

AWARD NUMBER: W81XWH-18-1-0135

TITLE: Development of Objective Electrophysiological Tests for Tinnitus Based on Long-Lasting After-Discharges in the Inferior Colliculus

PRINCIPAL INVESTIGATOR: Douglas L. Oliver

**CONTRACTING ORGANIZATION: University of Connecticut
Grant Administration**

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14. ABSTRACT Tinnitus is the sensation of ringing in the ears in the absence of a corresponding, physical sound and is a symptom of a pathological response of the auditory system. It is common in the aging population and in military veterans in particular. Most often, tinnitus is associated with hearing loss due to exposure to loud noise. There is no objective or diagnostic test for tinnitus, little understanding of its causes, and no curative treatment. This project's goal is to create an electrophysiological test for tinnitus. We have shown that there are measurable and detectable changes in the electrophysiological activity in the central auditory system in response to a long-duration sound; this is a long-lasting afterdischarge where neurons continue to fire 2-3 minutes after the sound has stopped. We test whether this type of afterdischarge behavior is pathologically modified in tinnitus subjects to become continuous, and doing so, it generates a signal that the brain mistakes for a phantom sound. The basis of our tinnitus test is a presumed difference in the electrophysiology in parts of the auditory system generating the tinnitus signal from other parts of the auditory system. Our current findings show that long-duration sounds produce afterdischarge behavior and can modify tone-evoked responses in subpopulations of neurons in normal hearing mice, and these patterns may change in mice with tinnitus. The results from the animal studies will be used in human subjects with and without tinnitus to investigate the sound-evoked auditory potentials before and after presentation of a long duration sound are related to tinnitus.					
15. SUBJECT TERMS Tinnitus, afterdischarge, inferior colliculus, mouse					
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1. INTRODUCTION:

The goal of this project is to develop an electrophysiological test for tinnitus that would allow its diagnosis without a verbal report from the subject in both military and civilian patients. Tinnitus is the sensation of ringing in the ears in the absence of a corresponding, physical sound and is a symptom of a pathological response of the auditory system. Unfortunately, we have little understanding of the causes of tinnitus, and there is no objective or diagnostic test for tinnitus. We postulate that there are measurable and detectable changes in the electrophysiological activity in the central auditory system in the brain that will reflect an abnormal pattern of neural activity in subjects with tinnitus, and this abnormal activity will be related to the parts of the system that process the frequencies of the perceived tinnitus sound. Such abnormal activity will be absent in other parts of the auditory system and in subjects without tinnitus. We discovered a new type of neural response to sound in the central auditory system, a long-lasting sound-evoked afterdischarge, in recordings of a subpopulation of neurons in the inferior colliculus. When presented with a sound of 30-90 seconds, they continue to fire for minutes after the offset of the sound. This may be a new type of neural plasticity in the normal brain. In normal mice, we have found that the responses to sound in deep brain recordings and the amplitudes of the evoked potentials in the brainstem are increased after a long-duration sound than before the sound. The premise of our experiments on tinnitus is that this type of plasticity is modified by tinnitus and may distinguish the part of the auditory system generating the tinnitus signal. This project uses a mouse model to investigate how the tinnitus and non-tinnitus animal differ in their electrophysiological responses to sound. In this project, we use continuous loud sounds to induce tinnitus in mice, and we also use the exposure to impulse noise to induce tinnitus as this type exposure is of particular concern for military personnel. Mice are behaviorally tested to determine if tinnitus has been induced. We then use both deep brain recordings in the inferior colliculus and auditory brainstem responses to compare the responses before and after a long-duration sound for sounds at or near the frequency of the tinnitus versus other sounds that are well separated from the tinnitus frequency. The paradigms in the animal studies that reveal differences between tinnitus and non-tinnitus subjects are being used to study sound evoked auditory potentials before and after a long-duration sound in human subjects with and without tinnitus. This forms the basis of our electrophysiological test for tinnitus.

2. KEYWORDS:

Tinnitus, afterdischarge, mouse, inferior colliculus, auditory, hearing, spontaneous activity

3. ACCOMPLISHMENTS:

What were the major goals of the project?

- Major Task 1: Electrophysiological testing of LSA in neurons in the inferior colliculus before and after administration of sodium salicylate in mice. Months 1-24. 60% complete
- Major Task 2: Develop noise/blast induced models of tinnitus in mice. Months 6-48. 90% complete
- Major Task 3: Behavioral testing for tinnitus in mice after noise/blast; Months 12-48. 80% complete
- Major Task 4: Electrophysiological testing of LSA in neurons in the inferior colliculus after noise exposure. Months 18-48. 75% complete
- Major Task 5: Evoked potential testing in mice. Months 12-48. 60% complete
- Major Task 6: Evoked potential testing in human subjects. Months 6-48. 30% complete

What was accomplished under these goals?

Major activity 1.

Develop noise/blast induced models of tinnitus in mice (Major tasks 2 and 3 above). The specific objectives are to induce tinnitus using continuous loud sound or by using impulse noise and determine the conditions and paradigms for optimal exposure to loud sound for the purposes of inducing tinnitus in mice. We continue to train more mice and expose them to sound trauma to induce tinnitus.

We presented a virtual paper at the Society for Neuroscience meeting on Nov 8-11, 2021.

Comparison of Active Avoidance and GPIAS for Tinnitus Assessment in Mice E. FABRIZIO-STOVER, A. L. BURGHARD, C. M. LEE, D. L. OLIVER. The meeting was a virtual meeting at which the presentations of “posters” were given as a recorded video. The script and the slides presented were the following:

Hi, my name is Emily Fabrizio-Stover, a graduate student in the Oliver lab at the university of Connecticut health center. I will be talking to you about comparing two different behavioral tests for tinnitus.

How can we diagnose animals with tinnitus? Chances are, if you’re researching tinnitus, you’re using a mouse model. Mice may have tinnitus, but they cannot communicate to us that they have tinnitus. So, we must infer their tinnitus status based on behavioral tasks. There are two general types of behavioral tasks: instinctual and learned. We wanted to train the same group of animals in both tests to help address the question ‘which test is best’.

Our instinctual test was gap-induced pre-pulse inhibition of acoustic startle. The mouse is presented with two different types of acoustic stimuli: startle only trials and gap trials. The startle only trials has a background narrowband noise meant to induce a startle response, which is then measured. The gap trial is the same with the addition of a 20 millisecond gap before the startle stimulus meant to attenuate the acoustic startle response. In tinnitus animals, when the tinnitus frequency is close to or centered at the background frequency, it’s possible that the tinnitus percept fills in the gap and reduce the attenuation of the startle.

The learned test we decided to use was active avoidance, where the mouse learned that the onset of noise was coupled with shock. So over time, the mouse learned that if sound is presented, it needs to move to avoid the shock. Sound is dangerous, associated with a shock, but silence is safe. The tinnitus animal no longer experiences silence, only it’s tinnitus percept. The tinnitus percept then becomes ‘safe’ and if a sound is presented at or close to the animal’s tinnitus percept, the mouse is less likely to move and avoid the shock.

We trained animals in active avoidance and GPIAS or only one test, sound exposed them with one ear plugged to preserve hearing, waited eight weeks for tinnitus induction, and then tested them in behavior again.

We found four general categories of animals with tinnitus behavior: positive in only one test, positive in both, and negative in both tests. Left graph is GPIAS, right graph is active avoidance. Tinnitus frequencies are indicated by arrows and asterixis. We wanted to quantify the number of animals positive in one test, both tests, or none.

This is the percentage tinnitus positive animals of mice tested in one test only. The number of active avoidance positive animals is higher than that of the GPIAS. This pattern carries over when looking at animals tested in two behaviors where the percentage of active avoidance tinnitus positive animals is higher than GPIAS positive animals. Furthermore, animals positive in GPIAS are more likely to be positive in active avoidance as well. This suggests that active avoidance may be more sensitive than GPIAS but may also have more false positives.

We also looked at the distribution of the tinnitus frequencies for both tests relative to our exposure frequency of 16 kHz. For active avoidance, the tinnitus frequencies are at or slightly above the exposure frequency, while the tinnitus frequencies for GPIAS are mostly at 32 kHz.

We were able to see differences between these two tests, we also wanted to see if these differences were reflected in spontaneous activity changes in the inferior colliculus. Increase spontaneous activity is associated with tinnitus. We looked at the spontaneous activity in the IC of mice that tested positive in active avoidance and looked at the characteristic frequency below, at, or above our exposure frequency. We found that active avoidance tinnitus positive animals had significantly higher spontaneous firing than the non-tinnitus and unexposed control animals, especially at 16 kHz. The same pattern is not seen for GPIAS positive animals, which had a lower spontaneous firing rate than non-tinnitus animals. This suggests that active avoidance behavior is more consistent with what we expect to see in tinnitus animals.

To conclude, active avoidance tinnitus is associated with an expected increase in spontaneous activity with GPIAS tinnitus is not and active avoidance is a more sensitive test for tinnitus but may have more false positives.

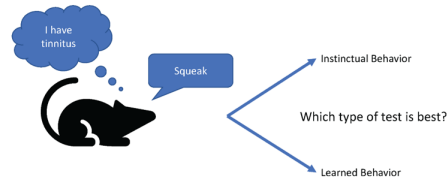
I want to give thanks to the Oliver lab and our funding from the department of defense. Thank you all for listening.

Comparison of Active Avoidance and GPIAS for Tinnitus Assessment in Mice

Emily Fabrizio-Stover
Douglas Oliver Lab, UConn Health
Farmington, CT, USA

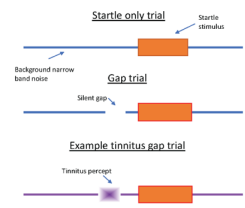


How can we diagnose animals with tinnitus?

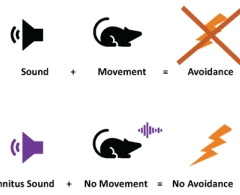


Instinctual Test and Learned Test

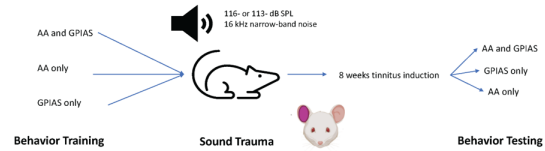
Gap-Induced Pre-Pulse Inhibition of Acoustic Startle (GPIAS)



Active Avoidance (AA)

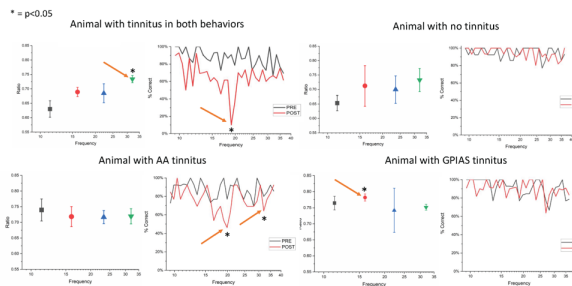


Do animals trained in both tests show the same tinnitus phenotypes?

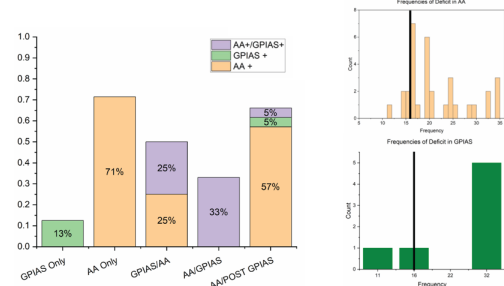


GPIAS only, AA only, GPIAS/AA, AA/GPIAS, AA/ POST GPIAS

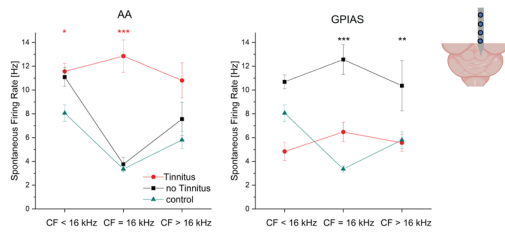
Four Categories of Tinnitus Behavior



Active Avoidance is More Sensitive Than GPIAS



Increase in Spontaneous Activity in the Inferior Colliculus is Associated with AA Tinnitus

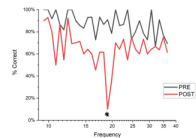


*p<0.05

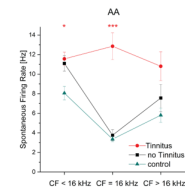
Diagram with bioRxiv

Conclusions

- AA tinnitus associated with expected increase in spontaneous activity while GPIAS tinnitus is not



- AA is more sensitive for tinnitus but may have more false positives



Major activity 2.

Deep brain 32-channel recordings of neurons in the inferior colliculus in normal hearing mice to characterize the conditions that alter spontaneous activity or evoked responses after a long-duration sound (LDS) (Major Tasks 1 and 4 above). *The specific objectives are:* 1) Determine the prevalence of neurons with long-duration sound-evoked afterdischarges by measuring the increase of spontaneous activity of neurons after an LDS \geq 60s in duration. 2) Measure the evoked responses to sound before and after an LDS and determine which acoustic parameters for LDS produce the largest change in the evoked response. 3) Determine the extent to which the increases in spontaneous activity and tone-evoked responses induced by an LDS occur in the same neurons.

This aim was completed and our manuscript "Long-Duration Sound-Induced Facilitation Changes Population Activity in the Inferior Colliculus" has been accepted for publication in *Frontiers in Systems Neuroscience*.

Major activity 3.

Deep brain recording in the inferior colliculus in mice previously exposed to noise trauma and behavioral testing for tinnitus (Major Tasks 1 and 4 above). *The specific objective* is to determine whether the prevalence of neurons with long-duration sound-evoked afterdischarges or the evoked responses to sound before and after an LDS changed in mice with behavioral evidence of tinnitus when compared to mice without tinnitus.

During the past year, we completed recordings and analysis of all mice with and without tinnitus. The results of these studies are summarized in this abstract to the Society for Neuroscience for the 2022 meeting.

Long-duration sound induced plasticity is altered differently in mice with tinnitus compared to without tinnitus.

A presentation of a long-duration sound (LDS) can lead to a change in both spontaneous activity as well as sound-driven activity in the inferior colliculus (IC) in mice. While the majority of sound-driven responses are suppressed, a subset is potentiated after the LDS. This potentiation is more likely in channels with higher spontaneous activity. Since tinnitus is associated with increased activity in the auditory system, we are hypothesizing that tinnitus animals will have more facilitation/less suppression than animals without tinnitus. Exposing awake CBA/CaJ mice to a unilateral sound exposure, resulted in mice with and without behavioral signs of tinnitus. Using multichannel electrodes, we recorded the activity in the inferior colliculus contra- and ipsi-lateral to the sound exposed ear. The spontaneous activity in the IC contralateral to the sound exposure was higher in the tinnitus group than in the sound-exposed non-tinnitus and the control (not sound-exposed) group. When comparing LDS-driven plasticity as a function of change in spiking activity during tone presentation in mice with and without behavioral signs of tinnitus, we find that the sound exposed non-tinnitus animals show more suppression than tinnitus animals exposed to the same sound. The tinnitus animals show a response that is more similar to control (not sound-exposed) animals. When comparing the change in spontaneous activity following an LDS presentation we find that tinnitus animals show less afterdischarge activity (significant increase in spontaneous activity) than both the control and the sound exposed non-tinnitus animals.

Taken together this indicates an LDS-induced difference in sound-exposed tinnitus vs non-tinnitus animals that might serve as an objective test to differentiate between hearing loss with or without tinnitus.

Major Activity 4a

Evoked potential recordings in mice. (Major task 5 above). The objective is to use electrophysiology to test for tinnitus in animal or human subjects. In the experiments on mice, we used needle electrodes to record the potentials evoked by sounds identical to those used in the deep brain recordings in Activity 3. The experiments to date have used 3 ms tone pips and responses were collected before and after an LDS. The frequency of the tone pips and the center of the long duration sound were the same. Our protocol is to first conduct the evoked potential recordings on sound exposed animals that are tinnitus positive and tinnitus negative, and then, a week later, perform terminal deep brain electrophysiology on the same animal as described in Major

Activity 3. This allows us to compare the results of the evoked potential and the deep brain experiments within subjects.

During the past year, we completed data collection from all mice that were available from the cohorts in Activity 3. This has resulted in the following abstract presented at the 2022 Society for Neuroscience meeting.

An objective, non-invasive electrophysiological test for tinnitus would allow for efficient diagnosis of tinnitus in laboratory animals and is needed for efficient tinnitus research. In CBA/CaJ mice of both sexes, a long-duration sound (LDS) can alter both spontaneous firing rate and responses to sound in the central nucleus of the inferior colliculus (ICC). Two sound-driven responses are seen after the LDS: suppression and facilitation. We believe that in tinnitus individuals, because of changes in neuronal excitability in the auditory system that may lead to tinnitus, LDS-generated changes will be less apparent than in non-tinnitus animals. Awake CBA/CaJ mice (n=44) received a unilateral sound exposure (16 kHz center, 2kHz wide, 113 dB SPL, 60 minutes) that resulted in mice with and without behavioral evidence of tinnitus in an active avoidance task. ABRs collected from each ear (exposed and unexposed) in sound exposed tinnitus (n=22), sound-exposed non-tinnitus (n=22), and unexposed control mice (n=10). ABRs to tone pips, chirps, and narrowband noise were collected before and after LDS. For each type of stimuli's, responses to three or more frequencies were collected including the frequency of the presumed tinnitus in mice with tinnitus.

Multiple analysis methods were used to analyze the ABR waveforms. First, we quantified the effect of LDS-changes and calculated a tinnitus score for each wave based on peak-trough amplitudes. Tone pip ABRs evoked by sounds in the exposed ear showed that non-tinnitus mice have lower scores than tinnitus mice. That is, non-tinnitus mice had more suppressed POST-LDS ABRs compared to tinnitus mice (One way ANOVA, Bonferroni post-hoc test, p=0.020). This effect was most obvious in tone evoked ABRs and not chirp or narrowband noise ABRs. A correlation analysis comparing PRE-LDS and POST-LDS tone pip evoked waveforms showed that non-tinnitus mice were more significantly affected by the LDS than tinnitus mice (1 way ANOVA, Bonferroni pos-hoc test, p=0.046). A differential time frequency analysis showed that in the spectrum of the ABR waveforms over time there was a tinnitus specific 'hotspot' of increased activity at frequency of the tinnitus. These 'hotspots' were not seen for non-tinnitus animals or for non-tinnitus frequencies.

Taken together, our data suggest that ABRs collected before and after an LDS may be a viable method for developing a non-invasive, electrophysiological test for tinnitus.

Major Activity 4b

Evoked potential recordings in human subjects. (Major task 6 above). The ultimate goal of the project is to use the sound-evoked electrophysiological responses before and after a long duration sound to distinguish subjects with tinnitus from those without. We are currently conducting tests for tinnitus in the Otolaryngology clinic at UConn Health on Tuesday afternoons in a sound chamber usually used for audiology. Subjects are tested for hearing loss, discomfort level, and pitch matching. We then record sound-evoked potentials using a configuration typically used for auditory brainstem responses – a vertex electrode on the forehead or top of head and reference and ground electrodes on the two earlobes. We then record the potentials evoked by acoustic stimuli PRE-LDS and POST-LDS. These sounds are at or near the tinnitus frequency, and the responses to those will be compared to the responses to other sounds ~ 1 octave distant from the tinnitus frequency. Sessions are limited to 2 hr in duration, but most subjects return and participate in multiple sessions.

We are currently in phase 1 of the testing in which we compare the responses of subjects known to have tinnitus to control subjects that do not have tinnitus. We have four cohorts of subjects: no tinnitus- no hearing loss; no tinnitus with moderate hearing loss; tinnitus with no hearing loss and tinnitus with moderate hearing loss. This phase of testing also involves using different types of acoustic stimuli to evoke potentials. We are comparing responses to clicks, 3 ms tone pips, 3 ms chirps, and 3 ms narrowband noise. All but the

clicks are tested with a long duration sound that is centered at the same frequency as the 3 ms acoustic stimulus.

In the case of clicks, we use long duration sounds at different center frequencies. Each type of stimulus is tested at two or more center frequencies, one at or near the frequency of the tinnitus as determined by the pitch matching task, and the other is at a lower frequency at least one octave below the first. We test each subject in two or three sessions and repeat the same stimulus parameters in the same subject several times on different days.

Our preliminary results suggest that there are marked changes in the POST-LDS responses of tinnitus subjects when compared to non-tinnitus subjects. There may be less suppression of the POST-LDS response with tinnitus than with no-tinnitus. Analysis will continue.

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

Drs. Burghard and Lee and Ms. Fabrizio-Stover participated in the 2021 and 2022 online meetings of the Association for Research in Otolaryngology. They also participated in the 2022 online meeting of the Society for Neuroscience.

How were the results disseminated to communities of interest?

Alice L. Burghard, Christopher M. Lee, Emily M. Fabrizio-Stover, Douglas L. Oliver, *Long-Duration Sound-Induced Potentiation Changes Population Activity in the Inferior Colliculus*. *Frontiers in System Neuroscience*. In Press.

P436.06 - Comparison of Active Avoidance and GPIAS for Tinnitus Assessment in Mice

E. FABRIZIO-STOVER, A. L. BURGHARD, C. M. LEE, *D. L. OLIVER; Society for Neuroscience Annual Meeting, Nov. 11, 2021, 2:15 PM - 3:15 PM

Evidence of Tinnitus-Specific Differences in Stimulus Evoked Brainstem Potentials

Emily Fabrizio-Stover*, Christopher Lee, Alice Burghard, Douglas Oliver. Association for Research in Otolaryngology, 45th Annual Meeting, 2022. p. 253.

What do you plan to do during the next reporting period to accomplish the goals?

1. This will be a no-cost extension period of our funding.
2. We have completed all mouse training, testing, and recording. We will focus on the completion of our data analysis and the composition of reports of this research to be submitted to scientific journals.
3. We will continue phase 1 of our human study to record auditory brainstem responses with scalp electrodes in subjects with and without tinnitus. We will also begin phase 2 of testing where the investigators are blind to the tinnitus status of the subjects, and thus validate our test for tinnitus.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our initial findings suggest that we will be able to use the responses of the brain to sound to identify animal and human subjects with tinnitus. This will be a new test since it is based on how the brain changes its response after hearing a long-duration sound and how the tinnitus brain may be different from the normal brain.

What was the impact on other disciplines?

"Nothing to Report."

What was the impact on technology transfer?

Douglas L. Oliver Tinnitus Treatment and Analysis. Patent Application Filing #17554973. Attorney Docket #38100.0034U3; 17-Dec-2021;

What was the impact on society beyond science and technology?

If the project is successful, it may lead to an objective clinical test for tinnitus. This could also benefit the discovery of drugs to treat tinnitus.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

We have abandoned work on the sodium salicylate model of tinnitus since it is less relevant to the types of noise-induced hearing loss and tinnitus experienced by military personnel and veterans.

Actual or anticipated problems or delays and actions or plans to resolve them

No problems are anticipated at this time.

Changes that had a significant impact on expenditures

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

IRB Number: 21-150-2. Project Title: Development of Objective Electrophysiological Tests for Tinnitus Based on Long-Lasting After-Discharges in the Inferior Colliculus. Approved March 29, 2021. Report amendments submitted to the IRB and USAMRMC HRPO for review:

IRB approval to add personnel: 5/24/2021

IRB Continuation approved: 12/23/2021

Significant changes in use or care of vertebrate animals

UConn Health IACUC protocol AP-200048-0623 "Development of Objective Electrophysiological Tests for Tinnitus Based on Long-Lasting After-Discharges in the Inferior Colliculus"

- New 3-yr protocol renewal approved by the University of Connecticut Health Center IACUC on 9-SEP-2020.
- New 3-yr protocol renewal approved by the USAMRMC Animal Care and Use Review Office (ACURO) as of 9-OCT-2020
- Modified 5/28/2021 to add/remove personnel
- IACUC Continuation Approved 8/25/2021

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers;*

status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Alice L. Burghard, Christopher M. Lee, Emily M. Fabrizio-Stover, Douglas L. Oliver, *Long-Duration Sound-Induced Potentiation Changes Population Activity in the Inferior Colliculus*. *Frontiers in System Neuroscience*. In Press.

Federal support acknowledged

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Invention entitled "Electrophysiological Test for Tinnitus Based on Sound-Evoked Afterdischarge Activity," UConn reference 18-028.

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Douglas Oliver No Change

Name: Alice Burghard No Change

Name: Christopher Lee No Change

Name: Emily Fabrizio-Stover No Change

Name: Qayyoom Kasliwala

Project Role: Research Assistant

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 3

Contribution to Project: Behavioral testing of mice and assistance with electrophysiological experiments

Name: Avni Jain

Project Role: Research Assistant

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 11
Contribution to Project: Behavioral testing of mice and assistance with electrophysiological experiments
Funding Support: This project

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

"Nothing to Report."

What other organizations were involved as partners?

Organization Name: Naval Submarine Medical Research Laboratory

Location of Organization: Groton CT

Partner's contribution to the project: Dr. Casper Brandon and colleagues joined the project as collaborators; subcontractor on project

Organization Name: Dr. Brad May, Johns Hopkins School of Medicine

Location of Organization: Baltimore MD

Partner's contribution to the project: In kind support; supplied software for active avoidance; consultant on project

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.*

- 9. APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*