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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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CONTRACTING ORGANIZATION:

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14. ABSTRACT

Long-duration sound-evoked after-discharges (LSA) may be a new form of plasticity with intermediate-duration potentiation in the auditory midbrain. We studied the properties of LSA in response to different sounds of long duration. Multi-channel single shank electrodes recorded spontaneous firing before and after the presentation of a long duration sound (LDS, ≥ 60s) from several frequency laminae in the inferior colliculus simultaneously. LSA was defined as firing after LDS that was two standard deviations above the mean spontaneous firing rate before LDS. We found LSA in at least ~23% of the recording sites. We could identify two basic response patterns: immediate onset after LDS or delayed onset (build-up) after LDS that began as late as 20 s after the LDS offset. Both response patterns could last for over a minute. The response type, immediate vs build-up, could not be predicted by the neural response during the long duration sound. Since the observed firing after the sound offset resembles acoustically driven activity, LSA may be related to tinnitus, a disease that is defined as the perception of a sound in the absence of an external sound stimulus.

15. SUBJECT TERMS
Tinnitus, afterdischarge, inferior colliculus, mouse

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The objective of this project is to develop an electrophysiological test for tinnitus based on a newly discovered neural response to sound in the central auditory system, a long-lasting sound-evoked afterdischarge (LSA). Tinnitus is the sensation of ringing in the ears in the absence of a corresponding, physical sound. It is a symptom of a pathological response of the auditory system. Unfortunately, the underlying cause of tinnitus is not known, and there is no objective electrophysiological test for tinnitus. The sound-evoked afterdischarge behavior in the auditory system is activity in the brain that continues after the end of a very long duration sound. In auditory brainstem responses, we have found that the amplitude of the evoked potentials in the brainstem is larger immediately after a long-duration sound than before the sound. This suggests that the brainstem response reflects the time-course of the afterdischarge behavior in single neurons. The main idea being tested in these experiments is that in tinnitus, the afterdischarge behavior becomes continuous, and this would eliminate any difference in the amplitude of the response before and after a long-duration sound. This project will investigate how the afterdischarge activity in the brain is changed with tinnitus. Two animal models will be used, tinnitus caused by overdose of salicylate (a component of aspirin) and tinnitus caused by noise damage. The results from the animal studies will be used to investigate the brain activity potentially related to tinnitus in human subjects with and without tinnitus. The ultimate goal is to develop an objective electrophysiological test for tinnitus that can be used clinically for both military and civilian patients. A secondary goal of the project will be to develop an animal model of tinnitus induced by exposure to impulse noise as this type exposure is of particular concern for military personnel.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

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

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

- 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. 25% complete
- Major Task 2: Develop noise/blast induced models of tinnitus in mice. Months 6-36. 25% complete
- Major Task 3: Behavioral testing for tinnitus in mice after noise/blast; Months 12-42. 20% complete
- Major Task 4: Electrophysiological testing of LSA in neurons in the inferior colliculus after noise exposure. Months 18-42. 25% complete
- Major Task 5: Evoked potential testing in mice. Months 1-42. 5% complete
- Major Task 6: Evoked potential testing in human subjects. Months 6-48. 5% complete

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major activity 1. Development of code for the simultaneous collection of neural data from multichannel electrodes in deep brain recordings of the inferior colliculus and analyze this data (Major Tasks 1 and 4 above). Our specific objective was to install and program Tucker-Davis Technologies RZ5 and RZ6 processor in order to present sounds and collect responses from many neurons simultaneously to probe for neural responses related to tinnitus. A second specific objective was to implement spike sorting code to identify single neurons in the multichannel recordings. These were major undertakings. The key outcome this year is that the RZ6-RZ5 system is fully functional, and spike sorting of data collected during the past 4 months had begun.

Major activity 2. Electrophysiological 16-channel recordings of neurons in the inferior colliculus in normal hearing mice began in November 2018 (Major Tasks 1 and 4 above). The specific objective is to locate, identify, and determine the prevalence of neurons with long-duration sound-evoked afterdischarges (LSA). This is done by measuring the spontaneous activity of neurons before and after a long duration sound (LDS) that is greater than 20 seconds in duration. A second objective is to determine which acoustic parameters for LDS are most effective in producing an afterdischarge. This is done by comparing both the local field potentials and the spike activity before and after LDS. A third objective is to determine the effects of LSA on sound-evoked activity immediately following LDS. This is done by measuring the firing in response to acoustic stimuli before and after LDS. Outcomes: This past year, the experiments have focused on mice with normal hearing in order to establish normative baseline data before the investigation of tinnitus animals. Our results show that neurons with a sustained response to sound are highly likely to be LSA neurons and produce an afterdischarge. With the 16-channel probes, we are able to identify multiple LSA neurons at the same time and compare their responses. By inserting the 16-channel probe perpendicular to the frequency-band laminae in the inferior colliculus, we are able to examine and compare LSA in neurons with different best frequency. This will be important to test for a change in LSA at the tinnitus frequency in comparison to other non-tinnitus frequencies. We also continue to test amplitude modulated and unmodulated LDS stimuli for their efficacy in eliciting an afterdischarge. These results will determine which LDS stimuli will be used in tinnitus subjects. We have just begun testing the firing of neurons to 3 ms tone pips before and after LDS. Post-stimulus time histograms of these responses resemble the auditory brainstem response and may prove useful in predicting optimal conditions to use for scalp recordings.

Major activity 3. Develop noise/blast induced models of tinnitus in mice (Major task 2 above). The specific objective is to induce tinnitus using continuous loud sound or by using impulse noise. Outcome: During the first year we have acquired designed a system to deliver 116 dB continuous uncorrelated narrow-band noise through two super tweeter speakers to awake mice. The main effort has been in the selection and testing of different speakers. While this occurred, the mice to be exposed to sound were being trained (see activity 4). After a delay in funding our subcontractors at the Naval Submarine Medical Research Laboratory, the reconfiguration of the equipment to deliver impulse noise stimulation has begun.

Major activity 4. Behavioral testing for tinnitus in mice after noise/blast (Major task 3 above).
The first specific objective is to develop active avoidance as a reliable behavioral task to determine if a mouse has tinnitus. This is in collaboration with Dr. Brad May (Johns Hopkins). A second objective is to establish gap prepulse inhibition of acoustic startle (GPIAS) in our laboratory to determine if a mouse has tinnitus. This is a more common test for tinnitus in animals, but it have come into questions since humans with tinnitus can still hear gaps in noise. Key outcomes: Three 5-mice cohorts have been trained in the active avoidance task to avoid shock when they hear a pure tones of random frequency and intensity played in a shuttle box. The mice tend to learn the task very quickly and can maintain a performance level of 80-90% success in avoiding a shock. The first cohort was then tested in a pseudo-tinnitus paradigm where their performance was measured while a continuous 16 kHz tone was presented outside of the shuttle box. Over 5 days of pseudo-tinnitus testing, these mice showed increased errors at 16 kHz but not at other frequencies, and this is a proof of principal that this task will identify animals with tinnitus. They also underwent extinction training in which the pseudo-tinnitus frequency was also evident. The second and third cohort of mice have undergone active avoidance training and now await sound stimulation to induce tinnitus. In late April, the GPIAS system was delivered and installed.

Major Activity 5: Evoked potential testing in human subjects. (Major task 6 above). The aim is to use electrophysiology to test for tinnitus in human subjects. Key outcome: A first step that will allow testing in human subjects is the recruitment of subjects. Dr. Roberts, our neurotology collaborator, established a patient registry for the Ear, Nose, and Throat Clinic that will allow them to be informed patients of our study and others that may be relevant to their medical history.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Dr. Alice Burghard received training in our laboratory in methods to obtain and analyze data from multichannel recordings in the inferior colliculus. Grace Nichols, Nazli Morel, and Jamie Corcoran, all undergraduates from the University of Connecticut working in our laboratory received training in methods to conduct and analyze active avoidance behavior in mice. Nichols and Corcoran were also training the using the amplitude modulation following response to establish the audiogram of the mouse.

Drs. Oliver, Burghard, Osman, and Lee all received training in the use of Synapse from Tucker Davis Technologies in courses held in Nashville and Washington.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project

activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The PI produced a TED-Ed lesson on the auditory system https://ed.ted.com/lessons/the-science-of-hearing-douglas-l-oliver

The University of Connecticut Health Center produced a Faculty spotlight on the PI and the DOD award. https://www.youtube.com/watch?time_continue=4&v=NlaGzcTm_MA

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."
Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Briefly, over the next year we will begin recording from the inferior colliculus in animals with and without tinnitus. We will expose mice to continuous loud noise to induce tinnitus and test them behaviorally to determine whether or not they have tinnitus. We also plan to install and begin using our shock tube system in order to induce tinnitus with impulse noise. We will begin coding the electrophysiological test to be used with scalp electrodes. Finally, we will begin to screen human subjects for testing.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

An invention disclosure was reported. This may result in a patent application and could lead to a startup company.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

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

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

There has been a delay in receiving custom 32 channel probes from Neuronexus Inc due to the sale and relocation of the company. We expect the delivery of the probes shortly.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

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

None

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  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).*

  Nothing to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

The University of Connecticut Health Center produced a Faculty spotlight on the PI and the DOD award. https://www.youtube.com/watch?time_continue=4&v=NlaGzcTm_MA

An abstract was submitted to the Society of Neuroscience Annual Meeting for 2019.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

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

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention,

diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

Name:	Douglas Oliver	No Change
Name:	Alice Burghard	No Change
Name:	Chris Lee	No Change

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

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

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.*