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

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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?

Note: Our lab was closed on March 23, 2020 due to the COVID-19 pandemic. It reopened for behavioral testing May 25, 2020 and other testing resumed in June on a limited basis.

Major activity 1.

Software development (Major Tasks 1, 4-6 above). The specific objective is to generate all software necessary for this project so that we can study spontaneous or sound-evoked responses before (PRE) and after (POST) a long-duration sound (LDS). Our software includes that used in mice to make 32 channel, deep-brain recordings

in the inferior colliculus (IC) and to record sound-evoked potentials from scalp electrodes. For the human studies, similar software has been developed to record sound-evoked potentials before and after an LDS in order to test for tinnitus. In addition to the electrophysiological software, we have completed software for use in human subjects to test hearing threshold, discomfort level, and match the pitch of tinnitus. Further software development has occurred to add new acoustic stimuli for stimulation of evoked potentials in both mice and human subjects. This includes frequency modulated sounds (chirps) and narrow-band noise pulses. These stimuli are being used in both animal and human studies.

The software for analysis of the sound evoked potentials PRE and POST an LDS has been improved remarkably with the implementation of a bootstrap analysis. With this, the cycle-by-cycle variability of the PRE evoked responses are compared to each other and then the cycle-by-cycle variability of the PRE vs POST evoked responses are compared. This is a fast and objective method with which we can observe and quantify the effects of the long-duration sound on the evoked responses. This analysis is used in both animal and human studies.

Major activity 2.
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 use two behavioral tests active avoidance (AA) and gap prepulse inhibition of acoustic startle (GPIAS) to test for a tinnitus behavioral phenotype. A study to compare the two behavioral tests is part of a doctoral dissertation for Ms. Emily Fabrizio-Stover and is nearing completion. The study suggests that the two test do not produce comparable results. We have determined that in the same animals, under the same sound exposure conditions, tinnitus frequencies are different in each test. There are also more animals positive for tinnitus in the AA test, suggesting that the GPIAS test is not accurate for assessing tinnitus. Consequently, all mice in the study now undergo the same procedure with AA training and testing. The hearing is assessed with auditory brainstem responses and amplitude modulation following responses before the beginning of training and at least 2 weeks after sound trauma exposure. Training of mice is ongoing for mice before and after sound trauma. In order to increase the throughput of mice in this procedure, we purchased a second AA setup which is now in place.

We are testing two different methods of sound exposure to induce tinnitus. We continue to use narrow-band noise (16 kHz center, 2 kHz wide, 113 db SPL) to generate mice for our electrophysiological testing. In addition, we have begun a study to develop a mouse model of tinnitus using impulse noise. Mice are exposed to ~167 dB SPL impulse noises from our 6 sound cannons. These are either one round of 6 impulses at 10 Hz or two rounds of 6 impulses at 10 Hz separated by about 15 minutes. This was an undergraduate thesis project for Ms Avni Jain completed May 1, 2021. We have exposed 13 mice at this point and assessed their hearing loss. Some have continued on for AA behavioral testing. Thus far, we can say that the repeated rounds of impulses increase the amount of hearing loss, and the loss appears to be broader in frequency that that generated by a narrow-band sound. Some of the mice show behavioral evidence of tinnitus, but it is premature to conclude which conditions are optimal for this animal model of tinnitus.

Major activity 3.
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 ≥ 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. These objectives have been met, and we have submitted the results to the Journal of Neuroscience for publication.

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. The behavioral tests included both of those described below in Major Activity 2.

During the past year, we completed recordings and analysis of two cohorts of mice with and without tinnitus. We had exposed awake mice to a continuous 1/2 octave or 2 kHz-wide narrowband noise centered at 16 kHz at either 113- or 116-dB SPL for one hour. In total, we have examined 15 mice total in our first two cohorts, nine showed behavioral evidence of tinnitus and this was unrelated to the loudness of the sound exposure. These mice had one ear plugged and the other one was exposed. The unexposed ear had normal hearing sensitivity and allowed the mice to perform the behavioral test despite the hearing loss in the exposed ear. We recorded responses from the inferior colliculus on both sides of the brain, opposite the good ear and opposite the exposed ear. Stimuli were sounds centered at frequencies at, above, and below the frequency of the tinnitus in tinnitus mice and comparable frequencies in non-tinnitus mice. This data is still being analyzed. At this point, we can say that the responses to tones after an LDS were significantly changed in the inferior colliculus opposite the sound exposed ear in mice with evidence of tinnitus. This was not seen in the colliculus opposite the good ear or in the mice without behavioral evidence of tinnitus. There was a marked increase in the proportion of neurons with increases of spectral power opposite the exposed ear in 8 of 9 mice with evidence for tinnitus. That increase usually was seen at a frequency of sound not used to induce tinnitus, most often a higher frequency. These data suggest that the more prevalent, facilitated, stronger evoked response to sound after an LDS in the tinnitus brain may be a useful biomarker for tinnitus that is discoverable with our electrophysiological test for tinnitus.

Major Activity 4: Evoked potential recordings. (Major tasks 5 & 6 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 mice that were available from the cohorts in Activity 3. We compared the auditory brainstem responses evoked by sounds while we plugged either the sound exposed ear or the good ear. As in the deep brain recordings, we used sounds centered at the frequency corresponding to the tinnitus as well as two other dissimilar frequencies. Our main result is an amplification in evoked potentials after the LDS when 3 ms tone pips are delivered to the sound exposed ear in tinnitus mice. This is more obvious in later waves that may correspond to the lateral lemniscus and inferior colliculus. This data is currently being analyzed with our new bootstrap method to determine further differences between tinnitus and non-tinnitus animals.

In the past year, we also have initiated human testing. We had purchased a separate TDT system for sound generation, hearing tests, and electrophysiological recordings, and this is now installed in the Otolaryngology

clinic at UConn Health. We have received approvals from our local IRB and HRPO and have commenced recruiting and testing human subjects. In Phase 1, we will test subjects with known tinnitus or non-tinnitus conditions for hearing loss, discomfort level, and pitch matching. We will then record sound-evoked potentials using a configuration typically used for auditory brainstem responses – a vertex electrode on the forehead and reference and ground electrodes on the two earlobes. We will record the potentials evoked by different types of acoustic stimuli PRE and POST an LDS to determine which are most effective in producing strong evoked potentials in a variety of tinnitus and non-tinnitus subjects. These sounds will be at or near the tinnitus frequency, and the responses to those will be compared to the responses to other sounds distant from the tinnitus frequency.

What opportunities for training and professional development has the project provided?
Drs. Burghard and Lee and Ms. Fabrizio-Stover participated in the online meeting of the Association for Research in Otolaryngology.

Ms. Avni Jain, an undergraduate at the University of Connecticut, received a 2020 summer undergraduate research fellowship and initiated a project tinnitus induced by impulse noise. This project culminated in an undergraduate thesis on the project.

How were the results disseminated to communities of interest?
Ms. Avni Jain wrote an undergraduate thesis entitled: *Preliminary Findings in Impulse Noise Induced Tinnitus in Mice.*

We submitted for publication at paper: 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*. Under Review.

What do you plan to do during the next reporting period to accomplish the goals?
1. We will continue to record auditory brainstem responses with scalp electrodes before and after a long duration sound in mice with and without behavioral evidence of tinnitus. These will be mice exposed to continuous loud noise to induce tinnitus and tested behaviorally to determine whether or not they have tinnitus.
2. We will continue to study the same animals in #1 with and without tinnitus by making deep brain recordings in the inferior colliculus before and after a long duration sound.
3. We will use our sound cannons to induce tinnitus with impulse noise. We will behaviorally test these animals to determine the parameters that are most likely to produce tinnitus.
4. 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?
Our provisional patent application expired. We plan to file another as soon as the parameters for our test are determined to be successful in detecting tinnitus.

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 are likely to abandon 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. However, these experiments are included in our animal protocol and may be performed if there is time.

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

The COVID-19 pandemic reduced our expenditure for supplies and animals but not for personnel costs. The pace of experiments and expenditures was lower than normal due to the pandemic.

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

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.

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" approved by the IACUC on 3-Sep-2020.

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS:

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

Journal publications.

Alice L. Burghard, Christopher M. Lee, Emily M. Fabrizio-Stover, Douglas L. Oliver, 2021, *Long-Duration Sound-Induced Potentiation Changes Population Activity in the Inferior Colliculus*. Under Review.

Federal support acknowledged

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

Nothing to report

Other publications, conference papers and presentations.

C1ql1 is expressed in adult outer hair cells of the cochlea in a tonotopic gradient.
Biswas J, Pijewski RS, Makol R, Miramontes TG, Thompson BL, Kresic LC, Burghard AL, Oliver DL, Martinelli DC. PLoS One. 2021 May 12;16(5):e0251412. doi: 10.1371/journal.pone.0251412. eCollection 2021.PMID: 33979385

- **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:	Anika Makol	
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: Qayyoom Kasliwala
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): 10
Nearest person month worked:
Contribution to Project: Behavioral testing of mice and assistance with electrophysiological experiments

Name: Avni Jain
Project Role: Undergraduate Student
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 4
Contribution to Project: Behavioral testing of mice and assistance with electrophysiological experiments
Funding Support: SURF Award from the University of Connecticut

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:

QUAD CHARTS:

9. APPENDICES: