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14. ABSTRACT
Exposure to noise can cause damage to structures in the inner ear, often resulting in a loss of hearing. Recent findings in noise-exposed animals raise a new specter that even moderate noise exposures may result in damage specifically located in the synaptic region between the sensory cells in the cochlea and primary auditory neurons. There is no way currently that scientists and clinicians can diagnose possible auditory synaptic damage in humans, and diagnosis is critical for the development of innovative treatments. The objective of this project is to develop a statistical model that will accurately predict the likelihood of synaptopathy in humans who have had noise exposures in their lives. The development of the statistical model will be supported by collecting non-invasive measurements in both humans and guinea pigs. Findings from the animal testing have identified several metrics that show promise for differentiating noise-exposed from control animals, including newly created analyses of evoked potential and otoacoustic emission testing. These metrics will be tested further with increasing animal data to determine if they are candidates for inclusion in the statistical model of synaptopathy under development. Successful metrics will subsequently be applied to the human data to predict synaptopathy.

15. SUBJECT TERMS
Human hearing loss, guinea pig, Noise exposures, Neural deficits, Statistical model development

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1.Introduction

Exposure to noise has long been known to cause damage to structures in the inner ear, often resulting in a loss of hearing. This is a significant health concern for millions of people and it will intensify as the population ages and loud noises become a more prevalent feature of the soundscape. Recent findings in noise-exposed animals raise a new specter that even moderate noise exposures may result in damage specifically located in the synaptic region between the inner hair cells in the cochlea and primary auditory neurons. Although this synaptopathy has been demonstrated in several animal models, there is no way currently that scientists and clinicians can diagnose possible synaptic damage in humans, and diagnosis is critical for the development of new, innovative treatment plans. The objective of this project is to develop and populate a statistical model that is designed to accurately predict the presence and degree of synaptic damage resulting from noise exposure in guinea pigs, and subsequently to use that model to predict the likelihood of synaptopathy in humans who have had significant noise exposures in their lives. Auditory function will be assessed using a variety of non-invasive tests following noise exposure in non-human animals, and cochleae will subsequently be analyzed for evidence of synapse loss or damage. Performance on tests of auditory function in non-human animals will be compared with human performance to develop a predictive model that can be refined and extended by adding results from behavioral tests of temporal processing and speech perception in noise by human listeners. Finally, a comprehensive statistical predictive model will be developed to evaluate the likelihood and severity of synaptic damage in individual humans.

2.Keywords

Human hearing loss, guinea pig, Noise exposures, Neural deficits, Statistical model development

3.Accomplishments

What were the major goals of the project?

a. Establish and set up final electrophysiological and acoustic diagnostic tests. Milestone 1 to be completed by end of 4th month (i.e., January 31, 2020). This goal was fully realized in the Walsh (animal) lab in November 2020. Partial completion has been achieved in the Leek (human) lab (about 30%)

b. Acquire test data on 12 guinea pigs per quarter. This has been partially completed during this year of the award.

c. Perform anatomical assessment of 12 guinea pigs per quarter. This aspect of the project was started during this year of the award
.

d. Acquire physiological and behavioral data on 10 human subjects per quarter. This has not been completed during this year of the award.

What was accomplished under these goals?

a. While this goal has been accomplished in the Walsh (animal) lab, it has yet to be completed in the Leek (human) lab. Accomplishment of this goal over the past year in the human lab has largely been stymied by the lack of appropriate research space due to relocation of the lab, which is still not completed (see description below). Over the past year, we have scrounged for space to set up temporarily, at least, partial experimental equipment to prepare for a limited amount of data collection in humans, only to find such temporary space access revoked due to other patient care needs of the VA hospital.

   Regular on-line meetings of the entire research team have been held during this year to address issues involved in creating the most similar testing procedures between the two sides of the project. These discussions have been informed largely by the successful implementation of data collection in guinea pigs, as well as by new findings in the literature. Part of the improvements we are continually making in the experimental protocols, including stimulus parameters, is to identify efficiencies we can implement that will accelerate the rate of data collection in the two labs without penalty to the ultimate goals of the work.

b. Complete electrophysiology/acoustic data sets have been acquired from 16 animals and another 6 are currently under investigation. Findings based on data obtained from 9 noise-exposed animals compared to control data support our *a priori* view that a subset of auditory response elements reliably differentiate noise-exposed from control animals (see Appendix 1). Although it would be premature to draw definitive, long-term conclusions from this congregate data set, findings are nonetheless encouraging with regard to the successful development of the proposed multivariate statistical model with significant predictive/diagnostic power.

c. Progress was made on this aspect of the project during the past funding year as well. Thirty-two inner ear specimens (both right and left cochleae) have been prepared (perfused with fixative) for histological assessment of synapse pathology, and the assessment is complete in the case of three cochleae acquired from two noise-exposed and one control animal. Those data are consistent, tentatively speaking, with the expectation that ribbon synapses are lost at predictable levels in noise-exposed animals.

   Notwithstanding progress on the immunohistological work critical to this study, it has been slowed somewhat by unanticipated personnel reassignments. The scientist originally slotted to assume responsibility for these anatomical studies decided to withdraw from the project due to his other funded research commitments. He will remain less directly involved in the project as an unpaid consultant, providing guidance and discussion on technical aspects related to this aspect of the project. A dedicated research technician was assigned to this aspect of the project, working under the supervision of Dr. Walsh. She is undergoing training and will give top priority to the histology aims during the upcoming funding period. A second research technician was recruited to take up the data acquisition slack resulting from the reassignment of personnel necessary to complete immunohistochemistry work in the Walsh lab.

d. The human laboratory continues to be delayed due to relocation of the lab and the renovations necessary to make the proposed permanent new space appropriate for human testing. Some small amounts of progress were made, though, in a few temporary spaces available to our use. Unfortunately, none of this space could be used with most of our testing equipment and materials remaining in storage. One such space was a booth in the Audiology Clinic at the VA Loma Linda, loaned to us temporarily through the generosity of the Chief of Audiology. During the two months or so that we could occupy that space, we were able to perform some rudimentary calibrations and setup tasks to make that space appropriate for some aspects of our research. Toward the end of May we were able to recruit three human subjects and perform audiometric screening procedures with them. Unfortunately, soon thereafter, this space was incorporated back into audiology patient

Please see Appendices 1 and 2 for further details of progress in the animal and human labs.

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

Although this project was not intended to provide formal training and professional development opportunities, as a matter of course, the more senior members in each lab (Walsh and Leek labs) do encourage professional and scientific growth among the less senior team members. For example, Dr. Venezia, who is an accomplished statistician and modeler, has led our team in understanding the type of modeling we plan to successfully complete this study. He also has led the team in understanding his career development grant, which includes both behavioral testing of human subjects, as well as functional MRI techniques. The Research Audiologist, Dr. Whittle, has assisted us all in understanding and interpreting audiological behavioral and electrophysiology testing, both of which are cornerstones of this project. Members of the Leek lab have learned more about working with animal subjects, carrying out the regulatory procedures for animal work, and learning how animal electrophysiology will be performed to mimic (as much as possible) the human testing. It is our practice for the entire Auditory Research Group at the VA hospital (some 13 scientists and technicians) to meet every two weeks (currently virtually on Zoom) to hear about the ongoing research and data from each lab or to review a current article that is of interest to the labs. Because our group has a wide range of interests and expertise, this is an enjoyable and valuable learning experience, and encourages the social bonding of the group.

Specific career training and professional development has been implemented during the year in the Walsh lab, motivated primarily by personnel changes leading to reassignment of tasks. This training has expanded the skills and capabilities of Research Technician, Dr. Xiaouhi Lin, and introduced a new Research Technician, Ms. Ashley Vasquez, to this area of auditory research. Although Dr.Lin had previous experience in the auditory neurosciences, Drs. McGee and Walsh extended her training in both the electrophysiology and otoacoustic emission realms during the past funding period. The training was designed to enhance Dr. Lin's data acquisition and analyses capabilities with the goal of preparing her to efficiently operate the data acquisition system and to perform a subset of basic analyses, all the while remaining under the direct supervision of Drs. McGee and Walsh. Dr. Lin successfully acquired these skills and is now independently operating the data acquisition system. She is also learning to prepare inner ear specimens for histological analyses and will assume primary responsibility for morphological aspects of the project during the upcoming funding period. Ms. Ashley Vazquez, a second Research Technician in the Walsh lab, had no prior experience in the area, but has responded enthusiastically to training at the hands of Drs. Lin, Walsh, and McGee. As her experience and independence grows, the goal is for her to begin to take primary responsibility for the electrophysiological data acquisition effort, releasing Dr. Lin to focus on morphological analysis.

How were the results disseminated to communities of interest?

An abstract of a paper to be presented at the upcoming meeting of the Association for Research in Otolaryngology (see Appendix 3). Findings from this project associated with activities in the Walsh lab will be presented in a presentation entitled "*Long-Term Electrophysiological Correlates of Noise-Induced Temporary Threshold Elevations in Guinea Pigs."*

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

In the Walsh Lab (animal): Electrophysiology and acoustic testing in the Walsh lab is proceeding actively and findings thus far are highly promising with regard to the identification of auditory response features differentiating noise-exposed from control animals (see Appendix 1). During the next reporting period, the plan is to carry on with the essential aspects of the data acquisition protocol currently in use, and efforts to increase the throughput of the lab are underway. In addition, time and effort on the morphological aspect of the project will be notably ramped up as histology responsibilities are being transferred to Dr. Lin, who will devote the time and effort necessary to advance the morphology goals.

In the Leek lab (human): We hope and anticipate that we will soon be able to set up our experiments in permanent lab space with two functional sound booths. This will allow us to calibrate and verify equipment, and quickly begin actively testing subjects. Once we are underway, we will devote maximum time possible to collecting and organizing data. We may have to entertain a modification of our data collection plans to try to accelerate the testing. We will be guided in those decisions by the exciting analyses and preliminary findings emerging from the animal work on this project described in Appendix 1.

4.Impact

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

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5.Changes/Problems

Changes in approach and reasons for change

Nothing to Report.

Please continue to next page

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

In our previous progress reports we have described some significant barriers to implementing and progressing on this project as rapidly as we had envisioned. These delays have included major disruptions to both the animal and human work due to shutdowns and limited access to our labs and to human research subjects as a response to the COVID-19 pandemic, and extended delays in the availability of appropriate and functional lab space and equipment.

In the animal lab: Unavoidable delays associated with COVID-19 and other assorted impediments pushed back the actual start date of the project to February of 2021. Currently, the most significant problems facing the Walsh lab are twofold. The first is related to the capacity to acquire electrophysiology data and the second is related to the unanticipated need to reassign morphology data acquisition responsibilities.

At the start of the project, our expectation was that we would have access to a second sound-attenuating booth in addition to the new sound booth provided so that we could study two animals on any given day throughout the course of the project. A second sound booth is not yet available to this work for data acquisition, limiting lab output. In addition, efforts to match non-human animal and human testing strategies, as well as the desire to acquire as much data as reasonably possible from individual animals, have prevented us from acquiring data from two animals sequentially in a single booth. To resolve this problem, we are continuing our attempts to bring a second data acquisition system online during the upcoming reporting period and have added a second research technician to help with the accelerated data acquisition effort. Additional steps to improve lab throughput include the reduction of protocol complexity by refining/tweaking the number of stimulus paradigms tested based on a comprehensive assessment of findings/outcomes from the previous reporting period.

Diminished progress associated with the morphology goals of the project is a result of personnel changes as Dr. Li has withdrawn from active contribution to the project. The problem is being resolved by hiring a second research technician and transferring histology responsibilities to Dr. Lin. Ms. Vazquez will be taking over much of Dr. Lin's electrophysiology data collection activities going forward.

In the human lab: The building that housed our human subjects auditory research lab was scheduled for demolition in the spring/summer of 2020, not long after we had finally received all regulatory approvals to move forward with the study. We were required to pack all our equipment, files, books, documents, supplies for storage in various parts of the hospital. A permanent new laboratory space was identified within the main hospital, and contracts were awarded to move our two sound-attenuating booths into the new space and for the rooms to be renovated to be appropriate to a research lab. It was anticipated that these renovations would take 2-3 months, during which time the lab members occupied shared, temporary office space (but no lab space) in various rooms around the hospital. Multiple deadlines were not met over the following 14 months, during which time our lab belongings including equipment were moved over ten times within the hospital. Finally, in mid-August 2021, we were allowed to move into the renovated space, but soon found that it was not completed and could not be used for data collection. We are unable to fully occupy this space and begin the work of data collection. Workmen come and go and the people in charge are made aware (nearly daily) of the concerns for this space but so far the best guess from facilities staff is that it will not be completed until at least the end of November 2021. Although we have made some attempts over this year-plus of exile from a functional lab space to carry out some limited testing of human subjects, access to private unshared rooms to accomplish this is extremely limited. So, for now, we continue to search for adequate space to at least do the paper and pencil surveys and consenting, but we are mostly reduced to making sure we remain on the radar to get our new lab space fixed so that we can collect data for this project.

Changes that had a significant impact on expenditures

Nothing to Report.

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

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6.Products

Publications, conference papers, and presentations

Journal publications

Sargsyan L, Hetrick A, Leek MR, Martin GK, Li H (2021) "Effects of combined gentamicin and furosemide treatment on cochlear ribbon synapses", NeuroToxicology 84:73-83. doi: 10.1016/j.neuro.2021.02.007. Epub 2021 Mar 2.
[Please note: This article is not directly a result of this project, but it has important information about synaptopathy after ototoxicity, and includes as authors two of the key personnel on the present project. Interestingly the results suggest that synaptopathy after ototoxic treatments may be synaptic plasticity rather than a loss of synaptic ribbons. Both Dr. Li's and Dr. Leek's DoD grants are acknowledged for funding.] This publication was cited in the last Annual Progress Report as "under review". The published article is included here as Appendix 4.

Books or other non-periodical, one-time publications

Nothing to Report.

Other publications, conference paper, and presentations

During the pandemic lockdown occurring near the beginning of the first period of funding of this grant, the following invited chapter was completed. The chapter contents are highly relevant to this DoD project and the chapter citation is included here, although the grant was not identified as a funding source.

McGee, J. and Walsh, E.J. (2021). Cochlear transduction and the molecular basis of peripheral auditory pathology. In: P.W. Flint, H.W. Francis, B.H. Haughey, M.M. Lesperance, V.J. Lund, K.T. Robbins, and J.R. Thomas (Eds.), **Cummings Otolaryngology: Head and Neck Surgery**, 7th ed., Part VII, Otology, Neurotology, and Skull Base Surgery, Volume III, Section 5: Inner Ear, Chapter 148, Elsevier Inc., Philadelphia, pp. 2221-2268.

Conference paper:

McGee, J., Lin, X., Vazquez, A., Li, H., Venezia, J., Whittle, N. Leek, M., and Walsh, E.J. (2022). *Long-Term Electrophysiological Correlates of Noise-Induced Temporary Threshold Elevations in Guinea Pigs*, to be presented at the 45th Annual Mid-Winter Meeting of the Association for Research in Otolaryngology. (see Appendix 3)

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.

Please continue to next page

7.Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name	*Marjorie R. Leek, Ph.D.*
Project Role	*Principal Investigator*
Researcher Identifier	*None*
Nearest person month worked	*2*
Contributions To Project	*Developed experimental programming for testing protocols; reviewed literature for studies relevant to the present project; completed regulatory submissions; attended and contributed to team meetings and discussions*
Funding Support	*This project*

Name	*Edward J. Walsh, Ph.D.*
Project Role	*Co-Principal Investigator*
Researcher Identifier	*None*
Nearest person month worked	*5*
Contributions To Project	*Oversaw lab activities; provided education, ongoing training, and supervision to lab technicians; conducted relevant data analyses; searched and reviewed literature relevant to studies proposed in this grant; recruited and identified new staff members; participated in administrative meetings addressing a range of relevant program items.*
Funding Support	*This project*

Name	*JoAnn McGee, Ph.D.*
Project Role	*Co-Investigator*
Researcher Identifier	*None*
Nearest person month worked	*9*
Contributions To Project	*Participated in team meetings that address a range of essential program items including the refinement of experimental protocols to be used in both non-human animal and human studies, managed data acquisition activities, and refined and expanded data analysis protocols necessary to complete the investigation; participated in training lab technicians.*
Funding Support	*This project*

Name	*Jonathan H. Venezia, Ph.D.*
Project Role	*Co-investigator*
Researcher Identifier	*None*
Nearest person month worked	*1*
Contributions To Project	*Wrote experimental programming for testing protocols; generated stimuli and experimental programming for electrophysiological tests in human subjects; attended and contributed to team meetings and discussions*
Funding Support	*Institutional funds*

Name	*Hongzhe Li, Ph.D.*
Project Role	*Co-Investigator*
Researcher Identifier	*None*
Nearest person month worked	*1*
Contributions to Project	*Performed animal terminal procedures including cardiac perfusion, followed by subsequent cochlear tissue processing; microdissection of cochlear sample for confocal identification of ribbon synapses; confocal image processing and synapse quantification.*
Funding Support	*This project*

Name	*Xiaohui Lin, Ph.D.*
Project Role	*Research Technician*
Researcher Identifier	*None*
Nearest person month worked	*12*
Contributions To Project	*Assisted in data collection on guinea pig subjects; became familiar with animal literature relevant to this grant, took care of purchase orders for items needed in the laboratory, maintained forms for anesthesia and monitoring; trained to perform animal terminal procedures, including cardiac perfusion, tissue processing, microdissection and synapse quantification; participated in group meetings associated with this grant.*
Funding Support	*This project*

Name	*Nicole Whittle, AuD*
Project Role	*Research Audiologist*
Researcher Identifier	*None*
Nearest person month worked	*12*
Contributions to Project	*Coordinated with staff of animal testing lab to assure that parameters to be used in electrophysiological testing of human subjects were consistent with parameters used in guinea pigs; assisted with regulatory submissions; began recruiting potential human subjects for the study, assisted staff in piloting electrophysiological data collection; kept records and organized documents; attended and contributed to team meetings.*
Funding Support	*This project*

Name	*Ashley Vasquez*
Project Role	*Research Technician*
Researcher Identifier	*None*
Nearest person month worked	*3*
Contributions To Project	*Assisted in data collection on guinea pig subjects; familiarized herself with relevant data acquisition software. Participated in group meetings associated with this grant.*
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?

Nothing to Report.

8.Special Reporting Requirements

Collaborative Awards

Quad Charts

Quad chart is attached

9. Appendices

Appendix 1 Report of Guinea Pig Testing

Appendix 2 Report of Human Subjects Laboratory (repeated from Quarterly Report dated July 30, 2021)

Appendix 3 Abstract of conference paper: McGee, J., Lin, X., Vazquez, A., Li, H., Venezia, J., Whittle, N. Leek, M., and Walsh, E.J. (2022). *Long-Term Electrophysiological Correlates of Noise-Induced Temporary Threshold Elevations in Guinea Pigs*, to be presented at the 45th Annual Mid-Winter Meeting of the Association for Research in Otolaryngology.

Appendix 4 Sargsyan L, Hetrick A, Leek MR, Martin GK, Li H (2021) “Effects of combined gentamicin and furosemide treatment on cochlear ribbon synapses”, NeuroToxicology 84:73-83. doi: 10.1016/j.neuro.2021.02.007. Epub 2021 Mar 2. [previously submitted as manuscript under review]