

AWARD NUMBER: W81XWH-17-1-0172

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

PRINCIPAL INVESTIGATOR (Partnering PI): Dr. Avril Genene Holt

CONTRACTING ORGANIZATION: Wayne State University
Detroit, Michigan 48202-4050

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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 (Lieberman 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 decrease 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					
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1. INTRODUCTION:

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 Site 1 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. Site 2 characterized blast-noise, another military relevant noise, simulating two different exposure conditions. Blast-noise studies often focus on the overpressure that is produced without evaluation of spectral characteristics of the blast-noise. During the first year of studies Site 2 we used two configurations of the shocktube - blast-noise generator (described in the results section). The results show that not only can we change the overpressure of the blast, but the spectral content as well. The conclusion is that blast-noise can have measurable differences in blast content which can provide more reliable targets for treatment when compared to blast overpressure measures which can result in unpredictable damage. We will now be able to determine the effects of these conditions on cochlear damage. The major goal of both Sites, however, is to test if synaptic reconnection will decrease or prevent the later development of tinnitus and that is the focus of the next stages of our ongoing studies. For Site 1 studies are now underway to determine if this rapid reconnection from NT-3 treatment will decrease the incidence of noise induced tinnitus compared to noise exposed rats without treatment. For Site 2 studies have begun to determine if blast-noise exposed rats will cochlear damage that is specific to the spectral content of the blast-noise. If successful, our studies will provide a military relevant treatment to prevent and treat noise-induced tinnitus.

2. KEYWORDS:

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

3. MAJOR GOALS - from Statement of Work (SOW):

(Years 1-2)

TASK 4 (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 noise to prevent appearance of tinnitus based on Gap Detection & and behavioral (operant conditioning) metrics
- **Subtask 2:** Influence of Treatments on Noise-Induced changes to Inner Hair Cell –Auditory Nerve synaptic connections.

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

(Years 2-3)

TASK 6 (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 w:** NT-3 treatment six week after noise
(*Milestones: Break Code and determine treatment effects in Months 33-36*)

Expected Deliverables for Year One (from SOW): Because of blinding that will not be removed until the end of Aim 1A studies in Year Two, there will be no Results reported in Year One except the number of animals going through study and any unanticipated problems that might have occurred and how they are dealt with

3. ACCOMPLISHMENTS for Task 4 (Aim 1A) in Year One

Major Activities 1:

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

A shocktube for blast generation was modified and two configurations were compared to define the pressure and spectral characteristics of the subsequent blasts. The two configurations of the shock tube were a short shocktube and a long shocktube. Desired peak static overpressure was produced with compressed helium, and calibrated mylar sheets (GE Richards Graphics Supplies Inc., Landisville, PA) placed between the driver chamber and the first segment of the driven chamber. A pencil probe measured the side on blast overpressure. Pressure data were collected at a sampling rate of 100,000 Hz (DEWE-3050 data acquisition system, Dewetron) and analyzed for incident blast overpressure (DEWESoft 7.1.2) as well as spectral characteristics (BK Connect analysis software; Brüel & Kjær).

Unanticipated Problems:

There was more delay than anticipated receiving the sound booths for the set-up of the Lick Suppression (CLS) stations (operant conditioning) at WSU. There was also delay in receiving the new Gap Detection testing stations from Kinder Scientific and making these stations (with a new design) operational for testing for tinnitus. Setting up and testing the two configurations of the shocktube also took additional time. These tools are now all operational, and AIM 1A studies are fully underway, but the start of the animal studies was delayed by eight months.

Major Activities 2:

Because of this initial delay in not being able to use animals in the blast-noise experiments, Year One studies first focused on characterization and modulation of the blast-noise in Task 4, Subtask 3. The ability to measure as well as modulate the spectral content of the Blast may allow the generation of more relevant animal models with tinnitus and hearing deficits. We wanted to modulate the overpressure intensity in two different configurations of the shock tube and evaluate the effect on spectral characteristics of the blast-noise. In theory, by changing the length of the shocktube the frequency of the blast-noise wave should be modulated and would result in distinctive cochlear injury. We also wanted to determine whether the protocol for producing a blast overpressure wave of 22 psi (10+3 mil mylar) would be effective in both configurations. To that end, blast-noise generated with the Wayne State University shocktube was evaluated using two different configurations: 1) long shocktube - ~ 20' and 2) short shocktube - ~ 8', designed exclusively for this project. This study was successfully completed during Year One and the preliminary analysis and results were presented at the Military Health System Research Symposium in the Fall of 2018. During Years One and Two, the analysis will be completed and the manuscript will be prepared for publication.

RESULTS:

Much of the blast-noise literature is related to the intensity of the blast overpressure with no detailed information related to the spectral characteristics of the blast-noise. The ability to modulate as well as measure the spectral content of the blast-noise may allow generation of more relevant animal models for blast-induced tinnitus. The Wayne State University long shocktube configuration of approximately 20' (**Figure 1-inset**) produces peak sound pressure levels between 160-180 dB SPL when mylar sheets of 10 mil, 10+3 mil, or 10+3+3 mil are used (**Figure 1**) with frequencies ranging from ~50 - 500 Hz. From ~500 Hz to ~2.5 kHz, the sound pressure levels fall to ~100 dB SPL or below. The sound pressure levels fall further from ~2.5 to 5 kHz (Figure 1). When the short shocktube configuration is used (**Figure 2-inset**), peak sound pressure levels of ~160 dB SPL are produced when a 10+3 mylar is used with pencil probe placed 7" inches inside the shocktube to mimic the placement of the the long shocktube paradigm (**Figure 2**). However, sound pressure levels fall more rapidly to a level of ~80 dB SPL and are then maintained over a broader range of frequencies before returning to pre-blast levels. (**Figure 2**).

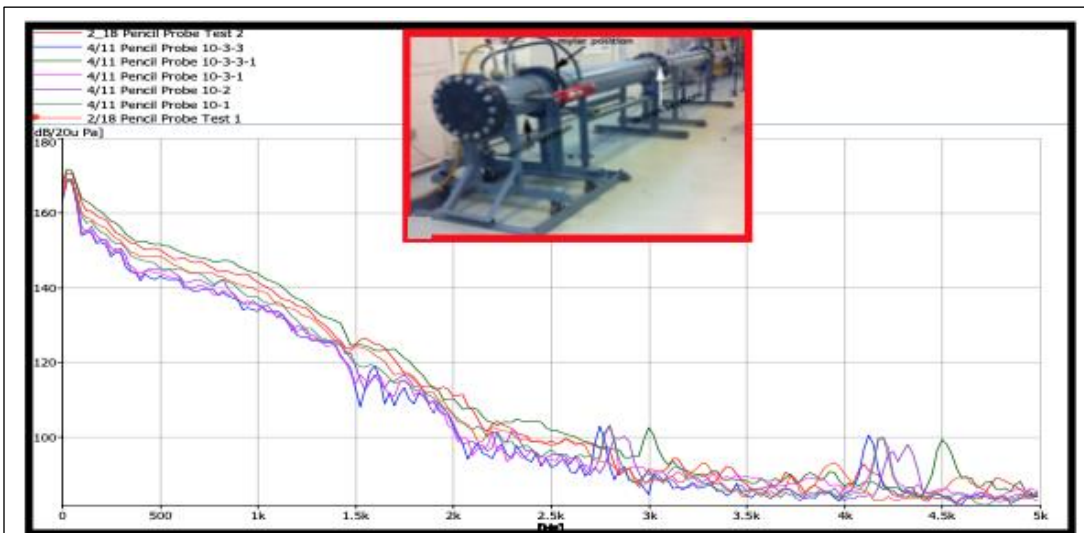
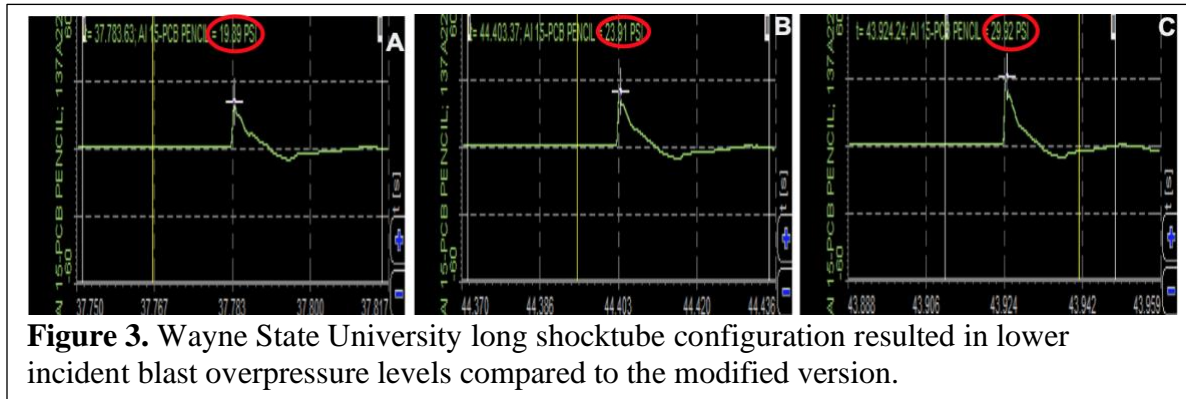


Figure 1. Wayne State University long shocktube configuration spectral characterization of blast-noise.

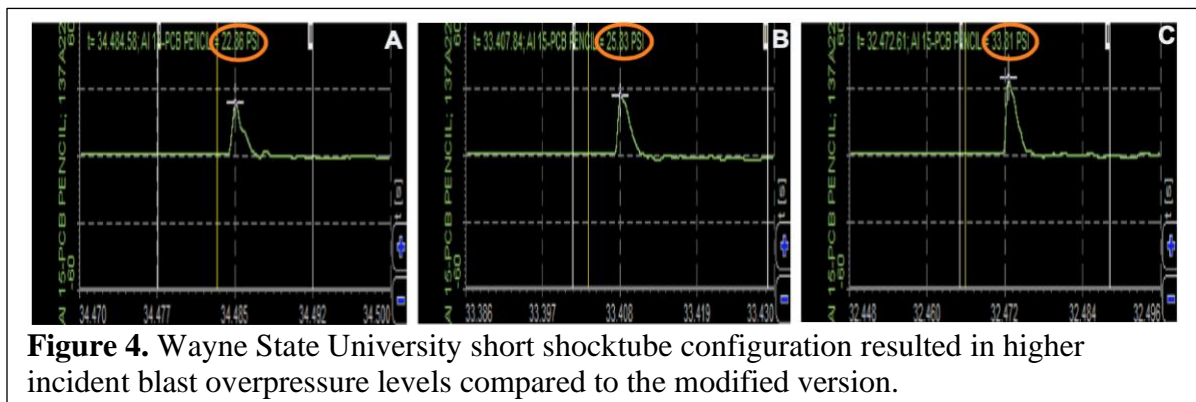


Figure 2. Shortening the length of the shock tube leads to sound pressure levels extending over wider frequencies at higher intensities.

The long shocktube configuration resulted in lower incident blast overpressure levels compared to the short shocktube configuration. Long shocktube configuration tests with a one 10 mil mylar sheet paradigm resulted in a peak overpressure of 19.89 psi (**Figure 3-A; red circle**); Testing with one 10 mil and a 3 mil mylar sheet resulted in a peak overpressure of 23.91 psi (**Figure 3-B; red circle**). Further testing with one 10 mil and two 3 mil mylar sheets resulted in a peak overpressure of 29.92 psi (Figure 3-C; red circle). In each case, the duration of the Blast was no more than 10 milliseconds. The white plus symbol in each panel indicates peak pressure (circled in red).



The short shocktube resulted in higher incident blast overpressure levels compared to the long shocktube configuration. Testing with one 10 mil and one 3 mil mylar sheets resulted in a peak overpressure of 22.86 psi (**Figure 4-A; orange circle**). However, testing with one 10 mil and one 3 mil mylar sheets with the pencil probe positioned 7” inside the shock tube to mimic the pencil placement in the long shocktube configuration resulted in a markedly higher peak overpressure of 25.83 (**Figure 4-B; orange circle**). The peak blast overpressure was even higher with one 10 mil and two 3 mil mylar sheets, resulting in 33.81 psi, compared to the long shocktube configuration using the same paradigm (**Figure 4-C; orange circle**).



Conclusions: Our results show that changing the length of the shock tube can alter multiple features of blast pressure. The closer the proximity to the blast pressure, the higher the experienced pressure level. While the maximum sound pressure levels were highest with the long shocktube configuration, the long shocktube configuration resulted in higher sound pressure levels across a wider range of frequencies. Blast-noise exposures containing different spectral characteristics would be expected to result in differential cochlear injury. The blast generation strategies and potential cochlear injury differences would be more applicable to those in the service with tinnitus than modulation of overpressure. Now our ongoing **Subtask 3** studies will assess whether varying spectral characteristics of blast-noise modifies changes cochlear injury.

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

Nothing to Report

How were the results disseminated to communities of interest?

- One Poster Presentation by Dr. Holt:

*Kallakuri S, Lewis R, Hali M, Cavanaugh JM, Braun R, **Holt AG**, Characterization and modulation of shock tube generated blast pressure. Military Health System Research Symposium (MHSRS), Gaylord Palms Resort and Convention Center, Kissimmee, FL, August 20-23, 2018.

*Kallakuri S, Cavanaugh JM, Holt AG, Blast overpressure induced cellular injury changes: relationship to pain and tinnitus. Military Health System Research Symposium (MHSRS), Gaylord Palms Resort and Convention Center, Kissimmee, FL, August 20-23, 2018.

Holt AG, Hali M, *Apawu A, Pace E, Zhang J, Altschuler RA, Noise and blast-induced tinnitus: Models of peripheral and central auditory damage in rodents. Military Health System Research Symposium (MHSRS), Gaylord Palms Resort and Convention Center, Kissimmee, FL, August 20-23, 2018.

* - Presenting author

- One Invited Platform Presentation by Dr. Holt:

“Forging a Path Towards Identifying Central Mechanisms and Developing Objective Measures for Tinnitus Diagnosis and Treatment” at the 25th Appalachian Spring Conference at the James Quillen VAMC in Johnson City, TN in June 2018.

Plans for next reporting period

Year Two will continue with Aim 1A started in Year One with rats being tested using different configurations of the shocktube generating blast-noise with different spectral characteristics. We will also begin testing the effects of post treatment with NT3 on cochlear injury, hearing dysfunction and tinnitus.

1. Before Blast, Immediately after Blast, and two weeks after Blast.
 - a. Baseline auditory brain stem response (ABR), and distortion product otoacoustic emission (DPOAE)

We will also begin testing the effects of post treatment with NT3 on cochlear injury, hearing dysfunction and tinnitus.

1. Base-line Auditory Brain Stem Response (ABR), Gap Detection (GD and Pre-Pulse Inhibition (PPI) of the Acoustic Startle Reflex (ASR) and distortion product otoacoustic emission (DPOAE) and operant conditioning training
2. Blast-noise exposure (or sham noise)
3. NT-3 treatment (or poloxamer only without NT-3) one day after the noise
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.

4. IMPACT:

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

- Our Year One studies showed that re-configuration of the shocktube for generation of blast-noise exposure produces a shift in the spectral characteristics of the blast with sustained loud noise at higher frequencies.
- We further demonstrated that a shift to higher frequencies correlated with a shift to more intense overpressure
- We have presented the initial findings from these studies at conferences and will now prepare the results showing that while changing the blast overpressure in a given shocktube does not alter the spectral content, modulating the configuration (length, width, etc...) for submission and publication.

While other studies have focused on changing overpressure resulting from blast, we have changed the spectral content which may be more relevant to those in the service with tinnitus and may offer promising targets for intervention. Our Year two studies will test the impact of these changes in blast-noise content on cochlear injury and hearing function. Treatment following blast-noise will be more applicable. Future studies will also test efficacy, with treatment at later times following blast-noise.

There is also more general impact as study results:

During Year 1 the contact PI -

- demonstrated NT-3 can induce re-connection of lost synapses
- showed effective anti-excitotoxicity treatments

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

CHANGES/PROBLEMS:

There were no significant changes other than a delay in being able to apply metrics for the presence of tinnitus, delaying tinnitus assessments.

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

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- 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

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Name:	Avril Gene Holt
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	avrilhol
Nearest person month worked:	3
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. She will interpret results, troubleshoot methods and make decisions on directions.
Funding Support:	Nothing to Report

Name:	John Cavanaugh
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Cavanaugh is an expert in the physics of blast overpressure. He will advise on shocktube configuration, design and analysis of blast experiments.
Funding Support:	W81XWH-17-1-0172 (Holt) 09/30/17 – 09/29/20 0.96 calendar months DOD \$167,074 Mechanism Based Prevention of Noise-Induced Tinnitus: Protection and Repair of Peripheral Auditory Neuropathy, PR160290 Our proposed studies will first test if repair /reconnection will prevent tinnitus from occurring. Next we will determine what is the time window for effective treatment, how long after the noise can reconnection be induced that will prevent or treat the progression of tinnitus.

Name:	Rod D. Braun
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Braun has substantial knowledge and expertise in analysis and mathematical modeling of biomedical data. He is developing unique methods for analyzing ASR and operant conditioning data for tinnitus.

Funding Support:	<p>W81XWH-17-1-0172 (Holt) 09/30/18 – 09/29/20 1.2 calendar months DOD \$167,074 Mechanism Based Prevention of Noise-Induced Tinnitus: Protection and Repair of Peripheral Auditory Neuropathy, PR160290 Our proposed studies will first test if repair /reconnection will prevent tinnitus from occurring. Next we will determine what is the time window for effective treatment, how long after the noise can reconnection be induced that will prevent or treat the progression of tinnitus.</p>
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Name:	Jinsheng Zhang
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	2
Contribution to Project:	Set-up operant conditioning. Provide advice and assist in interpretation of data
Funding Support:	<p>W81XWH-17-1-0172 (Holt) 09/30/17 – 04/01/19 2.4 calendar months DOD \$167,074 Mechanism Based Prevention of Noise-Induced Tinnitus: Protection and Repair of Peripheral Auditory Neuropathy, PR160290 Our proposed studies will first test if repair /reconnection will prevent tinnitus from occurring. Next we will determine what is the time window for effective treatment, how long after the noise can reconnection be induced that will prevent or treat the progression of tinnitus.</p>

Name:	Mirabela Hali
Project Role:	Research Lab Specialist - Research Assistant
Nearest person month worked:	12
Contribution to Project:	Ms. Hali is responsible for the Auditory Brain Stem Response (ABR) measures, DPOAE and assisting with the blast-noise exposures at WSU. She also trained with Diane Prieskorn at KHRI to learn the animal surgeries and drug delivery (by poloxamer on the round window niche). She also is responsible for animal terminations and fixation of cochleae.
Funding Support:	N/A

Name:	Andre Kuhl
Project Role:	Research Assistant
Nearest person month worked:	4

Contribution to Project:	Mr. Kuhl will be responsible for assisting with the Auditory Brain Stem Response (ABR), DPOAE measures and the blast-noise exposures at WSU. He will also be trained in the animal surgeries and drug delivery (by poloxamer on the round window niche). He will also assist in animal terminations and fixation of cochleae and some of the quantitative assessment of hair cells to generate cytochleograms and the quantitative assessment of IHC-AN synaptic connections at WSU.
Funding Support:	N/A

Name:	Srini Kallakuri
Project Role:	Research Lab Specialist Senior – Research Assistant Professor
Nearest person month worked:	4
Contribution to Project:	Dr. Kallakuri is responsible for the implementation of the blast-noise exposures and re-configuration of the shocktube at WSU. He also assists in ABR measures and assessment.
Funding Support:	N/A

Name:	Dr. Aaron Apawu
Project Role:	Post doctoral fellow
Nearest person month worked:	3
Contribution to Project:	Dr. Apawu is responsible for assisting in the implementation and assessment of Gap detection (ASR) and operant conditioning.
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. They will be sending in their collaborative Progress Report separately.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report

9. APPENDICES:

Nothing to attached