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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, West Lafayette, IN

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# REPORT DOCUMENTATION PAGE

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<b>6. AUTHOR(S)</b>  Edward Bartlett  Aravindakshan Parthasarathy  E-Mail: <a href="mailto:ebartle@purdue.edu">ebartle@purdue.edu</a>		<b>5d. PROJECT NUMBER</b>  RH200075
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<b>14. ABSTRACT</b>  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. <b>Our proposal aims to create a rich assessment of hearing, and the brain regions involved in hearing, in as short a time as possible.</b> 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

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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. 90% Completed*

### **Major Task 2: Running experiments for SA1**

*Milestones achieved: Collection of main physiological rodent data. Months 6-18. 25% 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. 10% completed*

### **Major Task 4: Cochlear histology**

*Milestones achieved: Data-driven anatomical model to discriminate groups. Months 6-20. 10% 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 this reporting period.

**How were the results disseminated to communities of interest?**

An abstract was submitted to the Association for Research in Otolaryngology.

**Rapid assessment of Temporal Processing from the Peripheral and Central Auditory Pathway using Dynamic Amplitude Modulated Stimuli**

Satyabrata Parida, Kimberly Yurasits, Victoria E. Cancel, Maggie E. Zink, Claire Mitchell, Meredith C. Ziliak, Audrey V. Harrison, Edward L. Bartlett, Aravindakshan Parthasarathy

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

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. Pilot and begin to collect data for the gunshot noise exposure
4. Begin cochlear histology and immunostaining of galactose and sham-injected cochleae
5. Collect human recordings and behavioral data using similar stimuli as in rodents

## DOD Annual Report 2022 Accomplishments

### What was accomplished under these goals?

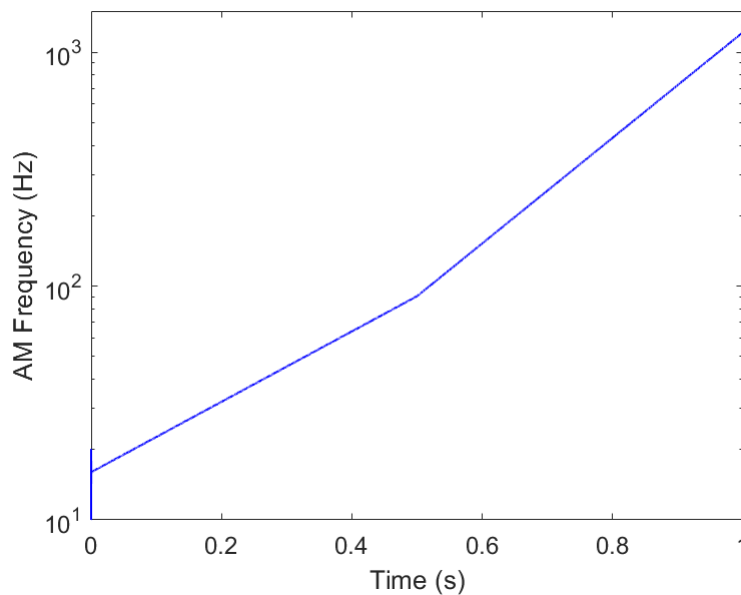
Major activities: In this first period, we focused on obtaining ACURO and HRPO approvals, and we began to collect human and animal electrophysiology data. We began analyses and coordination of analysis across human and rodent data sets. We began preparations for cochlear histology.

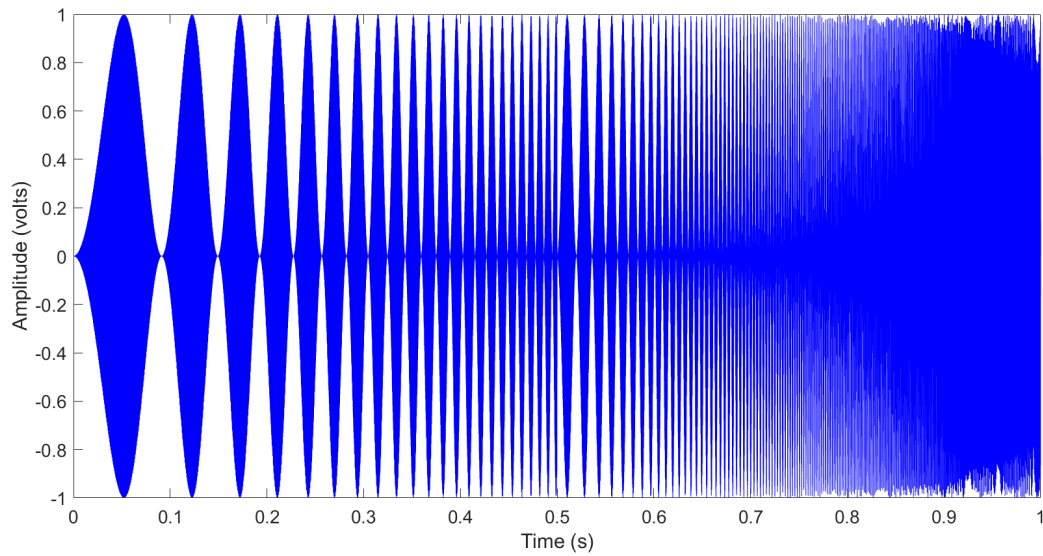
Specific objectives: Our specific objectives in this first reporting period were:

- 1) ACURO approval
- 2) HRPO approval
- 3) Pilot electrophysiological recordings in rodents and humans to test our sound battery.
- 4) Pilot first batch of animals tested with galactose injections and compare to sham (saline) injected animals.
- 5) Analysis of data from pilot recordings
- 6) Cochlear histology from pilot recordings

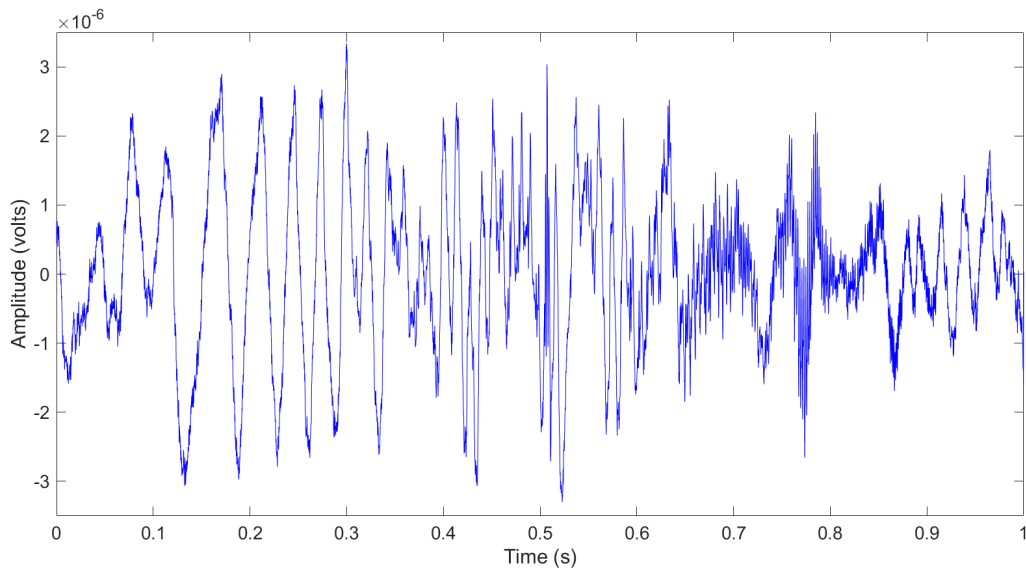
### Results:

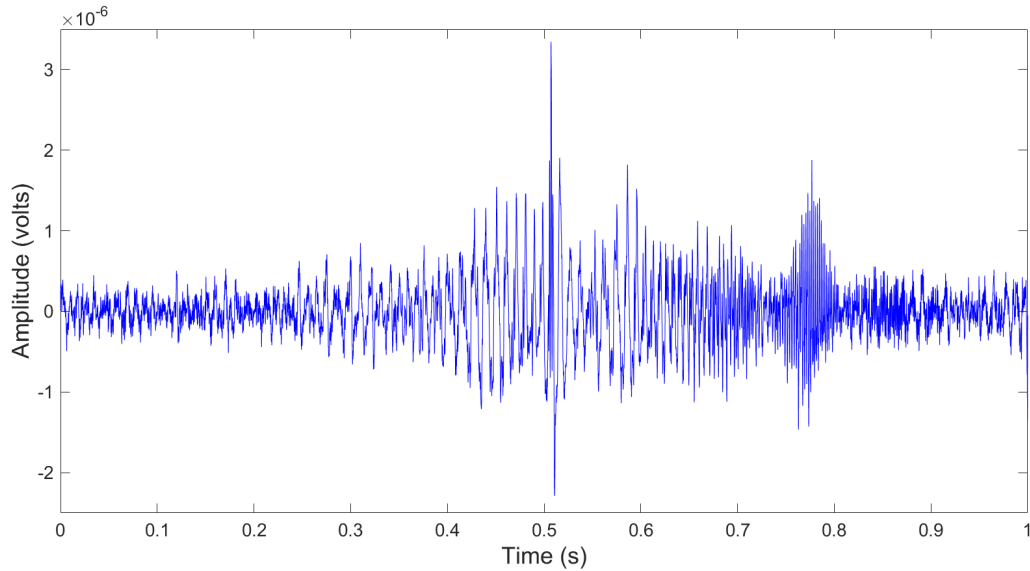
1. We obtained ACURO and HRPO approval.
2. We piloted our AM sweep stimuli and our rapid ABR stimuli, and we are in the process of analyzing those data for sham and galactose injected animals over the relevant time points. We have collected data from 10 animals so far as part of this protocol and are in the process of analyzing the data. Shown below are the AM frequency trajectory (top) and time waveform of the AM sweep stimulus (bottom) that was used.



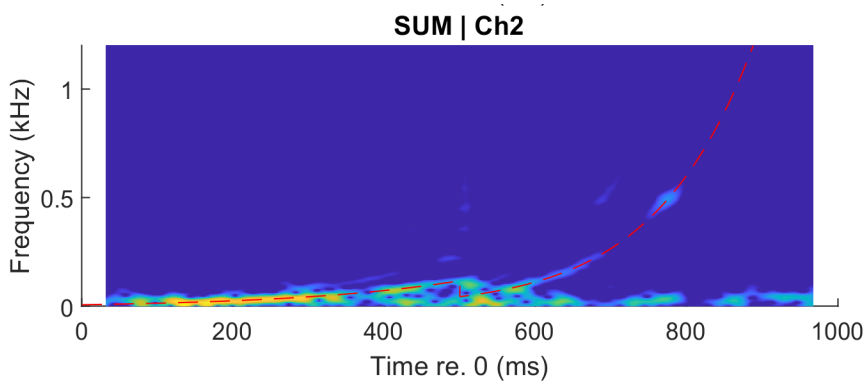


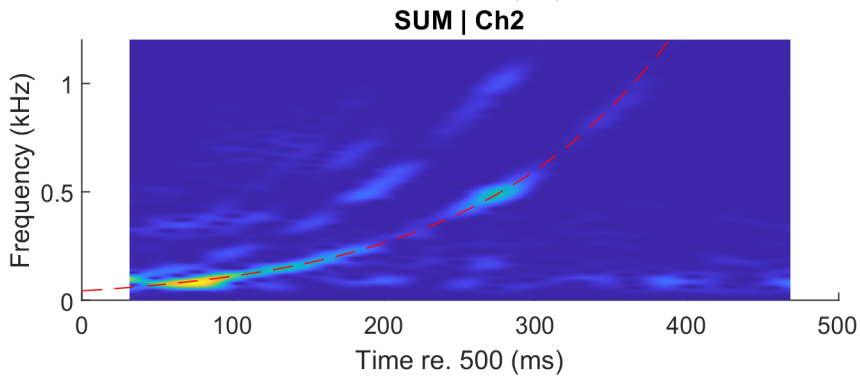
Example responses to this stimulus are shown in the figures below for a representative recording. These auditory evoked potentials were collected according to standard subdermal needle electrode recordings from our lab (e.g. Lai et al. 2022, *Neurobiology of Aging*, 117: 201-211). In the top figure, lower frequency modulations are clearly visible in the first half of the response, and low and high frequency temporal modulations are mixed in the second half (>.5 s). In the lower figure, the same trace has been high-pass filtered at 80 Hz to highlight the high frequency modulations for visualization and analysis.



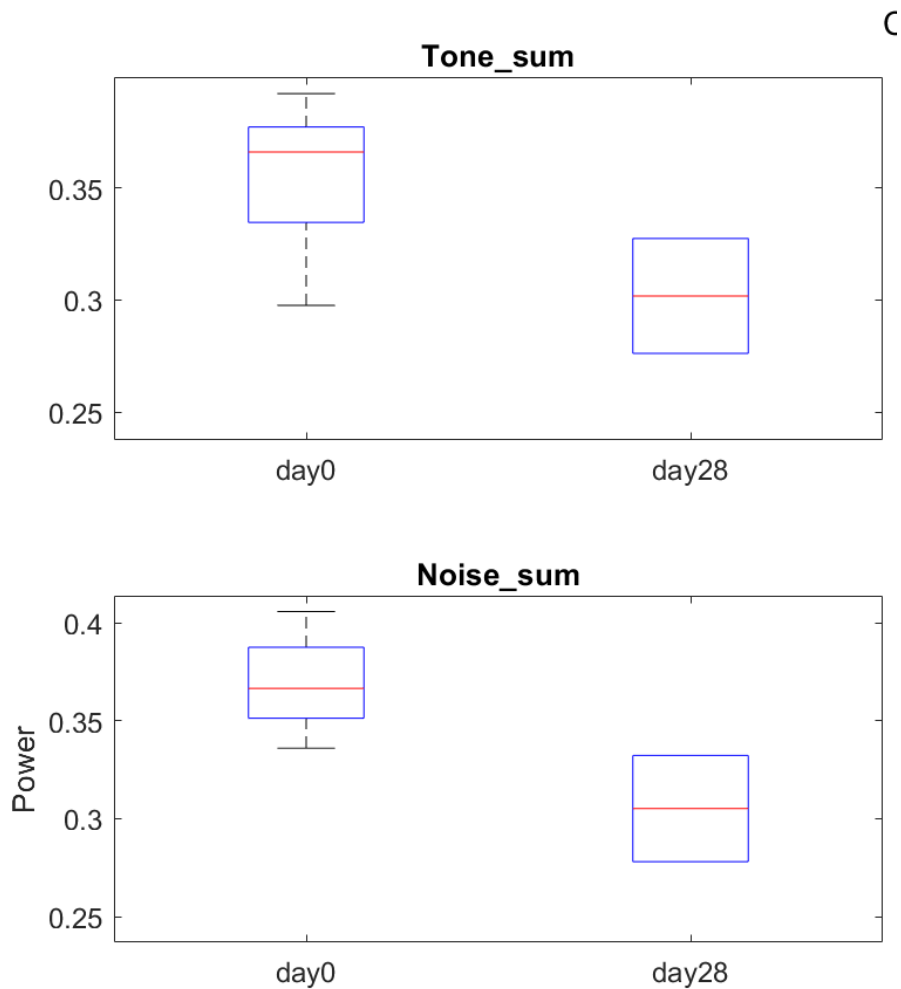


In order to quantify how well responses tracked the changing temporal modulation of the AM sweep, we have employed multiple analysis techniques, including short term Fast Fourier transforms, response autocorrelation, and, most recently, a tracking method based on spectral analysis of alternating polarity summed responses to isolate the modulation component (Parida et al. 2021, *PLOS Computational Biology*, 17(2): e1008155). Our results in the figures below demonstrate that modulations up through 100 Hz (top) and at least 900-1000 Hz (bottom) were reliably tracked using this method.





Tracking accuracy was quantified by measuring the proportion of response power that fell within 10 Hz of the stimulus AM frequency. Here, we quantified the tracking for both tone (top) and noise (bottom) carriers at two important time points – baseline and following 28 days of galactose injections. Our working hypothesis is that galactose injections will produce neuroinflammation that degrades neural responses to temporal modulation. Our initial results are consistent with this hypothesis, shown below.



- 
- 
3. Our initial focus has been to collect and analyze the initial set of recordings in galactose and sham-injected animals. We are in the process of setting up for cochlear histology, and that is the main listed objective where progress has been slow.

**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**

It took slightly longer than expected to pilot the galactose study and to train the students in recordings. That has been addressed, but it has delayed the beginning of cochlear histology, which we plan to do in the next reporting period. We have finished constructing a chamber than can hold 4 animals at once for the gunshot noise exposure, and we will begin those experiments during the next reporting period.

### **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 May 2023. 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.

#### **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.**

An abstract was submitted to the Association for Research in Otolaryngology.

**Rapid assessment of Temporal Processing from the Peripheral and Central Auditory Pathway using Dynamic Amplitude Modulated Stimuli**

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

**Other publications, conference papers and presentations.**

- **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 2022 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:**

**QUAD CHARTS:**

**9. APPENDICES:**