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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. Completion of this goal in the Leek (human) lab has been achieved during this reporting period.

b. Acquire test data on 12 guinea pigs per quarter. This has been partially completed (83%) during the current reporting period.

c. Perform anatomical assessment of 12 guinea pigs per quarter. This aspect of the project was partially completed during the current reporting period.
.

d. Acquire physiological and behavioral data on 10 human subjects per quarter. This has been partially completed during this year of the award.

What was accomplished under these goals?

a. This goal has been fully accomplished in both the Walsh (animal) lab and the Leek (human) lab. All testing protocols are in place and equipment set-up and calibration have been completed.

   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 and from human subjects, and by new findings in the literature. We are continuing to seek efficiencies to accelerate the rate of data collection in the two labs without penalty to the ultimate goals of the work.

b. Electrophysiology/acoustic data sets have been acquired from 56 animals and another 4 are currently under investigation. Findings based on data obtained from 31 noise-exposed animals and 25 controls support our *a priori* view that a subset of auditory response elements reliably differentiate noise-exposed from control animals (see Appendix 1). Findings from early efforts to lay out and consider a subset of essential elements of the proposed multivariate statistical model with significant predictive/diagnostic power during the current reporting period are highly encouraging.

c. Progress was made on this aspect of the project during the past funding year as well. One hundred and twelve 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 thirty-one cochleae acquired from seventeen noise-exposed and fourteen sham animals. Those data are consistent with the expectation that ribbon synapses are lost at predictable levels in noise-exposed animals.

d. Data collection continues in the human lab, although we have not been able to recruit as many subjects as we had planned. Subjects are asked to sit for eight 2-hour experimental sessions, which is a significant time commitment. So far, we have not completed all measurements for any subjects, but several are within one or two sessions of completing the testing. We currently have 11 participants enrolled and moving through the testing. During this reporting period, we have instituted new recruitment processes, including posting flyers in the audiology clinic, describing our work to the audiology staff and asking for referrals, and formally requesting that our recruitment advertisement be placed in the rotation of the informational closed-captioning televisions posted around the VA hospital.

Please see Appendices for further details of progress in the animal and human labs and preliminary work on model development.

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 during much of this year, Dr. Whittle, has assisted us all in understanding and interpreting audiological behavioral and electrophysiology testing, both of which are cornerstones of this project. Dr. Whittle decided to transfer to another VA during this year. Her replacement, Kelli Sugai has worked closely with other team members to learn how to work with human subjects on data collection, including enrollment procedures such as acquiring informed consent from subjects. She also has received training on how to carry out the various auditory tests required for this study. Two postdoctoral fellows in the lab, while not formally part of this study, are included in discussions of this work, and are being trained to recognize signs of hearing loss and other characteristics of audiological evaluation. 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 working with humans or with small animals) 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, as well as keeping us all up to speed on new developments in auditory research.

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 enhanced the skills and capabilities of Research Technician, Dr. Xiaohui Lin, and introduced a relatively new Research Technician, Ms. Ashley Vazquez, 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 year. The training was designed to enhance Dr. Lin's data acquisition and analyses capabilities with the goal of preparing her to efficiently and independently 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. She has also learned how to prepare and process inner ear specimens for immunohistochemical analyses and performing quantitative analyses for morphological aspects of the study. Ms. 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. She has also become proficient in conducting physiological data acquisition and basic analyses, as well as preparing and processing samples for immunohistochemical analyses and has taken primary responsibility for performing quantitative analyses of cochlear tissues.

How were the results disseminated to communities of interest?

A poster was presented virtually at the Association for Research in Otolaryngology meeting held in February 2022:

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*, presented at the 45th Annual Mid-Winter Meeting of the Association for Research in Otolaryngology, poster #471.

In addition, a poster was presented at the Spring (182nd) Meeting of the Acoustical Society of America held in Denver in May 2022:

McGee, J., Lin, X., Vazquez, A., Li, H., Leek, M.R., Venezia, J., Whittle, N., and Walsh, E.J. (2022). *A guinea pig model of hidden hearing loss: Prelude to the development of a human model*. J. Acoust. Soc. Am. 151(No. 4, Pt.2): A127, abstract # 2pPPb17.

Finally, a presentation regarding results of this project will be made at the Fall (183rd) Meeting of the Acoustical Society of America to be held in Nashville, TN in December 2022:

McGee, J., Lin, X., Vazquez, A., Li, H., Venezia, J., Leek, M.R., and Walsh, E.J. (2022). *Envelope following responses following partial cochlear deafferentation in guinea pigs*. J. Acoust. Soc. Am.

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

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. In addition, time and effort on model development aspects of the project will be continued.

Two major goals will be the focus of the next year of this grant (under a no-cost extension). First is to accelerate the recruitment and testing of human subjects. The second is to further develop the predictive model based on guinea pig data and extend it to test its value with human data. This, of course, is the major purpose of this work.

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.

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: Work on acquiring electrophysiological and acoustical data on guinea pigs and anatomically assessing hair cell survival and ribbon synapses in the cochlea will continue in the upcoming period.

In the human lab: We are delighted to be able to report that the renovated lab space became fully functional during this past year (January 2022), and we were able to begin recruiting research subjects actively and fully initiate data collection.

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

Nothing to report

Books or other non-periodical, one-time publications

Nothing to Report.

Other publications, conference paper, and presentations

Conference papers:

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*, presented at the 45th Annual Mid-Winter Meeting of the Association for Research in Otolaryngology, poster #471.

McGee, J., Lin, X., Vazquez, A., Li, H., Leek, M.R., Venezia, J., Whittle, N., and Walsh, E.J. (2022). *A guinea pig model of hidden hearing loss: Prelude to the development of a human model*. J. Acoust. Soc. Am. 151(No. 4, Pt.2): A127, abstract # 2pPPb17.

McGee,J., Lin, X., Vazquez, A, Li, H, Venezia, J., Leek, M, and Walsh, E.J. (2022).Envelope following responses following partial cochlear deafferentation in guinea pigs, abstract submitted during this year; to be presented at the 183rd Meeting of the Acoustical Society of America.

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	*Prepared experimental programming for testing protocols; generated stimuli for physiological and behavioral testing of humans, completed regulatory submissions; supervised research audiologist/research assistant, 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	*4.8*
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; participated in administrative meetings addressing a range of relevant program items. Participated in dissemination of project results.*
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, generated stimuli and protocols for data acquisition activities, and wrote and refined programs for data and statistical analyses; participated in training lab technicians; participated in dissemination activities for this project.*
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; developed initial modeling efforts based on guinea pig and human data; 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	*Took care of animal management and purchase orders for items needed in the laboratory; took responsibility for physiological data acquisition and a portion of anatomical and physiological analyses of data acquired in guinea pigs. 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	*6*
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	*Kelli Sugai*
Project Role	*Research Assistant*
Researcher Identifier	*None*
Nearest person month worked	*6*
Contributions to Project	*Continued recruiting potential human subjects for the study; enrolled subjects and collected behavioral and electrophysiological data record keeping/organizational documents; assisted with regulatory submissions; attended and contributed to team meetings.*
Funding Support	*This project*

Name	*Ashley Vazquez, B.S.*
Project Role	*Research Technician*
Researcher Identifier	*None*
Nearest person month worked	*12*
Contributions To Project	*Participated in animal management and took responsibility for anatomical preparation and imaging of cochlear specimens for quantitative analyses of hair cell numbers and ribbon synapses. Assisted in data collection and a portion of anatomical and physiological analyses of data acquired in guinea pigs. 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 Testing
Appendix 3	Report of Preliminary Model Development
Appendix 4	Abstracts submitted and Posters presented at national conferences