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TITLE: Research in Prevention and Treatment of Noise-Induced Hearing Loss (NIHL)

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<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> The current study will identify D-methionine (D-met) dose and delayed time otoprotective response from steady state and impulse noise-induced hearing loss (NIHL). The second year was dedicated to determining the latest time D-met may be administered for efficient protection. Groups of <i>Chinchillas laniger</i> (n = 10) received five intraperitoneal D-met (200 mg/kg/dose) every 12 hours beginning 7, 9, 12, 18, 34, 36, or 48 hours after steady state or impulse. D-met otoprotection was assessed by auditory brainstem response (ABR) analysis and outer hair cell (OHC) quantification. ABR analyses identified time-dependent D-met protection from steady state and impulse noise exposures when D-met administration began as late as 24 hours post-noise exposure. Histological cytocholeogram analysis is still in progress to confirm electrophysiological assessment. Statistical analysis is currently in progress.					
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## INTRODUCTION:

In military settings, noise exposure frequently exceeds the physical hearing protector capacity; resulting in Noise Induced Hearing Loss (NIHL). An oral antioxidant that could prevent permanent NIHL could allow more military personnel to be redeployed, to maintain the auditory capabilities needed in combat situations, and to keep our military personnel from having permanent disability. Currently, no pharmacologic protective agents exist in the clinical arena. We have developed an antioxidant, D-methionine (D-met), which has already shown promise in preventing NIHL in animals whether started before or after the noise exposure. In these studies we have performed dose timing studies in chinchillas to determine the latest time after noise exposure D-met may be administered to efficiently prevents impulse and steady state NIHL.

## BODY:

**Statement of Work (SOW): Year 2: Task:** To determine maximum time delay for effective D-met post-noise rescue protection from permanent NIHL using auditory brainstem response (ABR) threshold shift and outer hair cell (OHC) measurements.

### Methods:

Each animal group (9 groups for steady state noise and 9 groups for impulse noise) comprised 10 male *Chinchillas laniger*. To determine the latest protective D-met administration time post-noise, D-met started at time delays of 7, 9, 12, 18, 24, 36, 48, and 72 hours after completion of the steady state or impulse noise exposure and then another 48 hours BID. The control group received the placebo starting 7 hours after cessation of the noise. ABRs were measured at baseline, prior to any drug or noise exposure, and again 21 days after noise exposure cessation using tone-burst stimuli centered at the frequencies of 2, 4, 6, 8, 14 and 20 kHz. Threshold was defined as the lowest intensity capable of eliciting a replicable, visually detectable response.

Steady state noise exposure comprised a 105 dB SPL narrow band of noise centered at 4 kHz for 6 hours. Impulse noise consisted of simulated M-16 weapon fire at 155 dB peSPL 150 impulses at 2/s.

After the 21 day post-noise ABR assessment, each animal was sacrificed by decapitation and the cochleae harvested for OHC. Animals completing experimentation will be decapitated and cochleae perfused with fixative through the perilymphatic spaces. The primary fixative will be 10% formalin at 4 degrees C in PBS. A small hole was first hand-drilled into the otic capsule with a sharpened pick. *In vitro* perfusion was performed intermittently within 5 minutes of sacrifice through the small hole in the scala tympani, allowing the fluid to exit through the opened oval window. After perfusion fixation, the round window membrane was removed and the cochleae immersed in 10% formalin and placed in the refrigerator.

After cochlear preservation, the cochlear epithelium was carefully dissected in one half turn sections, stained with either hematoxylin (Eosin Y counterstain) or fluorescent markers (Alexafluor-488 Phalloidin Green), and mounted onto slides for cytochleogram analysis using Cytogram software (Kresge Hearing Research Institute, University of Michigan; Ann Arbor).

### Results:

#### *Year 2 Steady State and Impulse NIHL Studies:*

One hundred sixty of one hundred eighty of the scheduled animals were successfully tested for D-met protection from steady state and impulse NIHL. We determined no D-met protection with a 48 hour rescue dosing regimen and therefore did not test the 72 hour time point to prevent animal pain, suffering, and sacrifice.

Current steady state and impulse noise-induced threshold shift results at 21 day post-noise exposure (Figures 1 and 2, respectively) identifies D-met protection when rescue dosing was administered as late as 24 hours post-

noise exposure. D-met provided protection when it was administered between 7 and 24 hours after noise exposure. At 36 hours, threshold shifts from D-met-treated animals were still noticeably still less than control thresholds, but with little or no significance. Finally, the 48 hour rescue regimen measured very little protection compared to saline-injected controls.

Year 2 studies performed almost twice as many animal experiments than the previous year. Thus, statistical and histological analyses are pending and in progress. We will finalize Year 2 data while Year 3 experimentation planning and protocol approvals are developed and scheduled.

#### **KEY RESEARCH ACCOMPLISHMENTS:**

- 80/90 of the animals scheduled for testing D-met time-delayed protection from impulse NIHL were tested using saline or 200 mg/kg/dose D-met.
- 80/90 of the animals scheduled for testing D-met time-delayed protection from steady state NIHL were tested with either saline or 200 mg/kg/dose D-met.
- ABR analysis identifies time-delayed D-met protection from steady state and impulse NIHL when D-met administration begins as late as 24 hours post-noise exposure.
- Learned and developed a technique for cytochleogram quantitative analysis of chinchilla cochlear hair cells.
- Developed an animal model for chinchilla cytochleogram quantitative analysis.
- Dissected, stained and prepared slides for 40% of the specimens.
- Cochlear whole mounts and cytochleogram analyses are currently under development and in progress.

#### **REPORTABLE OUTCOMES:**

We have extended our published 7-hour D-met rescue from steady state noise exposure (Campbell et al. 2011) to protection from steady state and impulse NIHL when D-met administration begins at least 24 hours post-noise exposure. The results have just recently been analyzed, require statistical analysis, and have not yet been presented or published.

#### **CONCLUSION:**

We have identified a 24 hour time-delayed protective D-met dose from steady state and impulse NIHL. The 36 hour dosing regimen continues to yield threshold shifts lower than controls, but they are also greater than the 24-hour dosing group. Thus, the grant's third year will design animal experiments that test extended time-delayed D-met administration beginning between 24 and 36 hours, possibly at 28 and 32 hours, to refine the latest possible time D-met administration may begin to protect from steady state or impulse NIHL. These data will enable us to proceed to FDA approved clinical trials submit the data for publication and to the FDA.

#### **REFERENCES:**

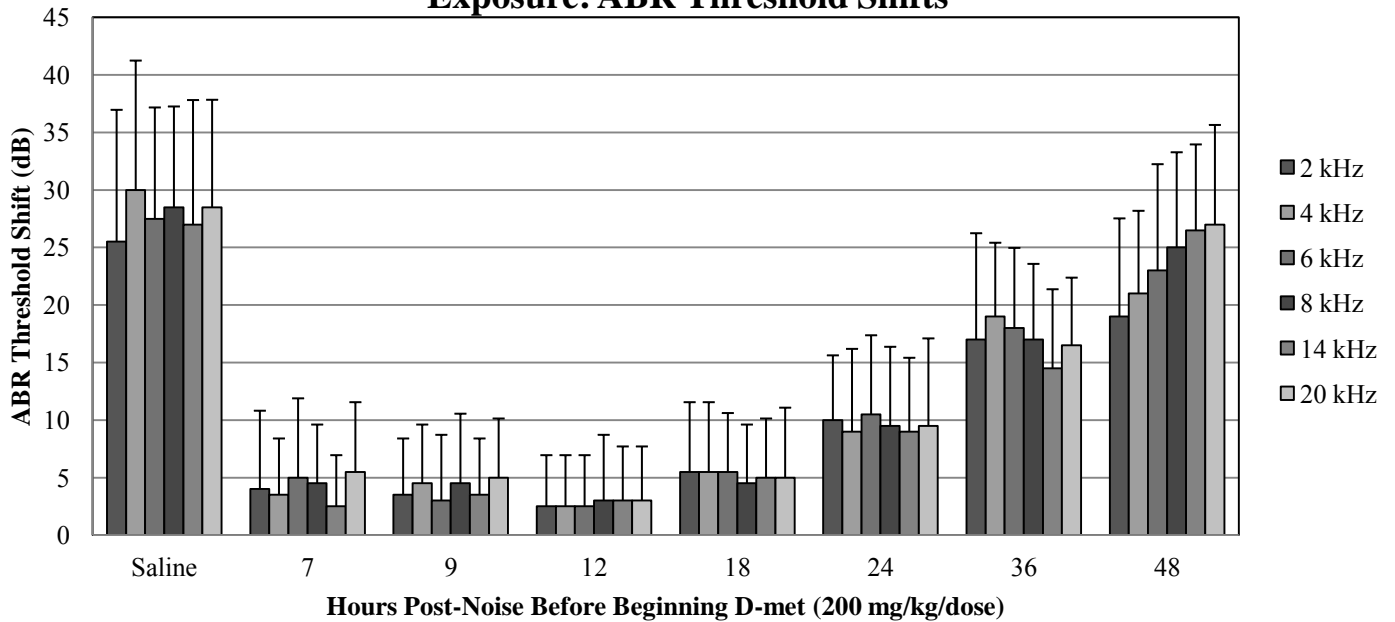
Campbell, K., Claussen, A., Verhulst, S., Fox, D., Hughes, L. 2011. D-methionine (D-met) significantly rescues noise-induced hearing loss: timing studies. *Hear Res.* Dec: 282(1-2): 138-44.

#### **APPENDICES:**

N/A

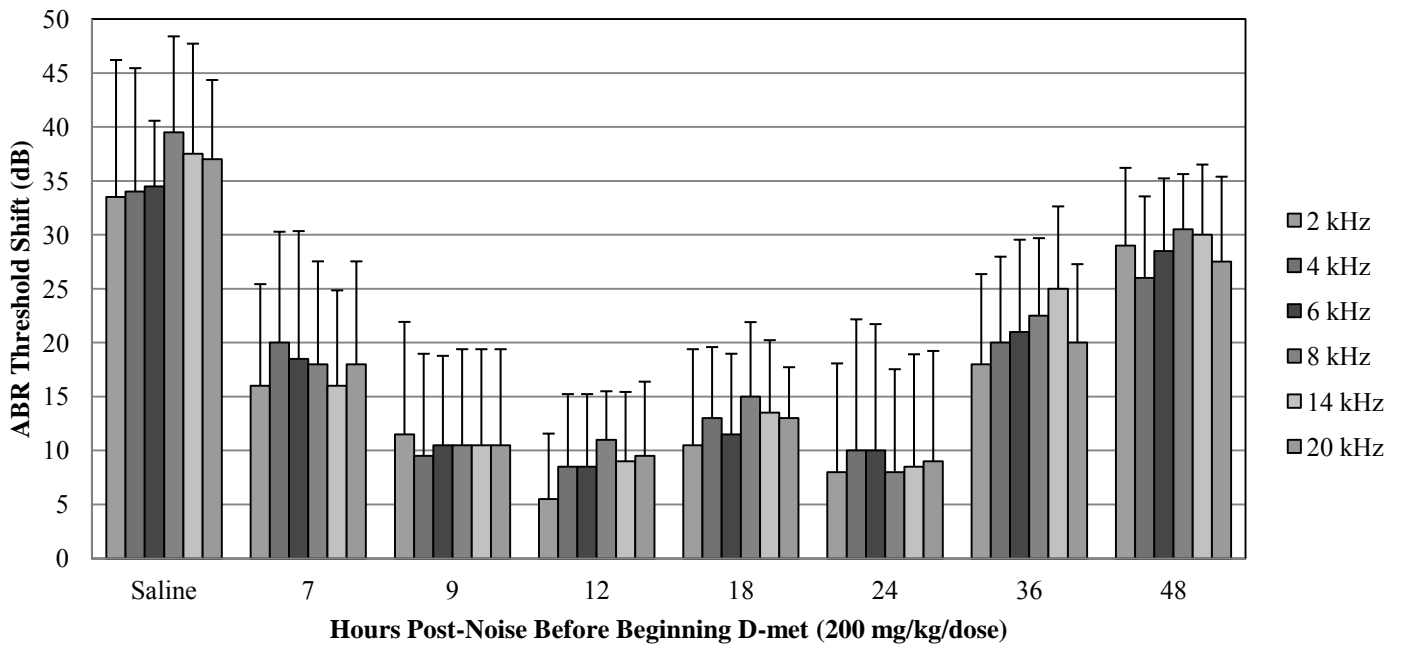
**SUPPORTING DATA:**

**Time-Delayed D-methionine (D-met) Rescue from Steady-State Noise  
Exposure: ABR Threshold Shifts**



**Figure 1.** Steady state noise-induced ABR threshold shifts in chinchillas 21 days after a 105dB peSPL 6 hour noise exposure centered at 4 kHz (n = 10 per group). D-met has provided protection when administration began as late as 24 hours post-noise exposure. Statistical analysis is currently in progress.

**Time-Delayed D-methionine (D-met) Rescue from Impulse Noise  
Exposure: ABR Threshold Shifts**



**Figure 2.** Impulse noise-induced ABR threshold shifts in chinchillas 21 days after simulated M-16 weapon fire at 155 dB peSPL 150 impulses at 2/s (n = 10 per group). D-met has provided protection when administration began as late as 24 hours post-noise exposure. Statistical analysis is currently in progress.