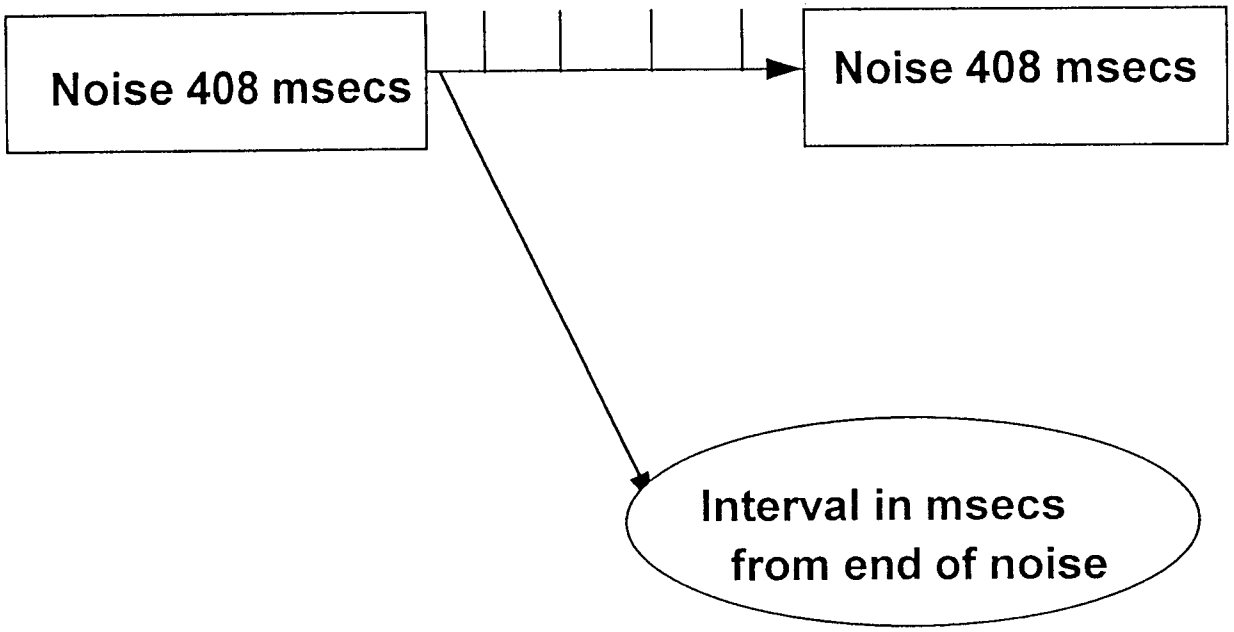


Figure 2.

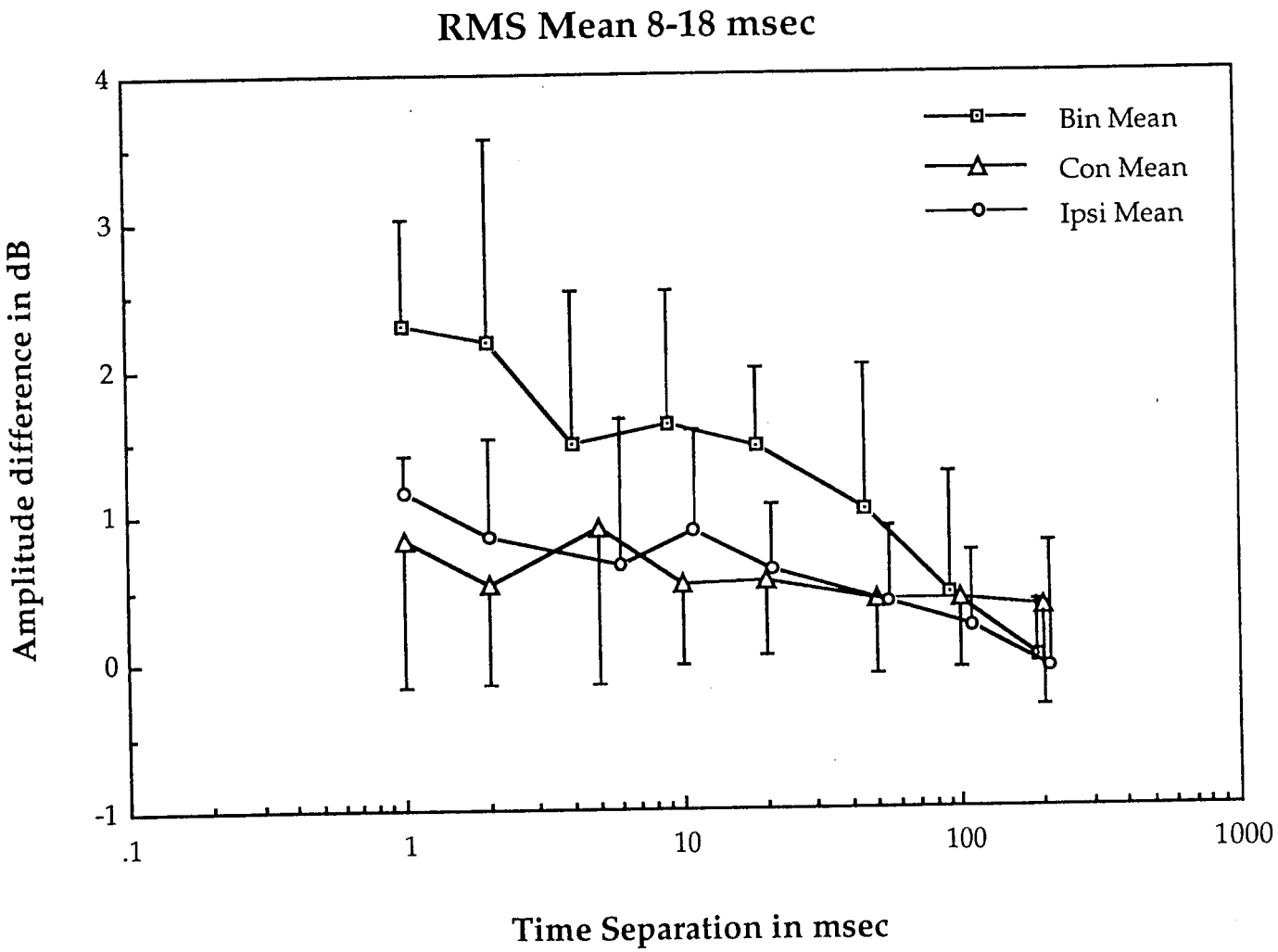

RMS Mean 8-18 msec
Bin Mean
Con Mean
Ipsi Mean
Amplitude difference in dB
4
3
2
1
0
-1
.1
1
10
100
1000
Time Separation in msec

Figure 3.

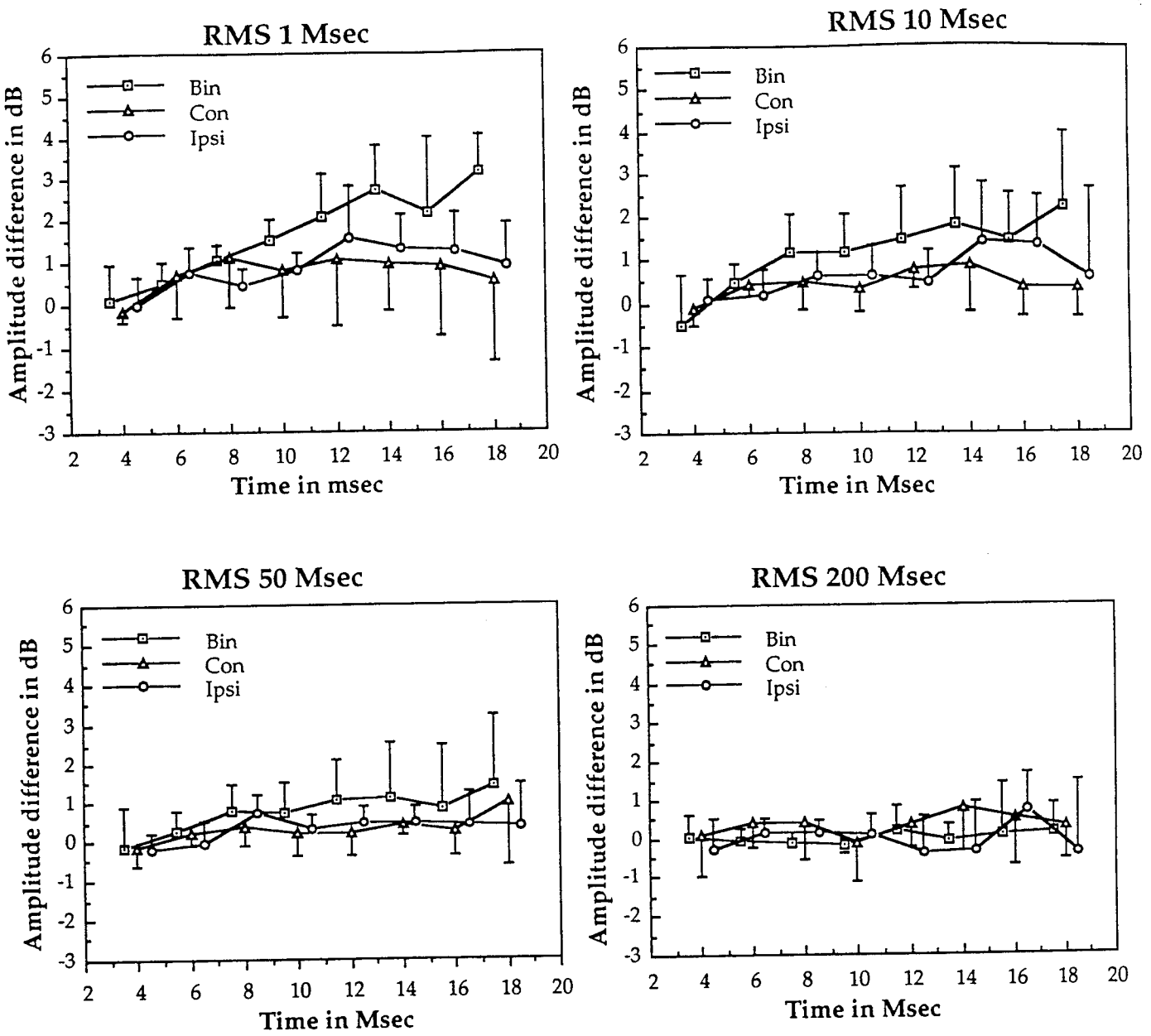

RMS 1 Msec
RMS 10 Msec
RMS 50 Msec
RMS 200 Msec
Bin
Con
Ipsi
Amplitude difference in dB
Time in msec
Time in Msec

Figure 4.

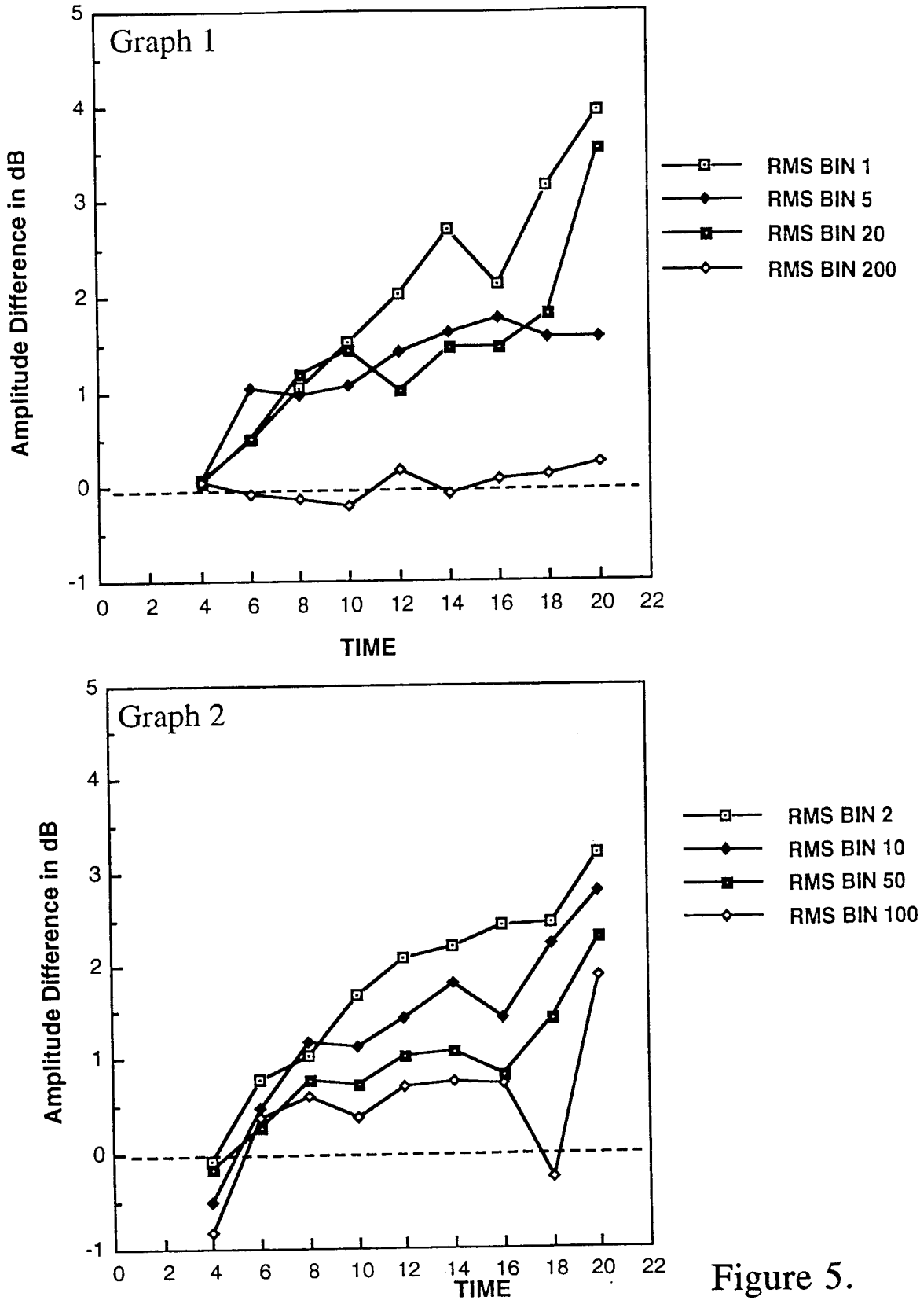

Graph 1
Amplitude Difference in dB
TIME
RMS BIN 1
RMS BIN 5
RMS BIN 20
RMS BIN 200
Graph 2
Amplitude Difference in dB
TIME
RMS BIN 2
RMS BIN 10
RMS BIN 50
RMS BIN 100

Figure 5.

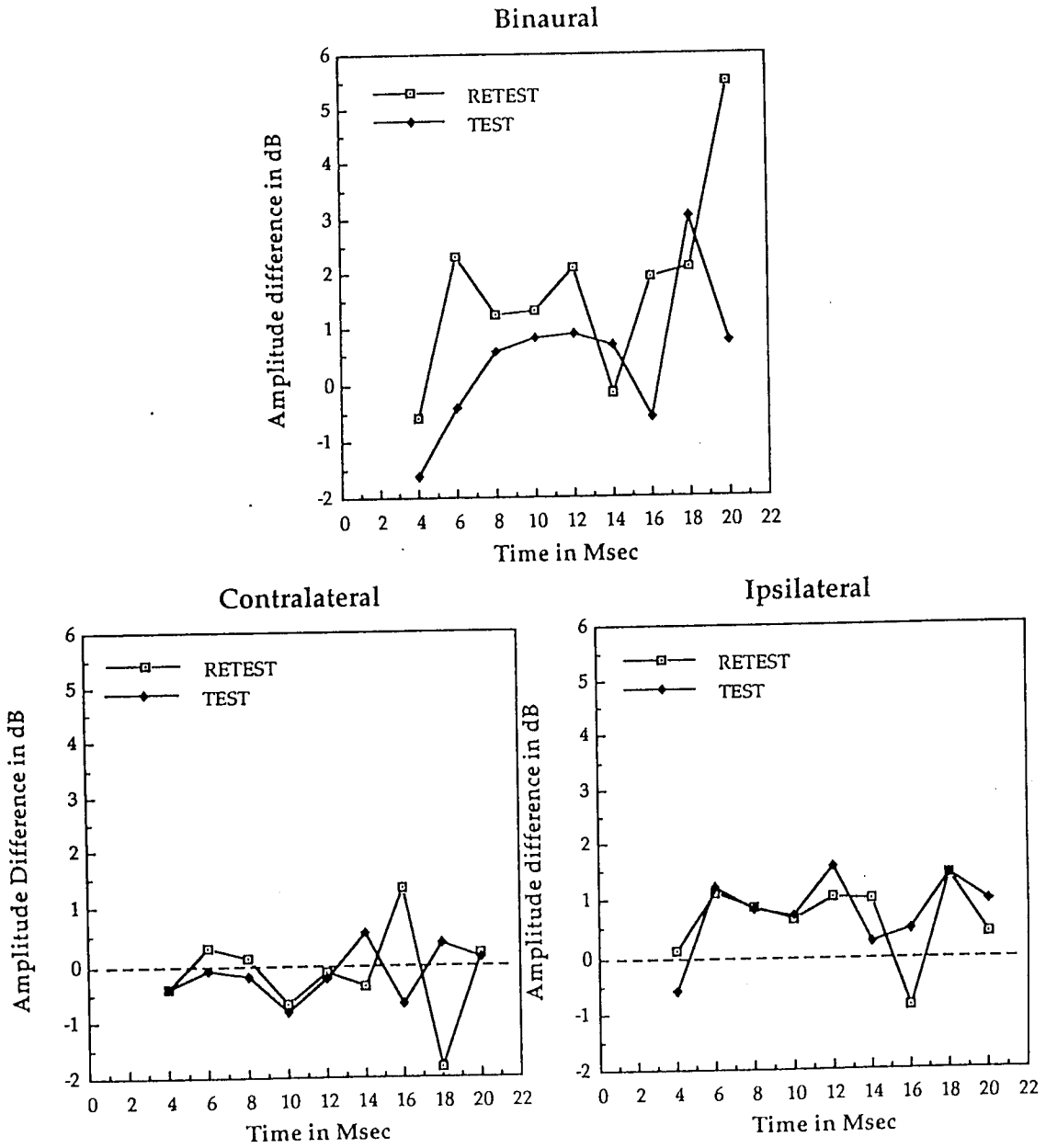

Binaural
RETEST
TEST
Amplitude difference in dB
Time in Msec
Contralateral
RETEST
TEST
Amplitude Difference in dB
Time in Msec
Ipsilateral
RETEST
TEST
Amplitude difference in dB
Time in Msec

Figure 6.

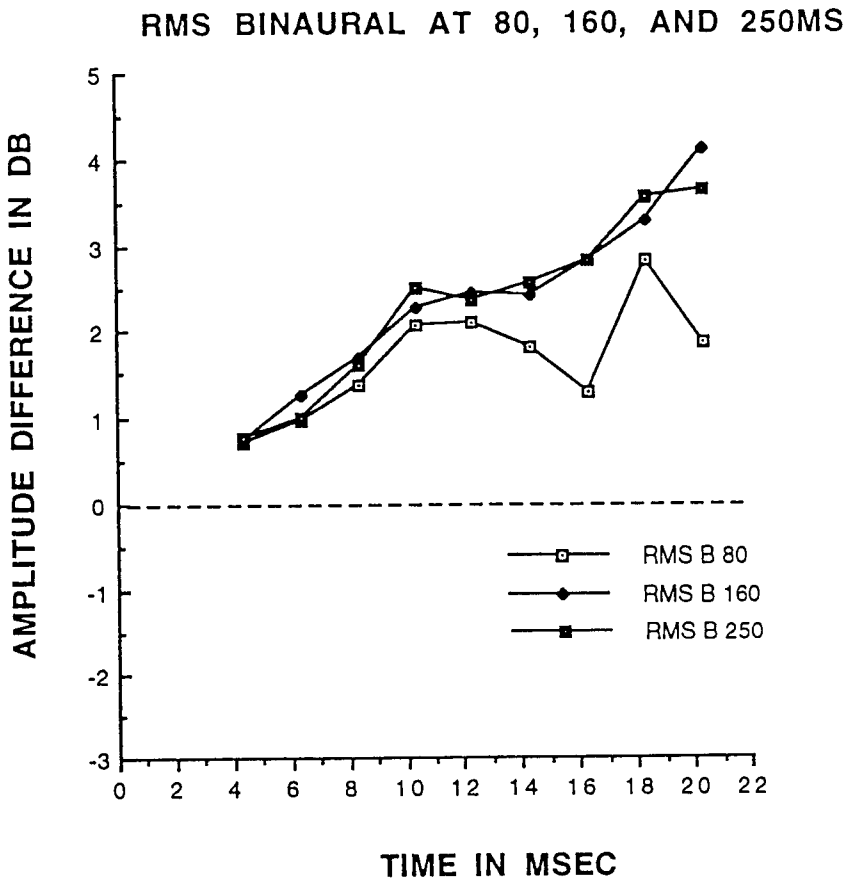
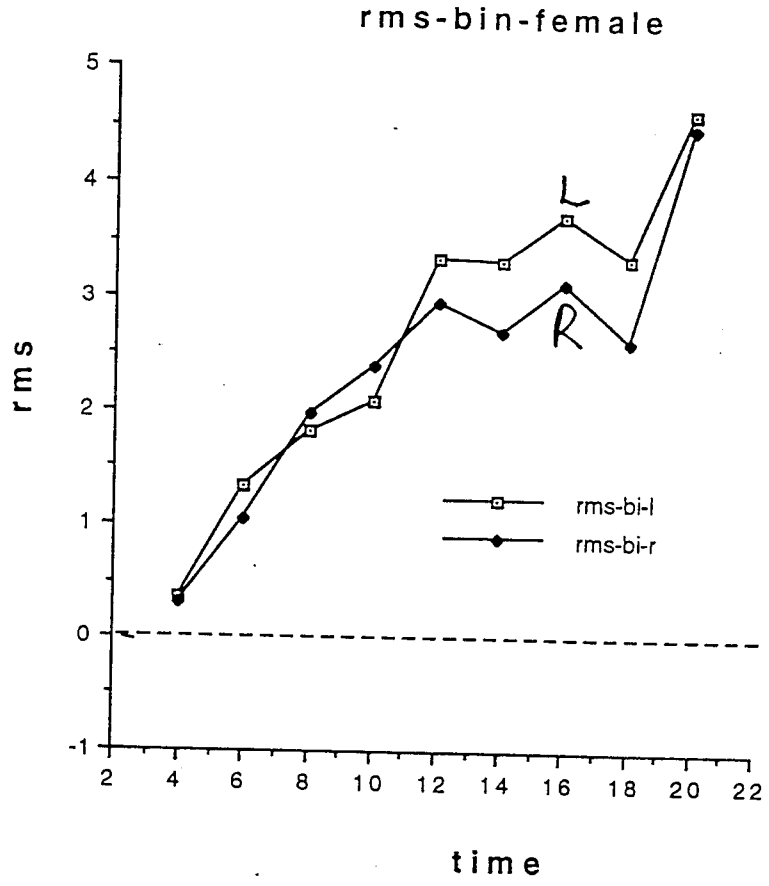

RMS BINAURAL AT 80, 160, AND 250MS
AMPLITUDE DIFFERENCE IN DB
5
4
3
2
1
0
-1
-2
-3
0
2
4
6
8
10
12
14
16
18
20
22
TIME IN MSEC
RMS B 80
RMS B 160
RMS B 250

Figure 7.


rms-bin-female
rms
time
rms-bi-l
rms-bi-r
L
R
5
4
3
2
1
0
-1
2
4
6
8
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22

Figure 8.

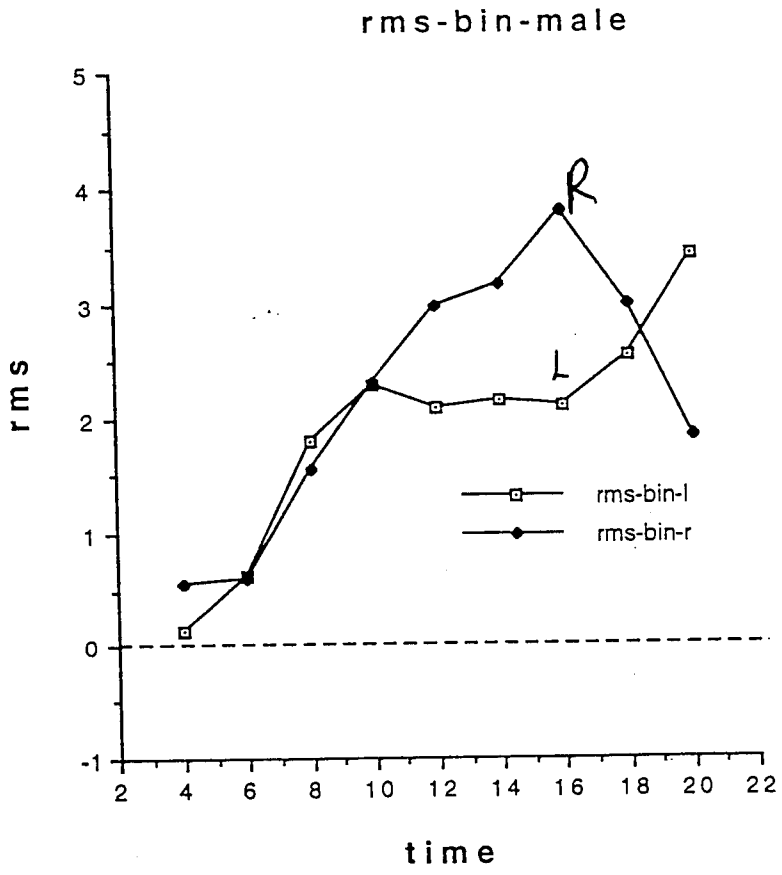
rms-bin-male
rms
time
rms-bin-l
rms-bin-r
R
L
5
4
3
2
1
0
-1
2
4
6
8
10
12
14
16
18
20
22

Figure 9.

APPENDIX 4

Berlin, C.I., Hood, L.J., Hurley, A.H., Wen, H., and Kemp, D.T., Binaural noise suppresses linear click-evoked otoacoustic emissions more than ipsilateral or contralateral noise.

CHAPTER: PROTECTING THE AUDITORY SYSTEM AND PREVENTION OF HEARING PROBLEMS

PROJECT DIRECTORS: RICHARD P. BOBBIN, PH.D.
CHARLES I. BERLIN, PH.D.

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Binaural Noise Suppresses Linear Click-evoked Otoacoustic Emissions More than Ipsilateral Or Contralateral Noise

Berlin, C.I., Hood. L.J., Hurley, A.H., Wen, H., and Kemp, D.T.*

LSU MEDICAL CENTER, DEPARTMENT OF OTORHINOLARYNGOLOGY AND BIOCOMMUNICATION, KRESGE HEARING RESEARCH LABORATORY OF THE SOUTH.

*INSTITUTE OF LARYNGOLOGY AND OTOLOGY, LONDON, ENGLAND.

Abstract:

Using a specially programmed ILO88 system, we stimulated left, right, or sometimes both, ears with 408 msecs of 65 dB SPL white noise. Each burst of noise preceded a series of four 80 usec 65 dB peak Sound Pressure clicks to the left ear only. The first click of the group followed the end of the noise by either 1, 2, 5, 10, 20, 50,100 or 200 msecs. Conditions were alternated so that a "without noise" condition preceded a "with noise" condition for three repetitions of 600 clicks per trial. Seven subjects participated in the study and three of the seven participated in a test-retest reliability study. Results showed the greatest suppression occurred following binaural stimulation ending within one to five msecs of the beginning of the pulse train. Less suppression occurred to ipsilateral or contralateral stimulation; in all cases there was little suppression when the end of the noise was 100 msecs or more away from the beginning of the click train.

Introduction

The medial olivocochlear system suppresses segments of outer hair cell activity when activated either contralaterally, ipsilaterally or bilaterally with an auditory stimulus of sufficient duration (Warr et al., 1986; Warr and Guinan, 1978; Puel and Rebillard, 1990; Liberman, 1989; Kujawa et al., 1991).

Previous experiments on suppressing otoacoustic emissions in humans have focused on the suppressive effects of continuous contralateral stimulation only (e.g., Collet et al. 1990; Ryan et al., 1991; Berlin et al., 1993 a, b; Berlin et al. 1994.)

In this work we used seven normal hearing human subjects to show the suppressive effects of binaural, ipsilateral, and also contralateral stimulation with 408 msecs of white noise in a forward masking paradigm.

Methods

One of us (D.K.) supplied the program for an ILO88 system to control the temporal interval between the offset of a duration-controlled noise stimulus and the onset of a 4-click train. We selected a 408 msec duration noise based on the work of Liberman (1989) and Huang et al. (1993) to maximize the likelihood of activating the efferent system. The emission-evoking 80 usec clicks were all 65 dB peak sound pressure and all of the same polarity (the

so-called linear mode in the ILO88 system). The first click of the group followed the end of the noise by either 1, 2, 5, 10, 20, 50, 100, or 200 msecs, whereas the final three clicks followed the first click by successive increments of 20 msecs. Figure 1 is an example of the paradigm. Conditions were alternated so that a "without noise" condition preceded a "with noise" condition for three complete trials. The order of ipsilateral vs. contralateral, vs. bilateral presentation of the noise was distributed equally throughout the 24 different listening conditions. Three subjects ran twice in the experiment at selected time separations to establish test-retest reliability.

Each set of experimental vs. control data consisted of the mean of three "without noise" trials, and three "with noise" trials, compared to one another using two separate quantification systems. In one case we compared the mean echoes to each other using the Kemp aggregate echo level number, which we called "dB ILO". This number represents the RMS value of the amplitude of the echoes calculated by the ILO88 system over a 20.48 msec window. The second method used the Kresge Echomaster system (Wen et al. 1992) which allows custom designed amplitude, time and frequency comparisons between means of control and experimental conditions. We chose to quantify the RMS differences between the echoes in two msec segments and labeled this number " dB-K" to differentiate it from the overall RMS number available from the ILO88 system. The Kresge Echomaster system also allowed us to make temporal comparison of

difference between segments of the control and experimental echoes in 40 usec steps . However, we analyzed the spectral differences between the echoes over the entire 20.48 msec window because of the paucity of digital addresses available within shorter epochs.

Results

The binaural noise condition generated 1.5 to 2 dB ILO of emission suppression when the noise preceded the first click in the train by one to twenty msecs. Thereafter the suppressive effects decreased as time-separation increased. The amount of suppression to binaural noise shown in Figure 2 is 2.5 to 4 dB K between 13 and 18 msecs after the click stimulation. The values for contralateral and ipsilateral suppression are considerably smaller in this forward masking paradigm. Figure 3 compares suppressive effects of binaural, ipislateral and contralateral noise at 1, 10, 50 and 200 msecs time separations between the end of the noise and start of the first click. The suppression is expressed in db K.

Figure 4 offers another view of the same data. Here we compare the data in dB K over the four to 20 msec period of the KEM 30 program analysis, showing the binaural data only at 1, 5, 20, and 200 msecs. Then in the companion figure we show similar binaural data for time-separations of 2, 10, 50 and 100 msecs. Again three primary trends are evident:

(1) The shorter the time-separation between the end of the noise and first click, the greater the suppressive effect.

(2) Suppression of 2.5 to 3.5 dB K takes place in the eight to eighteen msec zone after click onset.

(3) Binaural noise generates more efferent suppression than either ipsilateral or contralateral noise.

Figure 5 shows a sample of the spectral data through a Hanning window available in the KEM 30 program. Here we see figures 5a, 5b, and 5c displaying a gradual shift in the largest spectral difference (at 2344 Hz) from 6.328 dB of binaural-noise-induced-suppression when the first click followed the noise by only one msec, dropping to 2.267 dB at 100 msec time separation, and then down to 0.394 dB at 200 msecs time separation for one representative subject (CB).

We checked test-re-test reliability for three subjects. Data for one subject are shown in Figure 6. Group analysis shows reliability as high as .62 for the binaural data at 1 msec time-separation but only .13 for the ipsilateral data and .046 for the contralateral data. This observation suggests that the binaural effects are clearly more robust and more reliable than either ipsilateral or contralateral stimulation in this forward masking paradigm with humans.

Discussion

There are two major efferent systems which can affect the emissions reaching the recording microphone: the middle ear muscle reflex efferent motor loop, and the olivocochlear efferents. The click stimuli in this experiment were presented at 65 dB peak sound pressure, which was 22 dB HL, a level far too faint to evoke a clinically measurable middle ear muscle reflex. The threshold for the noise was 22 dB SPL; therefore, the 65 dB SPL noise was only 43 dB HL, also a level far too low to elicit a clinical middle ear muscle reflex.

The suppressive effects are actually largest when the clicks and the noise are at lower intensities.. We know this from separate work completed after this present experiment had started, which showed us that the suppressive effects of contralateral noise are largest when the clicks are at 55 dB peak sound pressure; the relative effects diminish when either the noise or the clicks are presented at higher intensities (Hood et al. 1994), a phenomenon also reported by Collet et al. (1990).

Finally, studies of people with no middle ear muscle function (e.g., Collet et al., 1990; Berlin et al., 1993) all suggest that the middle ear muscle reflex did not participate in this experiment, although of course it is impossible to completely rule out subclinical middle ear muscle contraction in any one subject.

Still another potential confounding problem in such experiments is acoustic crosstalk. If standard ear phones

such as TDH-39 with MX 41-AR cushions were to be used, crossover by bone conduction or even partly by air conduction could conceivably take place at levels as low as 40 dB Hearing Level. However, in this experiment we used insert earpieces from the Kemp system; the psychophysical crossover in similar insert earphones exceeds 70-90 dB in frequencies below 1000 Hz and 60-70 dB in frequencies above 1000 Hz .

Liberman and Brown (1986) showed almost no efferent suppression in cats stimulated by 25 msecs or less of noise stimulation; optimum durations to activate the efferents were reported to reach an asymptote between 50 and 500 msecs. Within the limits of the technical difficulties to be described, our findings suggest that the human efferent system overlaps at least one part of the time-frame seen in cats.

Binaural stimulation in the forward masking paradigm predictably elicited more robust and more reliable efferent suppression of evoked otoacoustic emissions than either ipsilateral or contralateral stimulation. In absolute numerical terms, however, more suppression is seen with continuous 60 dB SPL contralateral noise stimulation when the click is at 55 dB peak Sound Pressure (ca. 17 dB HL) than we see in the binaural condition in this forward masking experiment. (Hood et al., 1994; Berlin et al., 1994). This observation is to be expected because of the forward masking nature of the paradigm; the continuously running noise paradigm would confound

data collection during conditions of ipsilateral and binaural efferent stimulation.

Technical Difficulties:

We recognize several problems with the data presentation in this experiment . Because of constraints in the available software, the click stimuli could only be delivered in packets of four stimuli per stimulation unit. Note from Figure 1 that clicks 2, 3, and 4 followed the preceding pulses by 20 msecs respectively. Thus, when we described a click train as beginning "one msec after the end of the noise," it was only the first of the four clicks that was one msec away from the end of the noise. The other three pulses were 21, 41, and 61 msecs away from the end of the noise respectively. Yet each of the responses to the clicks is added into the average obtained by the ILO88. Similarly, in the fifty msec condition the last three clicks were presented 71, 91, and 111 msecs after the end of the noise. Thus, whatever efferent effects we report here are likely to have been attenuated because three-quarters of the 600 clicks used to comprise a single file were 20 to 60 msecs later than the intended time-relationship to the end of the noise. If we were to present only a single click after the end of the noise, the 80 msec window averaging paradigm used by the ILO88 would still include the data from the three subsequent empty "vacated" bins as "noise" and would attenuate the

apparent size of the averaged evoked emissions by a factor of three.

Secondly, the broad-band click and broad-band noises potentially ignore frequency specificity reported in experiments of this sort (e.g., Liberman et al. 1989). Thus an improved experiment would have all of the echo-evoking stimuli in the same time registration with respect to the end of the noise, would include the echoes from only a single 20.48 msec bin following the click, and would focus on various frequency bands, where presumably the effects might be even larger than we report here.

Of what value is a three to six dB effect in the auditory system?

A forward masking effect of 3 to 6 dB K suppression of hair cell activity, which we saw when both ears were exposed to approximately a half-second of noise, would be even larger if it could be measured while the noise were continuously active. This work supports Liberman's prediction that 200 msecs or more durations of noise would adapt outer hair cell function leading to a change in the excitation pattern of inner hair cells and single units. Liberman proposed that the presence of the noise probably changed the baseline operating characteristics of the outer hair cells and would adapt single units faster, in preparation for upcoming transients ; if such a shift were to be applied to the sharply rising edge of a speech

intelligibility curve such as the articulation index, it could conceivably improve connected speech intelligibility in a borderline noisy situation by as much as 40 to 60% (see for example Humes et al. 1986; Pavlovic, 1994; Hood, Berlin and Parkins, 1991). Thus, shifts in the baseline from which listening in noise takes place, or anti-masking phenomena as outlined by Hirsh (1948), Licklider (1948) Nieder and Nieder (1970), Kawase et al.,(1993) or Kawase and Liberman (1993), might arise from outer hair cell changes controlled by the efferent nervous systems which helps to facilitate listening in noise. We would not expect the effects of efferent function to be dramatic and outstanding in humans (cf. Scharf et al. 1994) without the conditions which mimic real-life listening conditions, including (preferably) binaural presence of long durations (200 msecs or more) of noise.

Conclusions

We studied the suppressive effects of binaural, contralateral and ipsilateral white noise on linear TEOAE's. Binaural stimulation elicits the most suppression of otoacoustic emissions in a forward masking paradigm when the onset of the click train is 20 msecs or less after the offset of a 408 msec white noise burst. Less suppression occurred to ipsilateral or contralateral stimulation, and the suppression disappeared when the end of the noise was 100 msecs or more away from the beginning of the click train.

Acknowledgments

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Figure Legends

Figure 1. Paradigm describing the temporal relationship between the click train and the noise bursts.

Figure 2. The effects of 408 msecs of binaural, contralateral, or ipsilateral noise preceding a left ear click train which started at 1, 2, 5, 10, 50, 100, and 200 msecs after the end of the noise burst. The mean and 1 Standard Deviation are presented using the amplitude difference data between 8-18 msecs from the Kresge Echomaster (KEM) 3.0 program.

Figure 3. The emission-suppressing effects of Binaural, ipsilateral and contralateral noise. Data, expressed in db-K are grouped by time intervals in which the noise preceded the click-evoked emission by either 1, 10, 50 or 200 msecs.

Figure 4. Shows Binaural noise suppression data for 1, 5, 20 and 200 msecs of time-separation. The companion figure shows the data for 2,10, 50 and 100 msecs. Data are expressed in db K.

Figures 5a, b and c. Show the successive frequency domain reduction in suppression caused by binaural noise 1 msec before the click train begins in a single subject. Changes in the entire emission spectrum over 20.48 msecs are viewed through a Hanning window. Other subjects show similar if not identical trends at slightly different places in the echo spectrum.

Figure 6 The test-retest reliability for the time separation of 1 msec with binaural, ipsilateral, and contralateral noise for the same subject.

Setting up the efferent suppression paradigm

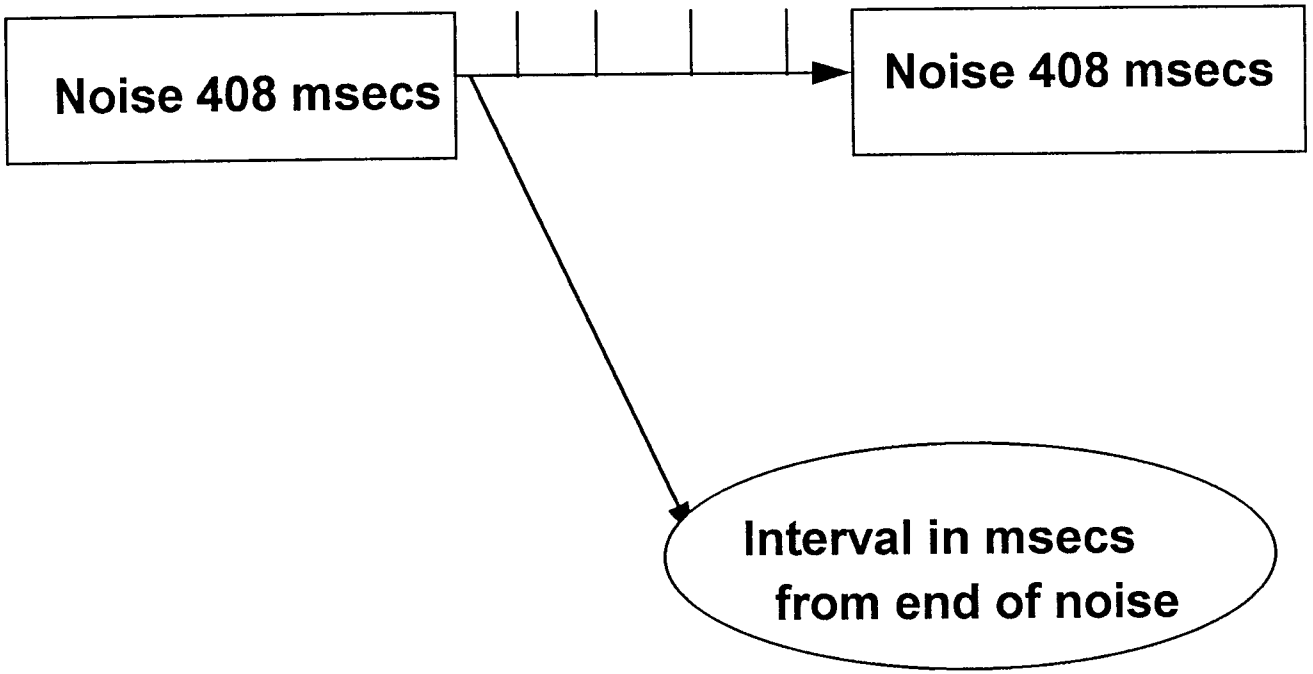


Figure 1

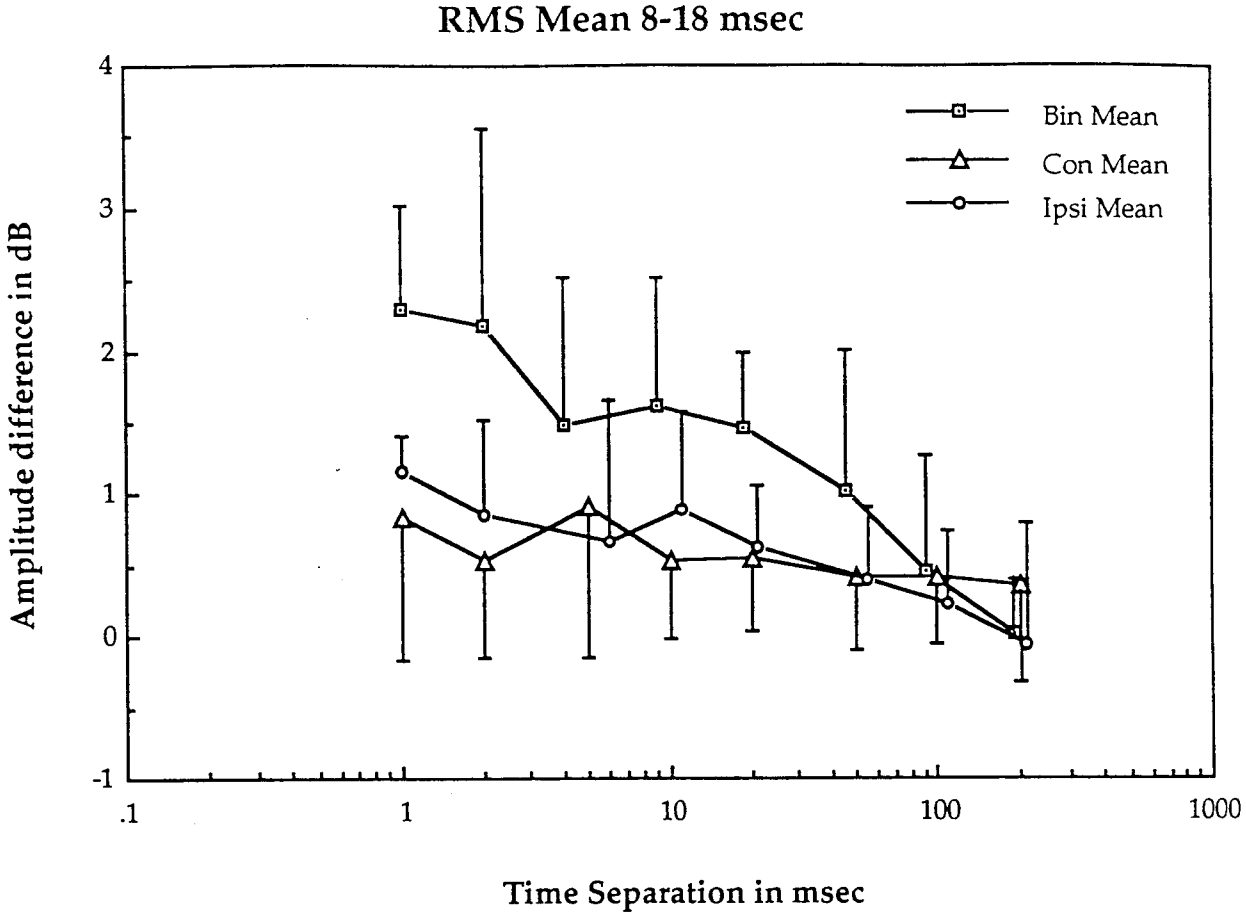


Figure 2 shows the mean and 1 standard deviation using the data between 8-18 msec from the Kresge Echomaster (KEM) 3.0 program as the metric for suppression. It is clear that the majority of suppression takes place in the first 1 to 100 msecs after the noise is terminated, and that binaural stimulation is far more effective than either ipsilateral or contralateral stimulation in keeping with our current understanding of the innervation density of the ipsilateral vs. contralateral medial olivocochlear system.

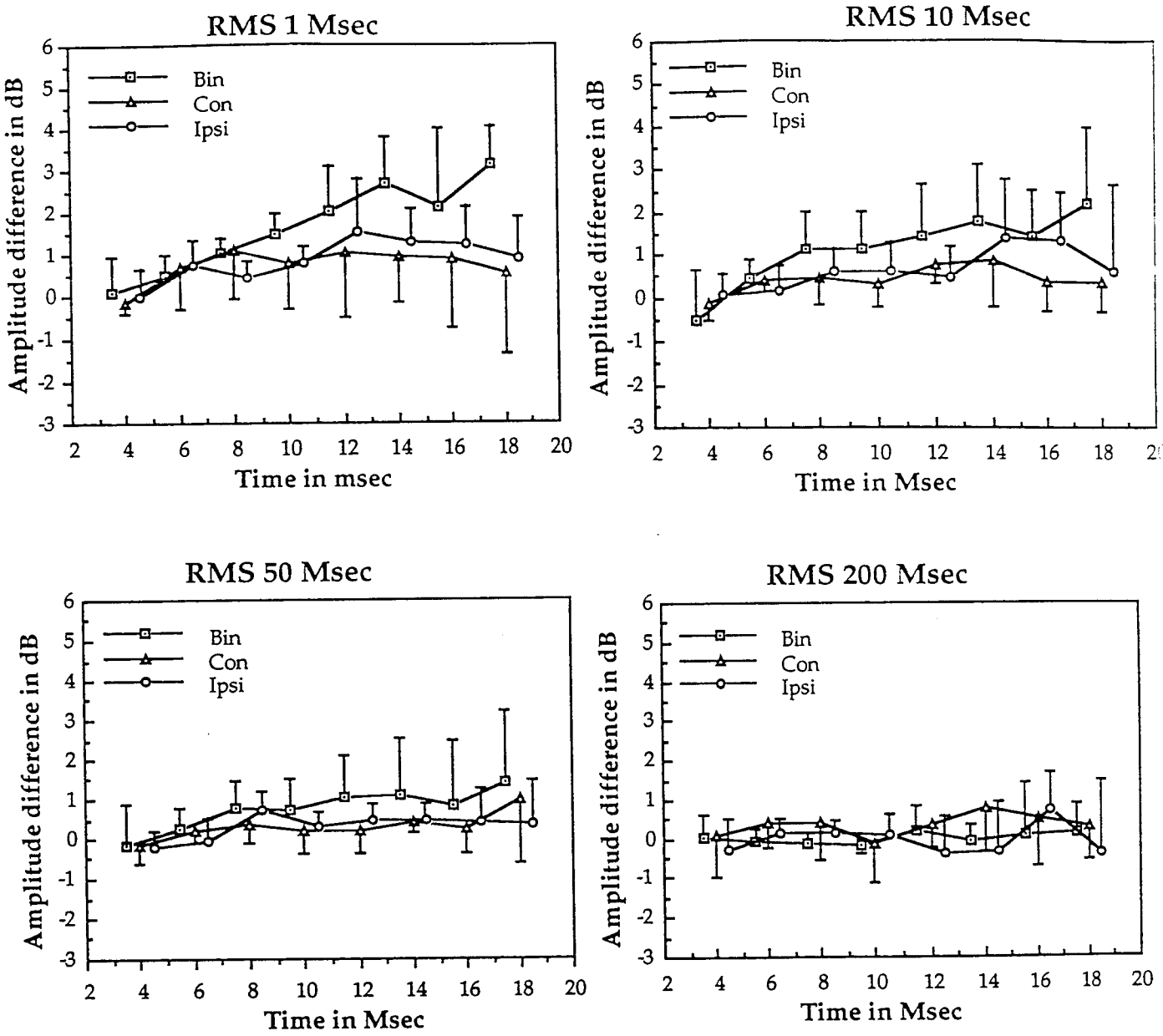
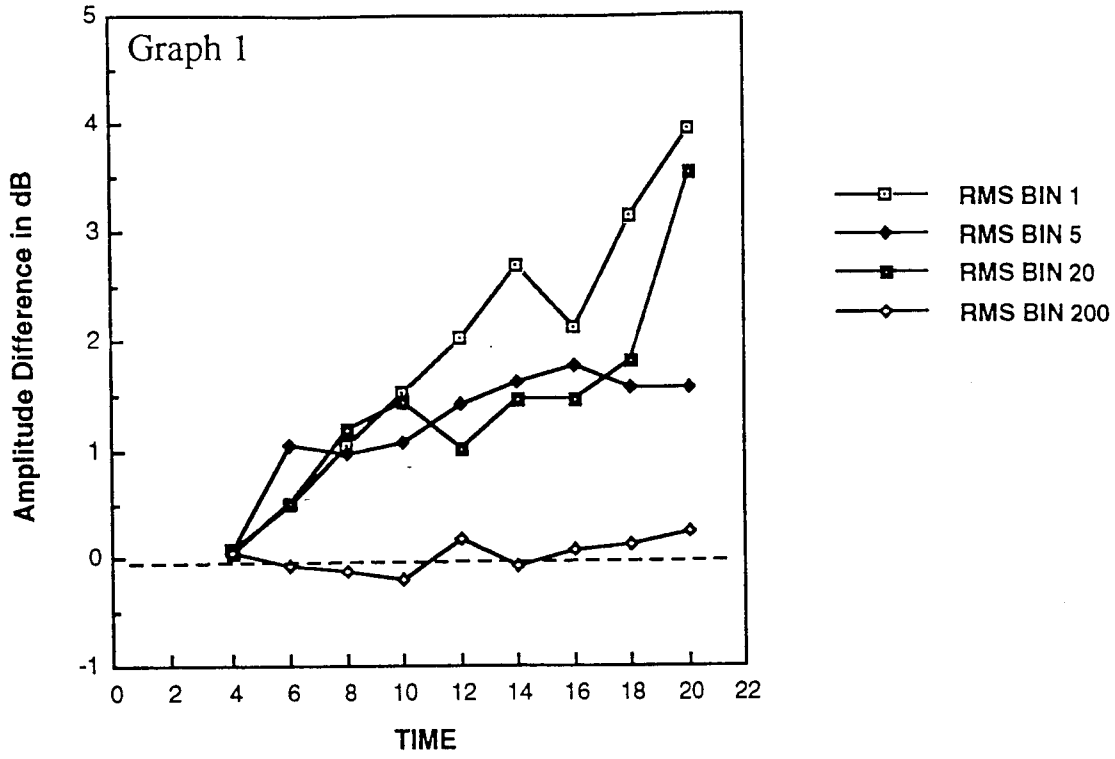


Figure 3 shows the effect of 1, 10, 50, and 200 msec time separations between the end of the noise and the start of the first click, with the analysis spread over the full 18 msecs of the emission.



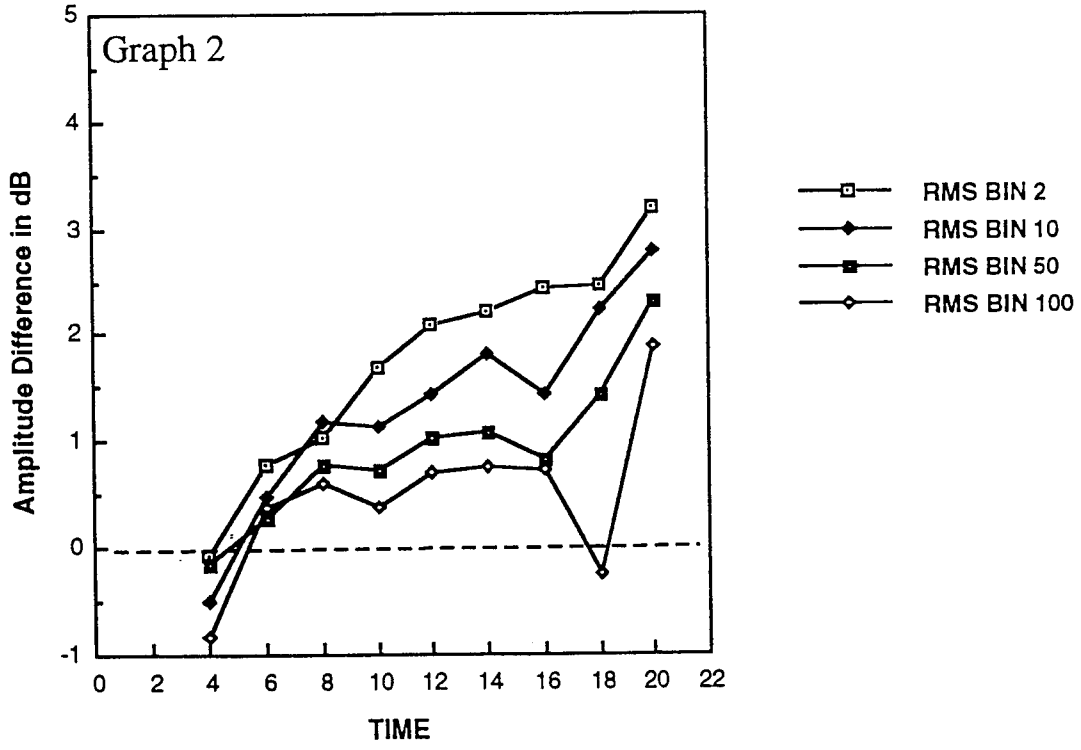
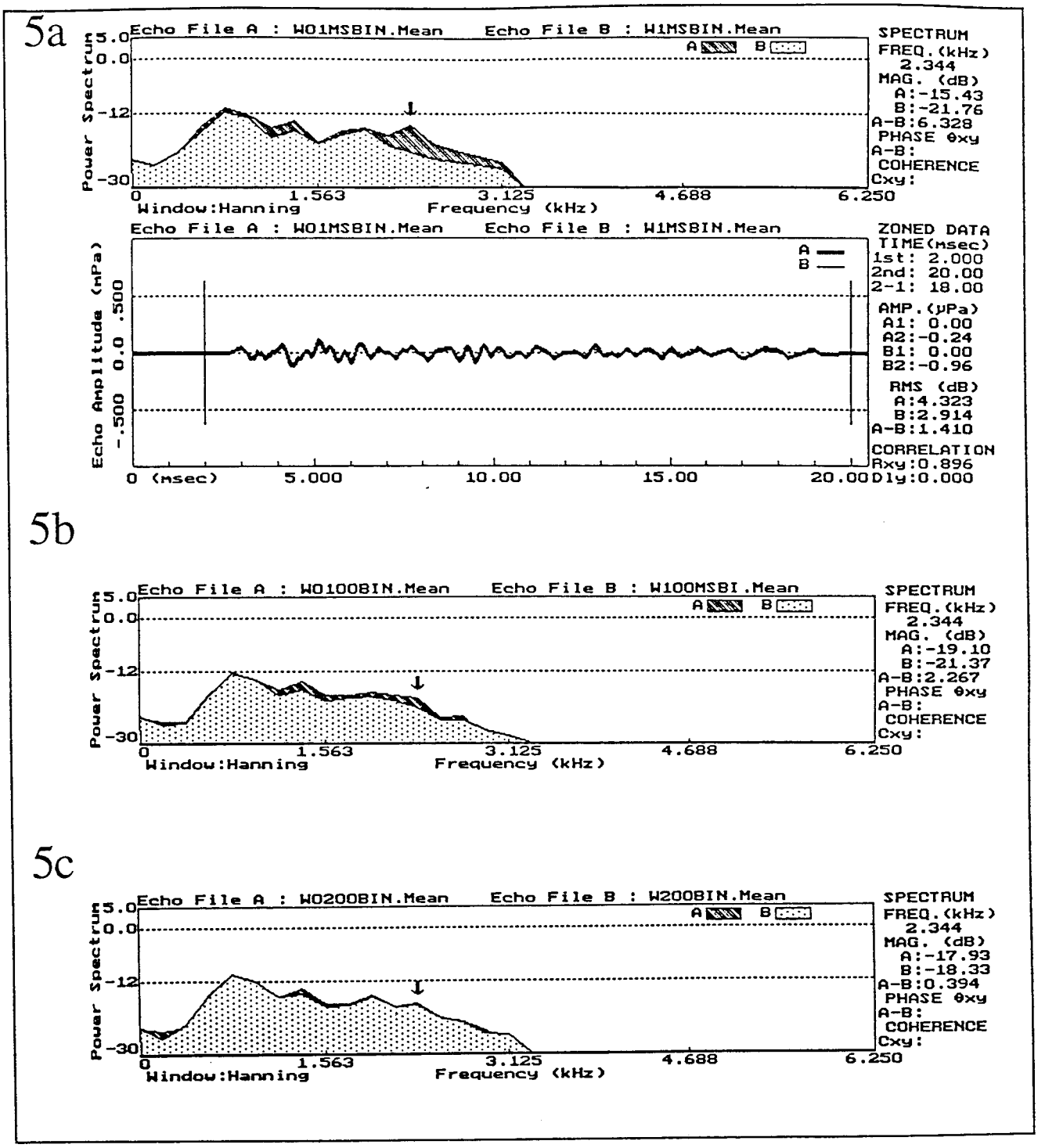


Figure 4 shows the differences in dB of suppression over 4 through 20 msecs of the emission with the time separations displayed of 1, 5, 20, and 200 in graph 1 and 2, 10, 50, and 100 in graph 2 for the binaural noise condition.



Figures 5a, 5b, and 5c show the gradual shift in the largest spectral difference (at 2344 Hz) from 6.33 dB of suppression at 1 msec (5a) to 2.27 dB at 100 msec (5b) down to .39 at 200 msec (5c) for a sample subject (CIB).

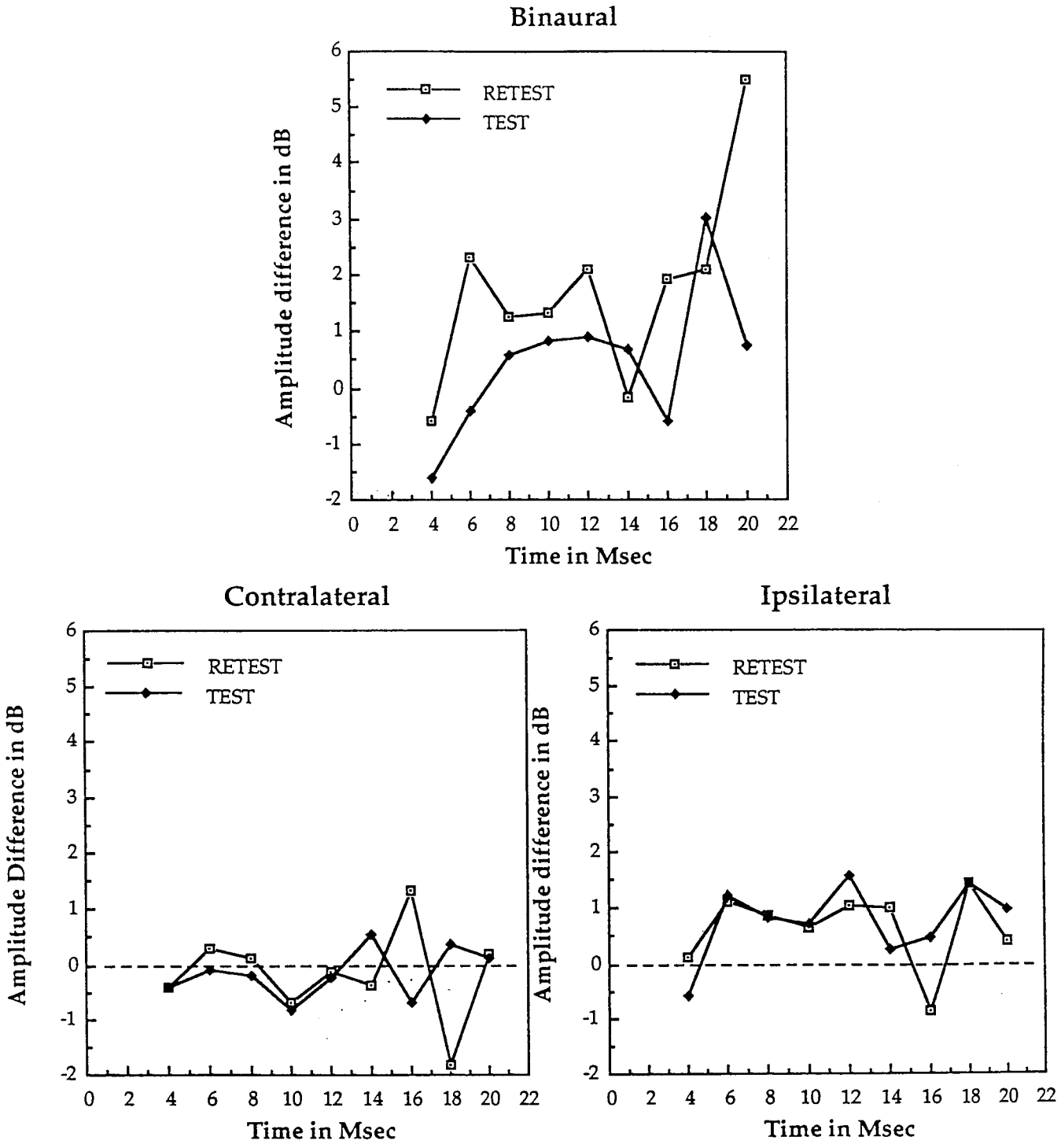


Figure 6 shows the test-retest reliability for the time separation of 1 msec with binaural, ipsilateral, and contralateral noise for one subject.