

## **Mentored Professional Enrichment Experience**

### **Applicant:**

**Name of Project/Experience:** An investigation into how long D-methionine delivery can be delayed post noise exposure and still provide protection from permanent noise induced hearing loss.

### **Location where Project/Experience will take place:**

Southern Illinois University School of Medicine – Springfield, IL

### **Mentor Name and Contact Information:**

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## **RATIONALE**

Noise-induced hearing loss (NIHL) is a type of sensorineural hearing loss caused by exposure to excessive amounts of noise. This harmful noise exposure may consist of a short impulse of intense sound or a prolonged exposure to sounds above 85 dB SPL<sup>1</sup>. The exposure to these high level noises may occur in a variety of recreational and occupational settings, including those for military personnel<sup>1,2</sup>. In some cases NIHL may be preventable by avoidance of high noise levels and by using hearing protective devices (such as earplugs). However, in some military settings noise exposure may exceed the protection of hearing protectors and there are still unexpected instances in which persons are exposed to high levels of noise<sup>1</sup>. Thus, an agent capable of preventing permanent NIHL post noise exposure would be useful for these applications. However the protective agent may not be immediately available. Thus having a protective agent that could be administered hours or perhaps even a day after the noise exposure may be clinically important.

Otoprotective agents are currently being researched to protect against NIHL as well as drug-induced hearing loss caused by aminoglycosides and platinum based cancer therapeutics. One such agent is D-methionine, the optical isomer of the essential amino acid, L-methionine. D-methionine's otoprotective effects may involve mitigating the oxidative stress in the inner ear induced by high level noise exposure through directly functioning as a free-radical scavenger and indirectly by increasing the intrinsic antioxidative defenses of cells<sup>3,4,5</sup>.

D-methionine's uses for protection from radiation-induced oral mucositis and protection from cisplatin- induced hearing loss are currently in clinical trials<sup>2</sup>. Translational research is also being performed to work towards FDA approval of D-methionine's use in prevention of NIHL. Campbell et al. (unpublished pilot data) has shown, using Chinchillas Laniger, that administration of D-Methionine, within 5 hours after 6 hour 105 dB SPL 4 kHz narrow band of noise exposure, may mitigate permanent outer hair cell loss (OHC) and changes in hearing, as measured by auditory brainstem response (ABR) threshold shifts, but more data is needed to confirm the effect and to reach statistical significance<sup>2</sup>. Determining the maximum time delay of post noise exposure administration of D-methionine, at which efficacy of NIHL prevention is retained could be important for several clinical settings. For example, military personnel on the battlefield exposed to sudden weapon fire may not have access to a protective

agent for several hours. Similarly fire fighters, or individuals in motor vehicle accidents where airbag detonation may cause noise induced hearing loss may have a time delay before an otoprotective agent could be available.

## GOALS

In the scope of this experiment, we hope to obtain sufficient data as to further address the question of: At what maximum time delay of post noise exposure administration of D-methionine can efficacy of the protection from permanent NIHL be preserved?

In a scientific sense, I am aiming to acquire proficiency in the necessary experimental techniques for this project, including performing Auditory Brainstem Response (ABR) measures and taking outer hair cell (OHC) counts via scanning electron microscopy. I am also trying to acquire general experience in the process of scientific research, including proposal writing, data presentation, and project planning, so that I may act more autonomously in future research endeavors.

Through this project, I will assess my interest in integrating research into my future medical career. I also hope to gain insight into otolaryngology through this project's relation to a relevant topic in this field.

## METHODS

Four groups of test subjects, consisting of ten male chinchillas each, will be tested. All groups will receive a 6 hour 105 DB SPL 4 kHz octave band noise exposure. The sound exposure will occur in isolated sound booths in the SIU Laboratory Animal Care Facility. The noise will be generated by a TDT GNS 40X white noise generator, and routed through an attenuator (TDT PA3), a filter (Krohn-Hite 3384) and a power amplifier (Sony 55ES) to a custom-built acoustic exponential horn with a maximum output at 4 kHz using an Altec 290E driver. Following noise exposure, D-methionine administration will be started ip at 200mg/kg at 3, 5, and 7 hours post noise cessation. Three of the four groups will each separately be assigned to one of this three dosing epochs. In addition, each animal will receive four additional doses of D-methionine that will follow in 12 hour intervals over two days. A fourth control group will also be noise-exposed with saline administration only.

Auditory brainstem response (ABR) will be used to assess changes in auditory threshold. ABRs will be performed just prior to the noise exposure and again on post-exposure days 1, 14, and 21. ABR thresholds will be obtained using tone bursts centered at the frequencies of 2, 4, 6, 8, and 14 kHz. Threshold is defined as the lowest intensity capable of eliciting a replicable, visually detectable response. An Intelligent Hearing Systems evoked potential unit will be used to collect the ABR data.

Histological analysis will be performed at the end of the three-week hearing assessment. The animals will be sacrificed and histological preparations of the cochlea will be made. Tissue preparations will be viewed through a Hitachi S3000N Variable Pressure Scanning Electron Microscope and photographs taken on Polaroid type 55 Film at 3,000X-4,000X magnification. A representative sample of 33 outer hair cells, or 11 per row, will be obtained at the 2, 4, 6, and 8 kHz regions on the cochlea. The number of damaged or missing outer hair cells in each sample will then be counted. The areas chosen for analysis are based on chinchilla cochlear frequency maps, and are meant to correspond to the frequency regions being tested in the ABR protocol. Using

## MPEE

the SEM techniques will provide a descriptive analysis of scarring and stereocilia bundles on the outer hair cells.

### ANALYSIS

Larry Hughes, Ph.D. will serve as statistical consultant for this project. The electrophysiologic data will be analyzed with a factorial ANOVA with between (rescue interval) and within (test days and test frequencies) factors. The histologic data will be analyzed with a factorial ANOVA with between (rescue interval) and within (cochlear region) factors. Overall error rate will be held at .05 using a Tukey post-hoc procedure. The goals will have been met if ABR threshold shift and outer hair cell loss are significantly less in the D-methionine rescue groups as opposed to the placebo group.

The scientific and personal goals will be accomplished as derivatives of a successful experiment.

### SUPPORT

1. Do you request support funds? Yes No
2. Would you be able to participate if a scholarship is not available? Yes No

### REFERENCES:

1. NIDCD Fact Sheet: Noise-Induced Hearing Loss. Bethesda, MD: *National Institutes on Deafness and Other Communication Disorders*, 2007.
2. Campbell KC, Meech RP, Larsen DL, Mitchell DL, Hughes LF. D-met Rescue from Noise-Induced Hearing Loss: Post Administration Intervals. Unpublished Pilot Data.
3. Campbell KC, Meech, RP, Klemens, JJ, Gerberi MT, Dyrstad SSW, Larsen DL, Mitchell DL, Mohammed E, Verhulst SJ, Hughes LF. Prevention of noise- and drug-induced hearing loss with D-methionine. *Hearing Research* 2007;226:92-103.
4. Le Prell CG, Yamashita D, Minami SB, Yamasoba T, Miller JM. Mechanisms of Noise-Induced Hearing Loss Indicate Multiple Methods of Prevention. *Hearing Research* 2007;226:22-43.
5. Kopke RD, Coleman KM, Liu J, Campbell KCM, Riffenburgh RH. Enhancing intrinsic cochlear stress defenses to reduce noise-induced hearing loss. *The Laryngoscope* 2002;112:1515-1532 .