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TITLE: Rapid Triage of Auditory Peripheral and Central Phenotypes Using a Brief but Rich Diagnostic Battery

PRINCIPAL INVESTIGATOR: Edward Bartlett

CONTRACTING ORGANIZATION: Purdue University

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Rapid Triage of Auditory Peripheral and Central Phenotypes Using a Brief but Rich Diagnostic Battery

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Hearing tests are an important part of assessing a person's health, whether they are service members being treated after noise damage, veterans having difficulties with conversations, or civilians. Hearing problems are associated with communicative, social, and cognitive difficulties. Despite the utility of current hearing tests, they have three connected problems. First, they are too simple and only provide information about significant losses in hearing sensitivity. Second, the testing focus on hearing sensitivity largely ignores any damage or compensation in brain regions that are responsible for making sense of sounds. Third, although current protocols could enable more complex evaluations, those evaluations would take too long to be useful. **Our proposal aims to create a rich assessment of hearing, and the brain regions involved in hearing, in as short a time as possible.** There can be multiple underlying causes of hearing difficulties in clinical populations, depending on an individual's genetics and history of noise exposure. Animal models, in contrast, offer a way to clearly dissociate and individually control these causes. In this study, we propose to design and refine our rapid hearing assessment battery in animal models where underlying causes can be explicitly confirmed. Damage to the early neural regions of the hearing system will be induced in the animal model by overexposure to repetitive noise resembling gunfire. Damage to the brain will be induced by injections of a chemical called D-galactose which causes the production of molecules damaging to cells (oxidative stress). We will then obtain neural responses to a test battery designed to emphasize the early and later neural pathways of the brain. We then propose to apply this test battery in a population of patients with hearing difficulties which cannot be currently diagnosed by any known tests. By using this translational approach, we aim to arrive at the optimal test battery which can provide the most information within the least amount of time. Our measurements of neural activities will be supplemented by measuring the extent to which the hearing organ, the cochlea, is damaged, and whether its connections to the rest of the brain are damaged, which is called cochlear synaptopathy or “hidden hearing loss”. We expect the successful completion of the experiments in this proposal to result in a rapid test battery that can classify, with confidence, the contributions of the different brain regions to a person’s hearing difficulties in the absence of any abnormal results using current clinical tests. By concentrating on tests that are modified from existing, mobile, clinical tests (such as the newborn hearing screening test), we can enable rapid translation to the field with minimal concerns about safety and regulatory clearances. We expect this test battery to help monitor hearing damage in active service personnel, inform deployment decisions, and create a new way to objectively diagnose hearing difficulties in veterans and the general American public.

15. SUBJECT TERMS
Hearing loss, aging, audiogram, envelope following response, cochlea, synaptopathy, neuroinflammation, inflammaging

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRDC
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1. INTRODUCTION:

Hearing tests are an important part of assessing a person's health, whether they are service members being treated after noise damage, veterans having difficulties with conversations, or civilians. Hearing problems are associated with communicative, social, and cognitive difficulties. Despite the utility of current hearing tests, they have three connected problems. First, they are too simple and only provide information about significant losses in hearing sensitivity. Second, the testing focus on hearing sensitivity largely ignores any damage or compensation in brain regions that are responsible for making

2. KEYWORDS:

Hearing loss, aging, audiogram, envelope following response, cochlea, synaptopathy, neuroinflammation, inflammaging

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Study preparation and pilot data
Milestones Achieved: IACUC approval at both sites, refinement of experimental protocols and analyses. Months 1-5. 95% Completed
Major Task 2: Running experiments for SA1
Milestones achieved: Collection of main physiological rodent data. Months 6-18. 40% completed.
Major Task 3: Analysis of physiology data and generation of predictive longitudinal response profiles.
Milestones achieved: Data-driven model to discriminate groups. Months 7-19. 15% completed
Major Task 4: Cochlear histology
Milestones achieved: Data-driven anatomical model to discriminate groups. Months 6-20. 20% completed.
Major Task 1: Study preparation and pilot data for SA2
Milestones Achieved: IRB approval, refinement of recruitment and experimental protocols and analyses. Months 1-5. 90% completed

What was accomplished under these goals?

See response on following pages.

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

One graduate student, Meredith Ziliak, was trained during the first reporting period. 4 undergraduates students, Audrey Harrison, Amanda Kenney, Shiv Shukla, and Paula Rivera Carrasquillo, were trained during the last reporting period. New undergraduates Emily Bell, Andres Navarro, Sahil Desai, and Valentina Micosilin were trained in the recent reporting period.

How were the results disseminated to communities of interest?

An abstract was submitted to the Association for Research in Otolaryngology and accepted for presentation at the 2024 Midwinter Meeting. ***"The Impact of Small Arms Fire-Like Noise on Temporal Processing of Simple and Complex Stimuli"*** Meredith C. Ziliak, Edward L. Bartlett

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.

For the next reporting period, we plan to:

1. Continue to collect data for the galactose injection portion of the project.
2. Refine analyses of neural data
3. Complete data collection for the gunshot noise exposure
4. Complete data analysis for the evoked potentials for the gunshot noise
5. Immunostaining of galactose, noise exposed and sham-injected cochleae
6. Immunostaining of galactose, noise-exposed, and sham-injected IC
7. Continue to collect human recordings and behavioral data using similar stimuli as in rodents
8. Correlate anatomy and physiology data into a predictive model for cochlear/IC damage

DOD Annual Report 2023 Accomplishments

What was accomplished under these goals?

Major activities: In this period, we focused on obtaining and analyzing electrophysiological for both galactose injected and noise-exposed rats, as well as continuing electrophysiological measurements in humans. We continued analyses and coordination of analysis across human and rodent data sets. We performed cochlear extractions to prepare fo cochlear histology.

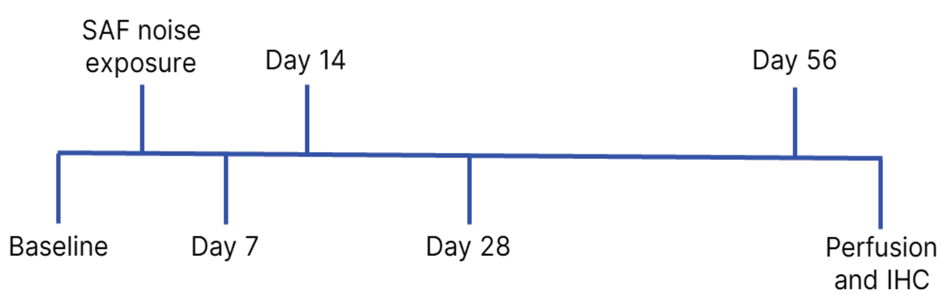
Specific objectives: Our specific objectives in this first reporting period were:

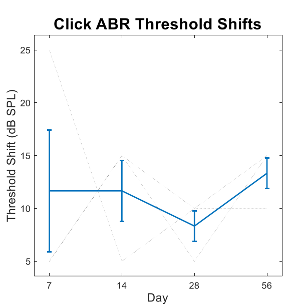
1) Pilot electrophysiological recordings in rodents and humans to test our sound battery.
2) Pilot first batch of animals tested with noise exposure
3) Learn and perform cochlear extraction surgeries
4) Fix brains for IC histology in 2024

Results:

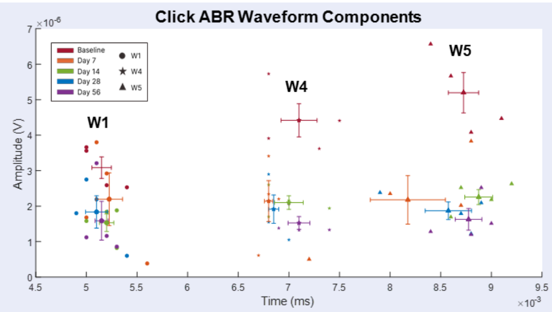
1. We did small-arms fire noise (SAF) exposure on 3 batches of rats.
2. We performed initial analyses of ABR threshold shifts and click MLR responses for SAF exposure.
3. We analyzed rapid ABR for detection of peaks and thresholds.

The figure shows the timeline of SAF exposure and recordings.

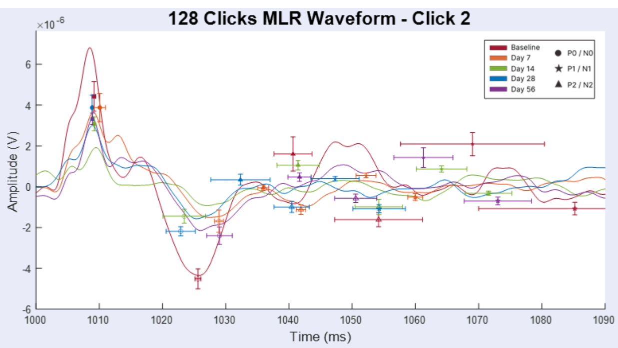


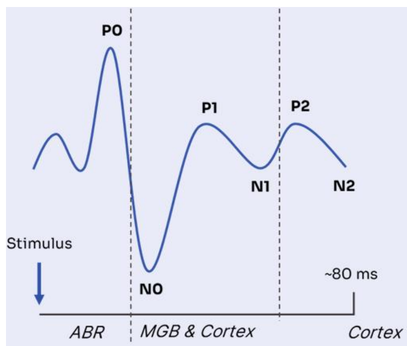


SAF induces threshold shift of 10-12 dB. This would be considered subclinical hearing loss in audiological measurements (20-25 dB considered clinically relevant).



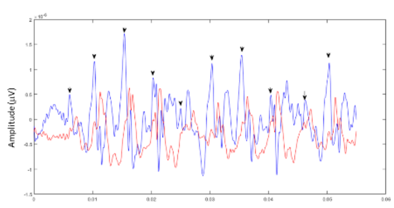
Here we show that SAF exposure reduces ABR waves, especially waves 4 and 5, over the 56 day window measured. The right shows the schematic of generators of



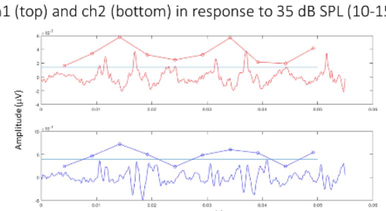


The middle latency auditory evoked response, which measures both subcortical and thalamcortical transmission, was significantly reduced by the SAF exposure for the entire 56 day window. There did not appear to be much cortical compensation within the window.

Wave 1 (auditory nerve) is easily detectable at 80 dB in ch2 (blue), and wave 3 (cochlear nuclei) is in ch1 (red)



Ch1 (top) and ch2 (bottom) in response to 35 dB SPL (10-15 SL)



From our SAF baseline recordings, we analyzed the ABR peaks evoked by rapid ABR testing, where we tested an audiogram spanning 5 octaves at ½ octave spacing at 1 minute per sound level, over an 80% reduction (improvement) in testing time. We show that the evoked ABRs are detectible at low sound levels even using simple measures. We plan to test combined amplitude/slope and amplitude/template matching measures to test detection closer to threshold.

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?

We have submitted an invention disclosure:
Rapid assessment of Temporal Processing from the Peripheral and Central Auditory Pathway using Dynamic Amplitude Modulated Stimuli" (Pitt ID 06185) from Dr. Aravindakshan Parthasarathy, Dr. Edward L. Bartlett, and Dr. Satyabrata Parida, co-inventors.

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Nothing to report

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

Changes that had a significant impact on expenditures

The main graduate student currently involved in the project, Meredith Ziliak, was awarded a fellowship that extends through August 2024. Therefore, we have not yet spent those budgeted funds. The delay in processing of cochlea for histology has delayed using those expenditures for antibodies and reagents. We plan to use those funds shortly.

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.

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.

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

Other publications, conference papers and presentations.

An abstract was submitted to the Association for Research in Otolaryngology and accepted for presentation at the 2024 Midwinter Meeting. *"The Impact of Small Arms Fire-Like Noise on Temporal Processing of Simple and Complex Stimuli"* Meredith C. Ziliak, Edward L. Bartlett

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

- **Technologies or techniques**

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

We have submitted an invention disclosure:
Rapid assessment of Temporal Processing from the Peripheral and Central Auditory Pathway using Dynamic Amplitude Modulated Stimuli" (Pitt ID 06185) from Dr. Aravindakshan Parthasarathy, Dr. Edward L. Bartlett, and Dr. Satyabrata Parida, co-inventors.

- **Other Products**

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

See following page.

DOD Annual Report 2023 Personnel

Name: *Edward Bartlett*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID): 0000-0002-9387-1854
Nearest person month worked: *3*
Contribution to Project: *Dr. Bartlett has piloted galactose injections, auditory evoked potential recordings and stimuli, and analysis. He has begun to set up the noise exposure and calibrate the exposure chamber.*
Funding Support: This grant, DOD W81XWH-21-1-0829 (Michael Heinz, PI), Showalter University Faculty Scholar (Purdue)

Name: *Aravindakshan Parthasarathy*
Project Role: *co-PI*
Researcher Identifier (e.g. ORCID ID): ORCID 0000-0002-4573-8004
Nearest person month worked: *3*
Contribution to Project: *Dr. Parthasarathy has written the has submitted the HRPO and obtained approval. He has begun to recruit human subjects, screen them, and collect pilot data.*
Funding Support: This grant, R21DC018882

Name: *Meredith C. Ziliak*
Project Role: *PhD student*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *3*
Contribution to Project: *Meredith has carried out most of the rat recordings at Purdue.*
Funding Source: Andrews Fellowship (Purdue)

Name: *Satyabrata Parida*
Project Role: *Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID): 0000-0002-2896-2522
Nearest person month worked: *0.5*
Contribution to Project: *Satya has contributed to the analyses of the AM sweeps.*
Funding Source: R01DC013315

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: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: *N/A*