Spring 2016

Reflection: Effect of age on auditory brainstem responses in mice with EphA4 mutations

Erica L. Hoogerland
James Madison University

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Reflection: Effect of Age on Auditory Brainstem Responses in Mice with EphA4 Mutations

An Honors Program Project Presented to the Faculty of the Undergraduate College of Health and Behavioral Sciences
James Madison University

by Erica Lynn Hoogerland

May 2016

Accepted by the faculty of the Department of Communication Sciences and Disorders, James Madison University, in partial fulfillment of the requirements for the Honors Program.

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Assistant Professor, Audiology Clinical Coordinator and Liaison, Communication Sciences and Disorders

PUBLIC PRESENTATION

This work is accepted for presentation, in part or in full, at Madison Union Ballroom on April 15, 2016.
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PREFACE

The purpose of this thesis is intended as a reflection on the research experience assisting, Melissa Teller, with her dissertation on presbycusis in mice Eph4A mutations. In addition, this present analysis will mention the process and analysis associated with the data collection, as well as an overview of the literature reviewed for this research opportunity.
ACKNOWLEDGEMENTS

My sincerest gratitude goes to my advisor, Dr. Lincoln Gray, who provided the opportunity to work with graduate student, Melissa Teller, as an undergraduate assistant in his (CS)$^2$D Lab. His guidance and encouragement made this research and thesis opportunity possible. I would also like to express gratitude towards Melissa Teller, for allowing me to work with her on the research portion of her doctoral dissertation: “Effect of age on auditory brainstem responses in mice with EphA4 mutations.” In addition, thank you to Anna Louthan for helping to care for the mice and analyzing the data as a second observer for increased validity and reliability of the test results. Many thanks to my thesis readers, Dr. Claire Jacobson and Dr. Chris Clinard for their support in completion of this project. Funding for Melissa Teller’s dissertation was provided by NIH grant DC012421-01. Thank you to both the James Madison Honors Program and Communication Sciences and Disorders Department for supporting this undergraduate research thesis opportunity.
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ABSTRACT

Presbycusis, or age-related hearing loss is a condition that affects approximately 40% of the population over 65 years of age (Gates & Mills, 2005). Studying the effects of EphA4 mutations (wild type, heterozygous, and homozygous) mice is fundamental in understanding the relationship between onset of age-related hearing loss, in both the mammalian population and the human population. In order to further understanding of age-related hearing loss, the researchers evaluated thirty-six mice in a preliminary study from two months to nine months of age. Following data analysis, the researchers confirmed the results by continuing a second round of testing. The hearing potential of nine mice in a longitudinal study evaluated thresholds, peak latencies and amplitudes of mice from six months of age to nine months of age. The researchers found a correlation between the delayed onset of hearing loss in the lower frequencies and broadband clicks of the heterozygous (EphA4lacZ/+ ) mice subject group. There is a positive relationship ($r^2 = 0.067$, $p < 0.001$) between the dB Threshold analysis of the independent observers. Additionally, there is a positive relationship ($r^2 = 0.334$, $p < 0.001$) between the independent observers’ data analyses of peak one latency. Based on a preliminary discussion with the director of the National Institute of Aging, there is reason to speculate a heterozygous advantage to delay the onset of age-related hearing loss.
Presbycusis

Presbycusis, more commonly known as age-related hearing loss (ARHL), is a progressive bilateral hearing loss that affects both speech discrimination and auditory processing (Gates & Mills, 2005). Although the disorder is commonly associated with aging, the term comprises all conditions related to the degeneration of the hearing system. This type of hearing loss progresses from the diminished ability to understand speech, to the more progressive stage, with the inability to localize and identify sounds. The hearing loss often originates with a decreased sensitivity to hearing pure tones, located in the higher acoustic frequencies, to the middle and lower frequencies (Villaume et al., 1997). Due to the nature of presbycusis, individuals experience great difficulty in distinguishing phonemes that are imperative for differentiating sounds within words. Some of the most problematic phonemes for individuals with presbycusis are located in the higher frequencies which include: “f,” “v,” “s,” “th,” and “k.” These consonants can create minor changes within particular words that often result in significant misinterpretations of speech. Comprehension of speech is further compounded by noisy acoustic environments.

According to Gates and Mills (2005) presbycusis affects approximately 40% of the aging population over 65 years of age. The hearing impairment has been linked to various psychological effects including social isolation, depression, and dementia due to the reduced ability to understand speech and participate in social situations. Villaume et al. (1997) notes that paralinguistic and emotional cues of speech are also lost with the reduced perception of phonemes. The reduced perception of phonemes is correlated with the age of the individual. Elderly individuals over the age of 75 typically have loss in both content and paralinguistic
communication, while elderly individuals younger than 70 years of age experience loss in the decoding of speech content.

**Mice as Subjects**

The degenerative pattern of hearing loss with presbycusis, evidenced in the human population, is also persistent in the aging mouse (Henry & McGinn, 1992). In the aging population of animals raised in quiet environments, such mice raised in a laboratory setting, hearing loss typically originates in the inner ear, specifically located at the base and apex of the cochlea (Gates & Mills, 2005). There are several advantages to studying the hearing of mice which include: biological, developmental, economic, and anatomical factors (Henry & McGinn, 1992). Most often hearing research is conducted using mice, since there is a large database of information regarding typical auditory function of the mouse. This is beneficial in case there are abnormalities, or marked differences in auditory function, it is easier to decipher these changes using previous research. Additionally, the mouse is the most economical animal to maintain and requires less expensive pharmaceutical drugs for testing.

Two of the main advantages to using mice for hearing models are their relatively short lifespan and their vulnerability to noise induced hearing loss (NIHL) (Henry & McGinn, 1992). During the sensitive period, shortly after birth, mice are susceptible to changes in acoustic input which make them more desirable for testing. The short lifespan of the mouse allows researchers to track hearing function from birth to death. The mouse experiences hearing loss in a similar manner to the human, with the initial loss of higher frequencies, middle and lower frequencies being the last to diminish. Anatomically, the mouse has a shorter cochlea with a shorter distance between the base and apex of the cochlea. This genetic difference requires less experiments to establish a trend (Ohlemiller, 2006). According to Ohlemiller (2006), four structures of the ear
including, the organ of corti, spiral ligament, limbus, and stria vascularis have been identified as producing subtle changes, that have contributed to the understanding of progressive hearing loss from age and noise damage.

**Mice with Eph Mutations**

As previously stated, the mouse has a similar anatomical cochlear structure and progressive hearing loss to the human, making the mouse the most reliable model for the study of presbycusis. In recent years, researchers have genetically modified breeds of mice in order to maintain specific gene mutations within the breed, more specifically selecting specific Eph mutations (Henry & McGinn, 1992). In one of the Eph mutated strains, C57BL/6J, the exact age hearing loss onset is somewhat speculative. According to Hunter and Willot (1987), the onset of high frequency hearing loss begins about four to five months of age, whereas Sponger, Flood, Frisia, and Salvi (1997) noted that the onset of hearing loss in C57 mice begins at approximately three to six months. According to Miko, Henkemeyer, and Cramer (2008), mice with Eph mutations show altered patterns of activation in the brainstem when presented with pure tones. Since it is evidenced that Eph genes have the potential to regulate patterns of the brainstem, further research in this area of study is important to understanding the relationship between genes and hearing loss.

**Research Study Purpose**

It has been suggested by Ohlemiller (2006) that understanding the relationship between genes and the aging process can potentially be a predictor of the injury process in acquiring an age-related hearing loss. Due to this background knowledge, James Madison University graduate student, Melissa Teller researched the effect of auditory brainstem responses in mice with EphA4 mutations, particularly the C57BL/6J strain. The purpose of the study was to
identify any correlations with hearing loss of wild type, heterozygous, and homozygous mice in relation to the aging process.

The preliminary findings have been linked to the heterozygous advantage theory. This theory refers to the evolutionary and genetic superiority of having heterozygous expressed genes over the dominant genes (Mitton, 2002). The principle of heterozygous advantage is that mutated genes balance natural selection, by maintaining a genetic variation that often is beneficial to the organism in the prevention of certain undesirable conditions. There are several conditions that have more advantageous effects for a person as a heterozygous copy of the gene such as cystic fibrosis and sickle cell anemia. According to Watts and Rizzo (2015), “cystic fibrosis (CF) is an inherited recessive condition—an individual has to have inherited two altered copies of the gene to be affected.” In the United Kingdom there is a one and twenty-five hundred chance of expressing the gene. It is more advantageous for the carrier of cystic fibrosis to have the heterozygous or homozygous gene, that is not expressed with disease activity.

Similarly, sickle cell anemia has a protective nature to the disease against malaria for carriers who are heterozygous with a single gene mutation (Aidoo et al., 2002). Individuals who express the homozygous gene have a shortened lifespan due to the nature of sickle cell disease. However, individuals who are carriers of the heterozygous gene do not experience the negative effects of sickle cell, while gaining a protective barrier against malaria. Understanding the heterozygous gene advantage across health fields can be beneficial to understanding other human conditions like presbycusis. Information gathered on specific gene mutations can potentially be used to create pharmaceutical drugs that prevent the onset of hearing loss for the general population that do not express the heterozygous gene.
Methods

The following information is intellectual property of Melissa Teller. Prior to beginning research in the James Madison University (CS)²D Laboratory, both researchers completed the James Madison University Institutional Animal Care and Use Committee (IACUC Protocol #A12-12 and #A16-01) training, see appendices 1-3. During the first round of auditory brainstem response testing, the researchers began testing thirty-six mice aged two to nine months, provided by Dr. Mark Gabriele’s Laboratory. Genotyping of the mice was completed using PCR analysis, provided by Dr. Mark Gabriele in the biology department at James Madison University, from the tail snips collected during anesthesia. In the first group of subjects, there were twenty-six wild type (EphA4⁺/⁺) mice, five heterozygous (EphA4lacZ/⁺) mice, and five homozygous (EphA4lacZ/lacZ) mice. Due to the inability to complete genotyping on five of the mice, these mice were excluded from the study. Refer to table 1 for the distribution of auditory brainstem response testing for each of the mice in round one.

Table 1
**Round One Testing (Wild Type, Heterozygous, and Homozygous Mice)**
Teller, Melissa (2016)

<table>
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<tr>
<th>Mouse Age (months)</th>
<th>EphA4⁺/⁺</th>
<th>EphA4lacZ/⁺</th>
<th>EphA4lacZ/lacZ</th>
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<tr>
<td>9</td>
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<tr>
<td><strong>Totals</strong></td>
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<td><strong>5</strong></td>
<td><strong>5</strong></td>
<td><strong>36</strong></td>
</tr>
</tbody>
</table>

The second round of testing, a longitudinal study of auditory brainstem response testing in mice with wild type EphA4⁺/⁺ and heterozygous EphA4lacZ/⁺ mutations was completed to
verify the findings from the first round of testing. In the second round of testing, there were nine mice total that were tested consecutively once a month for three months between the ages of six and eight months. In the second subject group there were six wild type (EphA4+/+) and three heterozygous (EphA4lacz+/+) mice. Refer to table 2 for the distribution of mice by gene and age in months they were assessed.

<table>
<thead>
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<th>Mouse Age (months)</th>
<th>EphA4+/+</th>
<th>EphA4lacz+/+</th>
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In both the first and second rounds of testing, the genotype and mutation type of each mouse was unknown to the researchers. This blind procedure increased the validity and reliability of the data gathered for each of the mice tested. In order to record auditory brainstem responses, the mice were administered an intramuscular (IM) or intraperitoneal (IP) injection of 150 mg/kg ketamine and 30 mg/kg xylazine. In accordance with the animal care protocols, each mouse was initially administered a two-thirds dose based on their weight. As needed and in order to maintain anesthesia, some mice received the additional one-third dose. Next mice were placed in a sound attenuated booth on a warming, temperature controlled blanket. Three subcutaneous electrodes were placed according to accepted impedance measures ≤ 1kΩ. The subcutaneous needles were placed at the base of the neck (ground), vertex (non-inverting), and mastoid (inverting), review image 1 below. Throughout testing, mice were monitored using an infrared camera.
A series of stimulus clicks between the frequencies of 12kHz and 8kHz were presented at decreasing intensity levels. In order to present the stimulus, electrodes were connected using the Tucker Davis RA4PA 4-channel Pre-amp. Stimuli were presented inside the pinna of one ear of the mouse through the TDT EC1 high-frequency electrostatic speaker and monitored using an Etymotic research ER-7C probe microphone, see image 2 below.
In order to maintain consistency and ensure accurate calibration, stimulus peak frequency, amplitude, and bandwidth were monitored throughout the duration of testing. As previously stated, three stimuli were presented at 8kHz, 12kHz, and a broadband click. These stimuli were generated using BioSig software and presented in a randomized order to the mouse. The clicks were presented with a decreasing intensity from a high of 90dBSPL to a low of 20dBSPL. The duration of the tone pips presented were at a rate of 5msec and broadband clicks at a rate of 0.1 msec. The auditory brainstem response thresholds were recorded using BioSig software.

**Results**

Following testing, the auditory brainstem responses were analyzed by two James Madison graduate level independent observers using MATLAB software. The visual analysis used tracings to determine thresholds for waves I through V, peak latencies and amplitudes. The thresholds were selected based on the lowest intensity, with identifiable peaks. The overall findings of the longitudinal study indicate a delayed onset of presbycusis in heterozygous mice with EphA4\textsuperscript{lacZ+/} mutations, as compared to mice of the homozygous and wild type genetic variations. This difference is noted in the comparison of dB thresholds between wild type and heterozygous mice. The data gathered strongly suggests a delayed onset of hearing loss in the lower (8kHz) tone frequencies and broadband click frequencies. As expected, both the wild type and heterozygous mice experienced loss in the higher frequencies (12kHz). The reliability of the dB threshold analysis between observers is a correlation coefficient of 0.067, p <0.001. Although there is also a positive relationship between the peak one latencies, it is more variable with less clarity on the relationship between peak latency and age. The correlation coefficient between
peak one latencies and time is 0.334. The results are summarized below, figures 1-5, provided by James Madison University graduate student, Melissa Teller.

Figure 1
Auditory Brainstem Response Thresholds in Comparison to Age
Teller, Melissa (2016)
Figure 2
Mean Average Latency Peak 1 of Wild Type and EphA4
Teller, Melissa (2016)

Wild Type

EphA4<sub>lacZ+/</sub>

Error Bars: +/- 1 SE
Figure 3

*Hearing Loss: Wild Type vs. EphA4 Mutations over Time*

Teller, Melissa (2016)
Figure 4
Peak 1 Reliability (correlation coefficient)
Teller, Melissa (2016)

Figure 5
dB Threshold Reliability (correlation coefficient)
Teller, Melissa (2016)
In conclusion, heterozygous mice with Eph, C57BL/6 strain mutations have a delayed onset of hearing loss in the lower frequencies. Based on the information gathered in this study there is reason to believe age-related hearing loss follows a heterozygous gene advantage model, similar to other conditions, like cystic fibrosis and sickle cell anemia. In the future this information could be used by other health professions to generate pharmaceutical drugs that prevent the onset of hearing loss in individuals who lack this particular gene mutation.
REFERENCES


APPENDICES

i. Occupational Health and Safety Curriculum Completion Report

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)

OCCUPATIONAL HEALTH AND SAFETY CURRICULUM COMPLETION REPORT
Printed on 02/06/2014

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OCCUPATIONAL HEALTH AND SAFETY

COURSE/STAGE: Lab Animal ResearchV1
PASSED ON: 02/06/2014
REFERENCE ID: 1230892

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Professor, University of Miami
Director Office of Research Education
CITI Program Course Coordinator
ii. Working with Mice in Research Settings Curriculum Completion Report

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)
WORKING WITH MICE IN RESEARCH SETTINGS CURRICULUM COMPLETION REPORT
Printed on 02/06/2014

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INSTITUTION
James Madison University

EXPIRATION DATE
02/05/2017

WORKING WITH MICE IN RESEARCH SETTINGS

COURSE/STAGE:
Lab Animal Research/1

PASSED ON
02/06/2014

REFERENCE ID:
12308901

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Introduction to Working with Mice in Research Settings | 02/06/14 | No Quiz
Research Mandates and Occupational Health Issues | 02/06/14 | 3/3 (100%)
Alternatives Searches, Humane Standards, Housing, and Acclimation and Quarantine | 02/06/14 | 2/2 (100%)
Detecting Pain and Distress, Genetics, and Biological Features | 02/06/14 | 3/3 (100%)
Injections, Blood Collection, and Antibody Production | 02/06/14 | 2/2 (100%)
Surgery, Supportive Care and Monitoring, Euthanasia, and References | 02/06/14 | 3/5 (60%)

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Director Office of Research Education
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### iii. Working with the IACUC Curriculum Completion Report

**COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)**

**WORKING WITH THE IACUC CURRICULUM COMPLETION REPORT**

Printed on 02/06/2014

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**INSTITUTION**
James Madison University

**EXPIRATION DATE**
02/06/2017

**WORKING WITH THE IACUC**:
The CITI Basic Course in Laboratory Animal Welfare for Investigators, Staff and Students.

**COURSE/STAGE**
Lab Animal Research/1

**PASSED ON**
02/06/2014

**REFERENCE ID**
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