AWARD NUMBER: W81XWH-17-1-0172

TITLE: Mechanism-Based Prevention of Noise-Induced Tinnitus: Protection and Repair of Peripheral Auditory Neuropathy

PRINCIPAL INVESTIGATOR: Dr. Avril Genene Holt

CONTRACTING ORGANIZATION: Wayne State University

REPORT DATE: JANUARY 2023

TYPE OF REPORT: Final Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JANUARY 2023	FINAL	30 Sept 2017 - 29 Sept 2022

4. TITLE AND SUBTITLE

Mechanism-Based Prevention of Noise-Induced Tinnitus: Protection and Repair of Peripheral Auditory Neuropathy

5a. CONTRACT NUMBER
W81XWH-17-1-0172

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dr. Avril Genene Holt, PhD
E-Mail: agholt@med.wayne.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Wayne State University
5057 Woodward Ave;
13th Floor, Suite 13202
Detroit, Michigan 48202-4050

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

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

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Studies test a potential treatment for noise-induced tinnitus in the rat model. We hypothesize that noise-induced loss of synaptic connection between Inner Hair Cells (IHC) and the auditory nerve (AN) contributes to the induction of tinnitus and rapidly repairing this loss will therefore decrease the incidence of tinnitus. Treatment with the neurotrophic factor NT-3 was previously shown by our consultant Dr. Corfas to induce significant IHC-AN synapse reconnection after a different type of noise in his mouse model (Suzuki et al., 2016). During the first year of studies we have found that we can duplicate these results using a more military relevant small arms fire (SAF)-like noise in the rat model, showing a large and significant re-connection (described later in Section 3 of the Results Section). NT-3 in poloxamer was applied to the round window with the trans-tympanic approach that has been successfully applied in people for other treatments. These results show that it is possible to reverse noise induced synaptic loss from a military relevant noise exposure with a treatment paradigm that can be applied to those in the service. Such noise-induced synapse loss can cause a "Hidden Hearing Loss" that can impair speech understanding (Liberman et al., 2016, 2017). Therefore the ability to repair and reverse Hidden Hearing Loss has immediate impact. The major goal, however, is to test if such reconnection will decrease or prevent the later development of tinnitus and that is the focus of the next stage of our ongoing studies. Studies are now underway to determine if this rapid reconnection from NT-3 treatment will decreases the incidence of noise induced tinnitus compared to noise exposed rats without treatment. If successful, this would provide a military relevant treatment to prevent and treat noise-induced tinnitus.

15. SUBJECT TERMS
Tinnitus, Deafness, Neurotrophins, NT-3, Synaptopathy, Noise, Small Arms Fire, Cochlea, Auditory, Hidden Hearing Loss

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	13	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Studies test an underlying mechanism and potential mechanism-based treatment for noise-induced tinnitus in the rat model. We hypothesize that noise-induced loss of synaptic connection between Inner Hair Cells (IHC) and the auditory nerve (AN) contributes to the induction of tinnitus and repairing this loss will decrease the incidence of tinnitus. Treatment with the neurotrophic factor NT-3 was previously shown by our consultant Dr. Corfas to induce significant IHC-AN synapse reconnection after a different type of noise in his mouse model (Suzuki et al., 2016). In our first phase of studies Site 1 reported that they can duplicate these results using a more military relevant small arms fire (SAF)-like noise in the rat model, showing a large and significant reconnection when this treatment is applied 1 day after the noise. During Year Two Site 1 has tested NT-3 treatment beginning after a delay of 1-2 weeks to determine if it remains effective in inducing re-connection of IHC-AN synapses. In Year One, Site 2 reported that we could generate military relevant blast-noise with different spectral characteristics. During Year Two, Site 2 has tested these different blast-noise in animal models, showing differences in cochlear injury and hearing function. Both of these results have relevance to those in the field that might not be able to get immediate treatment (Site 1) and those that are exposed to blast-noise (Site 2) which can vary in spectral content. Our initial results for Site 2 suggest that blast-noise with lower frequency spectral content have significantly different effects on cochlear damage and hearing when compared to exposure to blast-noise containing slightly higher frequencies. Year Two studies are also testing if the NT-3 poloxamer treatment given one day after blast-noise will also promote IHC-AN reconnection as it does after SAF-like noise exposure. These studies began in Year One and are still underway (and we remain blind as to the results). These assessments will continue into Year Three when we will "unblind" and report results. We will also begin testing efficacy of treatment 1 day after and 1-2 weeks after noise in reducing tinnitus in Year Three, continuing into a fourth year (we requested a no-cost extension). Reconnection could reduce the incidence of tinnitus but could also be effective in treating "Hidden Hearing Loss" that can impair speech understanding (Liberman et al., 2016, 2017). Either or both would provide benefit to Veterans and those in general population exposed to noise. **Nothing new to report due to pandemic related restrictions, equipment failure and lack of necessary funds to complete and/or publish studies. The current goal is to continue and complete data analysis.**

2. KEYWORDS:

Tinnitus, Deafness, Neurotrophins, NT-3, Synaptopathy, Noise, Small Arms Fire, Blast-noise, Cochlea, Auditory, Hidden Hearing Loss

3. MAJOR GOALS AND ACCOMPLISHMENTS

MAJOR GOALS - from Statement of Work (SOW):

YEARS THREE, FOUR, and FIVE:

Timeline & Milestones: Aim 1 studies continued (staggered start from Year One) into Year Two.

(Years 1-2)

Aim 1A: Determine if NT-3 treatment 1 day after a blast-noise will induce reconnection of lost IHC-AN synapses and prevent Tinnitus from appearing.

- ***Subtask 1***: Influence of NT-3 elevation 1 day after blast-noise to prevent appearance of tinnitus based on Gap Detection & and behavioral (operant conditioning) metrics
- ***Subtask 2***: Influence of Treatments on Blast-Induced changes to Inner Hair Cell –Auditory Nerve synaptic connections.

***(Milestones**: Break Code and determine treatment effects in Months 20-26)*

(Years 2-3)

Aim 2: Determine the influence of NT-3 treatment given at later times after a blast-noise on inducing re-connection of IHC-AN synaptic connections and preventing Tinnitus

- ***Subtask 1***: NT-3 treatment two weeks after noise
- ***Subtask 2***: NT-3 treatment six week after noise

Task 6 *Milestones*: *Break Code and determine treatment effects in Months 33-36*

1. Determine efficacy in re-connection (Year 2 and 3)
2. Determine treatment effects on tinnitus (Years 3 and 4)
3. *Complete analysis of re-connection data (Years 4 and 5)*

ACCOMPLISHMENTS:

Major Activities Task 4:

Aim 1 Group 1 – Blast Noise – no NT-3
Aim 1 Group 2 – Sham Noise – no NT-3
Aim 1 Group 3 – Blast Noise – NT-3 post-treatment 1 day after noise
Aim 1 Group 4 – Sham Noise – NT-3 post-treatment 1 day after noise

Major Activities Task 6:

Aim 2 Group 1 – Blast-Noise – no NT-3
Aim 2 Group 2 – Sham Noise – no NT-3
Aim 2 Group 15 – Sham Noise – NT-3 post-treatment 1-2 weeks after noise
Aim 2 Group 16 – Blast-Noise – NT-3 post-treatment 1-2 weeks after noise

Rats received base-line measures of auditory brain stem response (ABR) and Gap Detection (GD). Rats then received either operant conditioning or Gap Detection as metrics for later tinnitus testing. Animals were then randomly divided into groups receiving either Sham noise or blast-noise (one ear protected-plugged). Animals in each group were then again randomly divided and received either Poloxamer containing NT-3 or Poloxamer only (no NT-3), delivered with a trans-tympanic approach into the left middle ear by the round window, done one day after the noise or sham (Groups 3 & 4) or one to two weeks after the noise or sham (Groups 15 & 16). All animals then receive testing with either the GD and Operant Conditioning as metrics for the presence of tinnitus. After two months of assessments all rats are tested for ABR and then euthanized. Cochleae are processed to determine hair cell loss and loss of IHC-AN synaptic connections.

Unanticipated Problems: As previously mentioned in the Year One Progress Report, there was more delay than anticipated in building the new operant conditioning testing stations and generating the software necessary for testing rats for tinnitus in these stations. There was also delay in receiving the new Gap Detection testing stations from Kinder Scientific and making these stations (with new design) operational to test for tinnitus. New problems arose during Years One and Two in the testing. Test stations were not able to accommodate the increasing sizes of male rats during the course of their testing and required additional changes to allow their use for larger animals. The studies testing for tinnitus in the TTS-noise group, but the delayed and subsequent needs for changes in test stations have reduced the number of animals that could be tested. This delayed completion and "unblinding". Finishing Subtask 2 (that did not require testing for tinnitus) was possible and we have analyzed the effectiveness of NT-3 for functional reconnection of lost IHC-AN synapses (ABR with waveform analysis) after blast-noise. However, the influence of the immediate reconnection on incidence of tinnitus (Subtask 1) was not completed due to reduced staffing as noted below.

There was a "ramping down" of research activities at Wayne State University because of the COVID-19 pandemic starting in February 2020. We were unable to continue testing animals already in study due to the "stay at home order". While we were able to "ramp up" at Wayne State University, there were staffing constraints and therefore we needed to operate in person at a reduced level. While we were able to continue much of our testing procedures, analyses, dissemination, we still had some constraints on the number of staff allowed to be present in the laboratory at a given time and staff were given the option to work remotely for a number of hours each week. Therefore, we could not operate at full capacity. Therefore, full results were not known until Years Four and Five when unblinding was possible. Assessment of synapses for blast-noise

without tinnitus assessment was completed. Synaptic counts for blast-noise with tinnitus assessment and TTS-noise with tinnitus assessment and anti-excitotoxicity treatment (with sham controls), were not able to be completed due to challenges mentioned above. We will need to seek additional funds to complete the necessary anatomical and biochemical assessments.

Major Activities 2: Because of the delay (mentioned above) in being able to use the needed metrics to test rats for tinnitus, the studies first focused on the effect of blast-noise with distinct spectral content on cochlear damage, hearing, and synaptic connections, since these measures do not require tinnitus testing stations to be utilized to generate initial results. Studies first correlated spectral characteristics of the blast-noise with hearing thresholds, ABR wave 1 amplitude, distortion product of otoacoustic emissions (DPOAEs), hair cell loss, and synaptopathy. The effects of modulating spectral content of blast-noise on cochlear trauma and hearing are currently in the process of being written and submitted for publication. The studies next examined NT-3 treatment given one day after the blast-noise for repair of lost IHC-AN synapses (synaptopathy; Groups 1 – 4). The analysis examining anti-excitotoxicity treatment given after the blast-like noise are now completed.

RESULTS:

Studies examining pre-treatment with anti-excitoxicity agents using SAF-like noise were published by Site 1 (Altschuler et al., 2021). Studies comparing effects of delivering NT3-poloxamer to the inner ear to combat blast-noise induced cochlear trauma and hearing were completed and assessments were begum in Year Three and are summarized below.

Sprague Dawley rats were tested for ABR threshold, wave 1 amplitude, and latency. Only rats with normal responses were placed into the study. The blast-noise groups were exposed to 22 psi blast overpressure generated and delivered either by a 20' or an 8' configuration of the WSU shocktube, described in studies performed in Year One. They received a second ABR within one hour following the blast-noise. The left cochlea was infused with either poloxamer alone or poloxamer with an anti-excitotoxicity agent. Then a third ABR assessment was performed two weeks later and animals were then euthanized. Cochleae received intrascalar fixation with 4% paraformaldehyde fixative and were then processed for assessment of hair cell loss and loss of IHC-AN synaptic connections, using CTBP2 ribbon immunolabeling as a marker for IHC synapses. Blast exposure using the 20' configuration of the WSU shocktube (sound pressure levels – up to 180 dB SPL at 50-500 Hz with a precipitous decline in sound intensity from 2.5 – 3.5 kHz from 80-less than 40 dB SPL) resulted in a significant temporary threshold shift (**Year Two report**).

Configuration	# of 10 mil mylar	# of 3 mil mylar	# of Tests	peak pressure (psi)		peak avg pressure (psi)	BOP duration (ms)
Long Tube (all rats)							
694	1	1	1	25.362		23.1900	16.79
695	1	1	1	25.706		23.2220	15.15
741	1	1	1	20.526		19.99	13.8
742	1	1	1	20.526		19.96	13.84
average				23.03		21.5905	14.8950
STD				2.8947		1.8655	1.41045

Configuration	# of 10 mil mylar	# of 3 mil mylar	# of Tests	peak pressure (psi)	peak avg pressure (psi)	BOP duration (ms)
Short Tube (all rats)						
696	1	1	1	24.00437	22.2638	2.92
697	1	1	1	23.16916	21.62134	3.2400
743	1	1	1	29.82158	22.85	5.36
744	1	1	1	26.633711	22.08894	3.98
745	1	1	1	26.6234	22.5567	3.92
746	1	1	1	26.07691	21.52773	2.1500
average				26.05485517	22.1514	3.5950
STD				2.340031867	0.517452	1.0989768

Figure 1. Rats in long tube blast tube tests were exposed to prolonged BOP durations. Long tube configuration on the left and short tube configuration on the right.

Male SD rats were divided in to two groups and exposed to blast exposure using either the long or short tube configuration. In animals exposed to long tube BOP, the peak blast pressure was 23.03(± 2.9) compared to a much higher peak BOP of 26.05 (± 2.3) in the short tube exposed animals. However, the peak average pressures in both the set-ups were very similar with 21.59 (± 1.87) and 22.15 (± 0.5) respectively. Furthermore, BOP in the long tube tests persisted for a prolonged duration of 14.9 ms (± 1.4) compared to a very short duration BOP in short tube tests of 3.6 ms (±1.1). The prolonged BOP duration may be related to the differences in the pathology observed in the cochlea and the small, but permanent ABR threshold shift (Year Two report). We used the short tube configuration to determine whether an anti- excitotoxicity agent

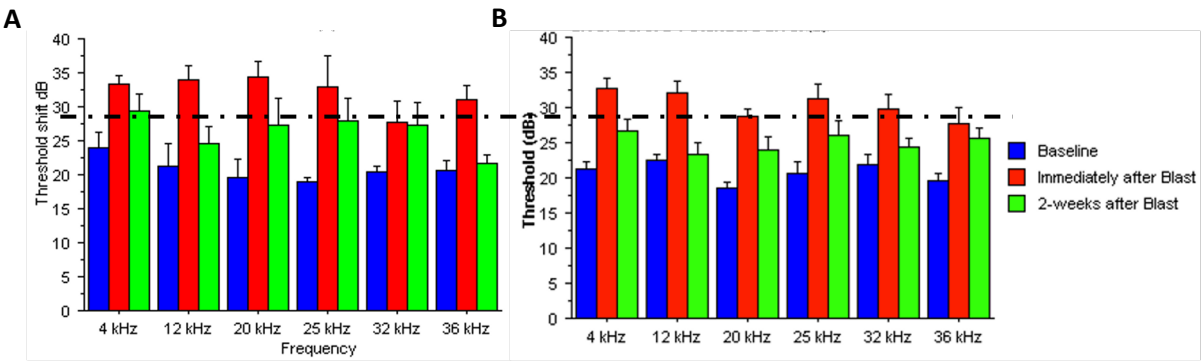

Figure 2. *Effects of inner ear poloxamer only (A) and Poloxamer + anti- excitotoxicity agent (B) on hearing thresholds after blast-noise exposure.*

would be an effective treatment for blast-noise induced hearing loss. Administering the poloxamer without the anti- excitotoxicity agent did not alter the normal progression of hearing loss after the blast-noise exposure (Fig 2A).

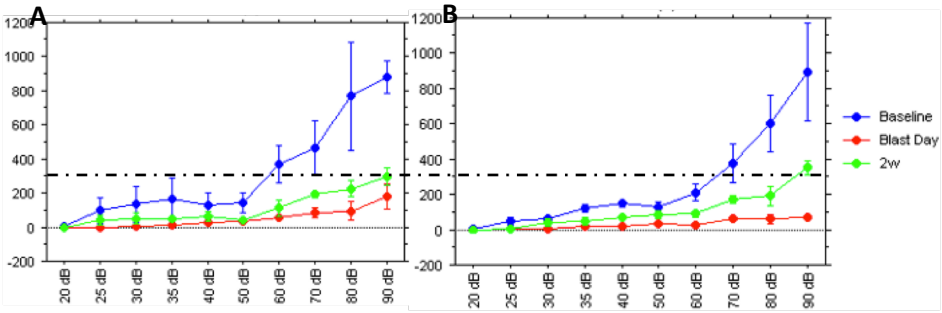

Figure 3. *Effects of inner ear poloxamer only (A) and Poloxamer + anti- excitotoxicity agent (B) on ABR wave I amplitude after blast-noise exposure.*

Our most recent analyses assessed effects of administering the poloxamer **with** the anti-excitotoxicity agent on the normal progression of hearing loss after the blast-noise exposure. We demonstrate that Poloxamer + anti-excitotoxicity agent improves thresholds two weeks after blast exposure (Fig. 2B). Our previous results demonstrated that animals exposed to blast-noise generated by the short shocktube configuration had fewer CtBP2 labeled puncta near the base of the cochlea. Therefore, in addition to thresholds we also assessed ABR wave I amplitude to determine both inner hair cell-auditory nerve reconnection and central auditory system effects. Our results suggest that the anti-excitotoxicity agent restores better function at this time point (Fig. 3B). We have directed some of our efforts towards studying the role of therapeutic agents in mitigating some of the observed pathological changes due to loss of synaptic connections (see presentations at ARO and SFN conferences, reported below). When examining functional changes at later time points following blast-noise exposure. Even one day after the blast-noise exposure, hearing loss was evident with thresholds elevated to 32 dB. Although this elevation was significantly reduced by 11 days, we found that hearing thresholds were not

at normal levels until 20 days after blast-noise exposure (Fig. 4A). We also have also used a measure of vestibular function VsEPs (vestibular short latency evoked potentials) to assess the impact of the blast-noise exposure on peripheral and central neuronal activity. One day after blast-noise exposure VsEP amplitudes are significantly blunted during moderate jerk stimulation of 4000 g/s (Fig. 4B). These and other related results were presented at several conferences listed below in the section titled: How were the results disseminated to communities of interest?

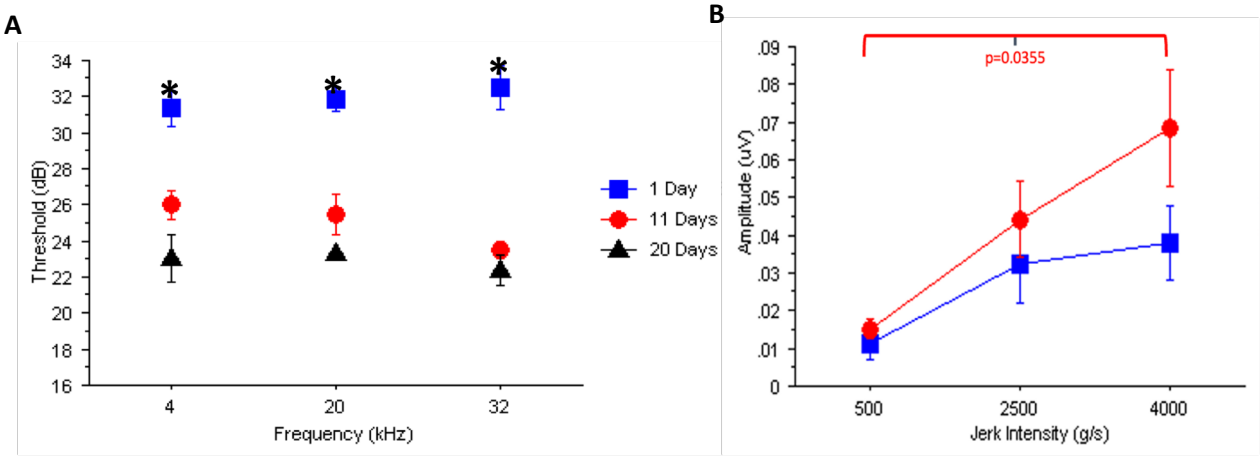

Figure 4. *Effects of blast-noise exposure on ABR hearing thresholds (A) and vestibular short latency evoke potentials - VsEPs (B).*

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

- Poster Presentations
    - King, S, Braun R, Anderson M, Bauer D, Naqvi D, Sepulveda AP, King WM, **Holt AG**. Irregular fiber stimulation and activation of central vestibular-related neurons, 46th Annual Mid-Winter Meeting of Association for Research in Otolaryngology, Orlando, FL. February 11-15, 2023.
    - Ponce Sepulveda A, Naqvi D, Braun R, **Holt AG**. The Effect of Overstimulation on Peripheral and Central Vestibular Function. Neuroscience 2022, Society for Neuroscience, San Diego, CA. November 11-15, 2022.
    - Yalcinoglu S, Kotcharian S, Braun R, **Holt AG**. Longitudinal analysis of the effect of noise exposure on neuronal activity and the role of the non-dihydropyridine L-type calcium channel blocker Verapamil. Neuroscience 2022, Society for Neuroscience, San Diego, CA. November 11-15, 2022.
    - Yalcinoglu S, Kotcharian S, Wattoo A, Braun R, **Holt AG**. The effect of non-dihydropyridine L-type calcium channel blocker Verapamil on noise induced hearing loss. 9th Annual Vision Research Workshop, Kresge Eye Institute, Detroit, MI. October 12, 2022.
    - Naqvi D, Braun R, **Holt AG**. The Effect of Repetitive Linear Acceleration on Gravity Receptor Function. 9th Annual Vision Research Workshop, Kresge Eye Institute, Detroit, MI. October 12, 2022.

- Yalcinoglu S, Kotcharian S, Wattoo A, Braun R, **Holt AG**. The effect of non-dihydropyridine L-type calcium channel blocker Verapamil on noise induced hearing loss. 9th Midwest Auditory Research Conference, Ann Arbor, MI. June 23-25, 2022.

- Naqvi D, Braun R, **Holt AG**. The Effect of Repetitive Linear Acceleration on Gravity Receptor Function. 45th Annual Mid-Winter Meeting of Association for Research in Otolaryngology, Virtual (planned for San Jose, CA). February 5-9, 2022.

- Braun RD, Kuhl A, Hali M, Kallakuri S, **Holt AG**, Noise impacts neuronal activity in vestibular pathways, Annual meeting of the Society for Neuroscience, Chicago, IL. October 19-23, 2019

- Invited Platform Presentations
  - Naqvi D, Braun R, **Holt AG**. The Effect of Blast Overpressure on Vestibular Pathway Function. 46th Annual Mid-Winter Meeting of Association for Research in Otolaryngology, Orlando, FL. February 11-15, 2023.

  - Yalcinoglu S, Braun R, **Holt AG**. Modulation of Voltage-Gated Calcium Channels following Noise Exposure: Impact of Synaptic Activity in the Auditory Pathway. 46th Annual Mid-Winter Meeting of Association for Research in Otolaryngology, Orlando, FL. February 11-15, 2023.

  - Naqvi D, Braun R, Ponce Sepulveda A, King S, **Holt AG**. The Effect of Repetitive Linear Acceleration on Gravity Receptor Function. 9th Midwest Auditory Research Conference, Ann Arbor, MI. June 23-25, 2022.

  - Yalcinoglu S, Wattoo A, Braun R, **Holt AG**. The Role of the Calcium Channel Blocker Verapamil on Hearing. 45th Annual Mid-Winter Meeting of Association for Research in Otolaryngology, Virtual (planned for San Jose, CA). February 5-9, 2022.

  - Naqvi S, Braun R, **Holt AG**, The Effect of Blast Overpressure on Vestibular Pathway Function. 46th Annual Mid-Winter Meeting of Association for Research in Otolaryngology, In-Person Orlando, FL. February 10-15, 2023

  - **Holt AG**, Kuhl A, Hali M, Braun RD Impact of Noise on Manganese Uptake Following Otolith Stimulation 47th Annual Scientific and Technology Meeting of the American Auditory Society, Scottsdale, AZ. March 5-7, 2020.

  - **Holt AG**, "Novel QUEST MRI In Vivo Measurement of Noise-induced Oxidative Stress in the Cochlea" at the Department of Otolaryngology, Wayne State University, December, 2019.

Plans for next reporting period

This is the final report. We have made substantial progress on the project, particularly given the challenges encountered during shut down and other fall-out from the pandemic. For Aim 1 (Task 4) and Aim 2 (Task 6) rats completed the "in-life phase" of blast-noise exposure (or sham) and NT-3 treatment 1 day after the blast-noise exposure (or no treatment). They have been euthanized and tissues collected. They will need to be assessed for hair cell loss and loss of Inner Hair Cell – Auditory Nerve synaptic (re)connections.

Aim 1

1. NT-3 treatment (or poloxamer only without NT-3) one day after the noise
2. Final ABR and DPOAE

Aim 2

1. Base-line Auditory Brain Stem Response (ABR), Gap Detection (GD and Pre-Pulse Inhibition (PPI) of the Acoustic Startle Reflex (ASR) and Distortion Production Oto Acoustic Emission (DPOAE) and training in operant conditioning.
2. Noise exposure (or sham noise)
3. Testing after treatment with an anti- excitotoxicity agent prior to noise exposure
4. Testing for tinnitus (using GD and Operant Conditioning) for two months
5. Final ABR and DPOAE

After the in-life phase animals are then euthanized and assessed for hair cell loss and loss of Inner Hair Cell – Auditory Nerve synaptic connections.

We will have written four manuscripts and are revising them for submission journals.

4. <u>IMPACT</u>:

There were three important results that impact hearing research and rehabilitation:

- Our studies show that spectral content of a military relevant blast-noise is highly relevant to the type of cochlear injury and hearing dysfunction that results. Focus only on the intensity of the blast overpressure risks missing important information about the cause of injury and potential treatment.
- We are in the process of preparing our studies for publication. The first study shows that modifying the shocktube (length) results in modification of the characteristics of the blast-noise. The second study demonstrates the effects of shifting these spectral characteristics on cochlear damage, hearing function, and synaptopathy. The third study demonstrates that an anti- excitotoxicity agent can restore moderate levels of hearing function after blast injury compared to no treatment. The fourth study shows how an anti- excitotoxicity agent can prevent both peripheral and central deficits that result from exposure to loud noise. In addition, we show that the anti- excitotoxicity agent reduces gap detection deficits (a measure of tinnitus). However, a single dose was insufficient to sustain the effect. We plan to apply for additional funding to follow-up on this result and to process the tissue collected from the studies above.
- Our more recent preliminary studies of assessing vestibular function one day following the blast-noise is promising. We plan to apply for additional funding to follow-up on these results by assessing additional time points, evaluating anti- excitotoxicity agents for treatment vs prevention of blast and noise induced hearing loss and tinnitus. Treatment following noise will be more applicable and the trans-tympanic middle ear approach to applying NT-3 is feasible for clinical application. We also plan to examine potential mechanisms of action that are suggested by our results. We have used physiological measures to determine the efficacy of NT-3 to induce re-connection and repair of the lost connections. However, anatomical assessment of the periphery and central auditory pathway is still needed for verification. Therefore, short term funding will be sought to complete these studies.

The more general impact of study results:

- Demonstrated frequency content of blast-noise is crucial for predicting hearing dysfunction
- Blast overpressure and spectral content impact cochlear damage
- Blast-noise can produce vestibular nerve hypofunction which may correlate with auditory dysfunction.

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

<u>REFERENCES</u>

Altschuler RA, Halsey K, Kanicki A, Martin C, Prieskorn D, DeRemer S, Dolan DF (2019) Small arms fire-like noise: Effects on hearing loss, gap detection and the influence of preventive treatment, Neuroscience 2019 May 21;407:32-40. PMID: 30053484

Liberman MC, Epstein MJ, Cleveland SS, Wang H, Maison SF. (2016) Toward a Differential Diagnosis of Hidden Hearing Loss in Humans. PLoS One. 11(9):e0162726. PMID:27618300

Liberman MC, Kujawa SG. (2017) Cochlear synaptopathy in acquired sensorineural hearing loss: Manifestations and mechanisms. Hear Res.;349:138-147. PMID:28087419

Suzuki J, Corfas G, Liberman MC. (2016) Round-window delivery of neurotrophin 3 regenerates cochlear synapses after acoustic overexposure. Sci Rep. 6:24907. PMID:2710859

5. CHANGES / PROBLEMS:

There was a delay in being able to apply metrics for the presence of tinnitus significantly delaying tinnitus assessments (outlined above) so that completion of Tasks were delayed. Because of these delays we were not able to process tissue that was collected for analysis of anatomical and biochemical evidence of synaptic re-connection after anti- excitotoxicity treatment following blast-noise or TTS noise. During the final year, the primary use of our time was for analysis of collected data and writing associated manuscripts.

6. PRODUCTS:

Other publications, conference papers, and presentations.

Nothing additional over what was reported above

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products *Nothing to report*

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Name:	Avril Genene Holt
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	avrilhol
Nearest person month worked:	.6
Contribution to Project:	Responsibility for the supervision of the histopathology, quantitative assessments of hair cells and the connection between hair cells and auditory nerve, auditory brain stem response (ABR) measures including thresholds and input-output function, DPOAE, and overseeing the assessment of gap detection and pre-pulse inhibition of the acoustic startle reflex as well as the

	assessment and interpretation of these results. Dr. Holt interpreted the results developed and refined methods and made decisions on directions.
Funding Support:	W81XWH-17-1-0172 (Holt) 09/30/18 – 09/29/21 .6 calendar months (cost share) DOD (NCE) Mechanism Based Prevention of Noise-Induced Tinnitus: Protection and Repair of Peripheral Auditory Neuropathy, PR160290

Name:	Rod D. Braun
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0
Contribution to Project:	Dr. Braun has substantial knowledge and expertise in analysis and mathematical modeling of biomedical data. He worked with the PI to develop unique methods for analyzing ASR data for tinnitus assessment.
Funding Support:	W81XWH-17-1-0172 (Holt) 09/30/18 – 09/29/20 1.2 calendar months DOD

Name:	Danial Syed Naqvi
Project Role:	Research Assistant/Graduate Student
Nearest person month worked:	N/A
Contribution to Project:	Mr. Naqvi was responsible for analysis of Auditory Brain Stem Response (ABR), and VsEP measures for blast-noise experiments. Danial was trained in animal surgeries and has assisted in animal terminations. Danial has learned biochemical techniques for assessing protein localization and distribution. Danial has been instrumental in all phases of analysing and writing of the manuscripts for the blast-noise studies. The plan includes Danial working with on quantitative assessment of hair cells to generate cytocochleograms and the quantitative assessment of IHC-AN synaptic connections at WSU once funding is obtained.
Funding Support:	N/A

Name:	Selin Yalcinoglu
Project Role:	Research Lab Specialist/student

Nearest person month worked:	N/A
Contribution to Project:	Selin worked was responsible for quantitative assessment of ABRs and ASRs related to the TTS-noise studies. Selin has been instrumental in all phases of analysis and writing of the manuscripts related to the TTS-noise studies.
Funding Support:	N/A

What other organizations were involved as partners?
University of Michigan, Ann Arbor Michigan, is the Corresponding Primary Institution to this project.

8. SPECIAL REPORTING REQUIREMENTS
Nothing to Report

9. APPENDICES: