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Prepulse inhibition of the acoustic startle reflex to obtain a psychometric function in mice

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Prepulse Inhibition of the Acoustic Startle Reflex to Obtain a Psychometric Function in Mice

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Abstract

The acoustic startle reflex (ASR) is an automated motor response to an unexpected and intense auditory stimulus (Ouagazzal, Reiss, & Romand, 2006). When an audible 'prepulse' stimulus is presented before the intense, startle-evoking stimulus (SES); the startle reflex response is reduced and this is known as prepulse inhibition (PPI). The degree of ASR inhibition serves as a measure of the behavioral salience of the prepulse (Carlson & Willott, 1996). This study aimed to obtain a psychometric function from the amount of PPI of the ASR that resulted from varying intensity levels of a prepulse stimulus (PPS).

Twelve mice were used for this study and each was tested twice. Six of the mice were of the C57BL/6J background (a common strain often used as a control) and six were wild-type offspring of mice that had a mutation of the ephrin (EphA4) gene (labeled as EphA4+/+$_{(WT)}$) and were expected to be normal aside from possible early rearing effects from their mutant parents.

An accelerometer measured amount of movement associated with the SES with and without the PPS. The PPS randomly varied between 13 different intensities in the range of 25 dB SPL to 75 dB SPL. In addition, there were two control trials of the SES with a PPS of 0 dB SPL and one random trial with no sound at all. Therefore, there were a total of 16 trials which were presented randomly in each of 11 blocks. For each test session, the PPS randomly varied by frequency filter; high-pass (HP) or band-pass (BP). The SES was presented at an intensity of 120 dB SPL for a duration of 15 ms and medium inter-stimulus interval (ISI) of 50 ms was used for all trials.

A psychometric function was successfully obtained. There was no significant difference between the two strains of mice (p=0.15) so data between the groups was pooled. A significant effect (p=0.04) of frequency filter was seen as more PPI was obtained with the HP vs. BP filter. The obtained threshold ranged from 19 dB SPL to 45.8 dB SPL depending on how threshold was defined.

Introduction

The Acoustic Startle Reflex (ASR)

The startle reflex is a brief motor response to an unexpected and intense auditory, tactile, or visual stimulus (Ouagazzal, Reiss & Romand, 2006). This automated response can be reliably observed and studied across mammalian species, allowing for it to be used in translational research (Braff, Geyer & Swerdlow, 2001). The startle reflex is thought to serve as a defensive protection from external threats. Reflex activation results in flexor contractions that begin at the head/neck and move through the body, resulting in a crouch-like posture (Hoffman & Ison, 1980).

When this reflex is initiated by an acoustic stimulus it is appropriately referred to as the acoustic startle reflex (ASR). The ASR is elicited by intense sounds (80 dB SPL or greater), has a short latency, and can be measured easily in small mammals using movement-sensitive devices such as an accelerometer (Koch, 1998; Willott et al., 2003; Yeomans & Frankland, 1996). Any stimulus frequency that is within the audible range of the subject can be used to elicit the response (Yeomans & Frankland, 1996; Koch, 1998). The transient motor response caused by the ASR creates a measureable spike on an accelerometer and is typically seen 9-12 ms following onset of the startle stimulus. However, ASR is often defined as the greatest peak-to-peak voltage deflection recorded within a time frame of approximately 30 ms after startle onset (Willott, Carlson & Chen, 1994).

Neural circuits within the lower brainstem are thought to be the origin of the acoustic startle reflex. Evidence suggests that transmission moves from the auditory nerve to the cochlear nucleus (CN) to brainstem neurons and the spinal cord; either indirectly (through the lateral lemniscus) or directly, to the caudal pontine reticular nucleus (PnC). The PnC is where sensory and motor neurons of the startle circuit interact (Basavaraj & Yan, 2012; Carlson & Willott, 1996; Davis et al., 1982; Leitner, Powers & Hoffman, 1980). It is not yet known which sub

regions of the PnC are involved (Koch, 1998). While the PnC may be the main brainstem site to evoke the ASR, other brainstem nuclei may also act as premotor relays that mediate the reflex (Koch, 1998; Yeomans & Frankland, 1996). Neuro-pharmacological studies reveal that glutamate is the likely excitatory transmitter of auditory input to PnC neurons and y-amino-butyric acid (GABA) has been indicated as the inhibitory transmitter on the ASR (Koch, 1998).

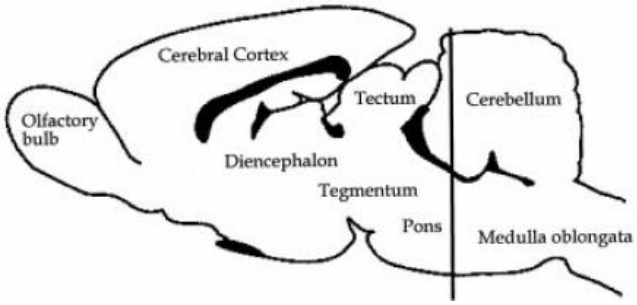

Figure 1. A diagram of a parasagittal section through the rat brain with the vertical line depicting the location of the caudal pontine reticular nucleus (PnC). It is hypothesized that the PnC may be the main brainstem site to evoke the ASR (Koch, 1999).

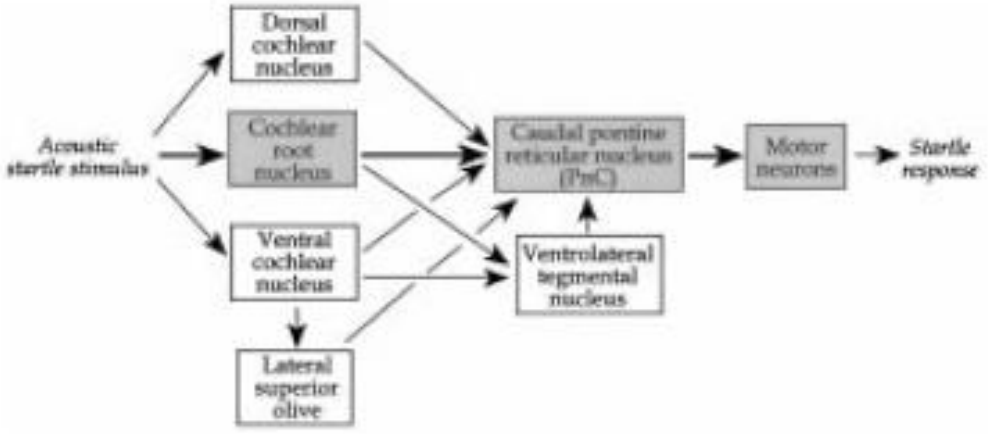

Figure 2. A hypothetical flow-chart depicting the primary ASR pathway. The bold arrows and shaded boxes demonstrate the most direct route of transmission of the acoustic stimulus into the motor response (Koch, 1998).

Startle amplitude can be affected by anything from changes in the startle-eliciting stimulus parameters, the surrounding sensory environment or subject state, to pharmaceutical use, genetic differences, and presentation of a pre-startle stimulus (Carlson & Willott, 1996; Davis & Gendelman, 1977; Hoffman & Searle, 1968; Koch, 1998). The widely varying methods for

modifying the amplitude of the acoustic startle are likely due to an increase or decrease in the transfer of information between the sensory and motor systems. This study focused on modification of the acoustic startle response as a result of the presentation of a pre-startle auditory stimulus.

Prepulse Inhibition (PPI)

A perceived disturbance in the environment immediately prior to presentation of a startle stimulus can affect expression of the reflex response (Hoffman & Ison, 1980). For example, an audible stimulus presented 30-500 ms before an intense, startle-evoking stimulus causes reduction of the ASR (Braff, Geyer, & Swerdlow, 2001). As the intensity of the prepulse stimulus becomes greater, the amount of inhibition of the ASR also increases (Reijmers, 1994). This phenomenon is a natural, unlearned response and is known as prepulse inhibition (PPI).

Optimal PPI is typically obtained when the prepulse stimulus is presented 80-120 ms before the startle sound. Conversely, a prepulse that is presented more than 500 ms prior to the startle stimulus could cause an increase in ASR amplitude; known as prepulse facilitation (Basavaraj & Yan, 2012). PPI reliably occurs in all mammals without prior conditioning and does not exhibit habituation in humans (Koch, 1998).

Amount of PPI can be quantified as a relative difference between ASR magnitude with and without presence of a prepulse (Koch, 1998). It is generally tabulated as either a percentage or as an absolute reduction in the magnitude of the ASR (Swerdlow, Geyer & Braff, 2001).

Factors that have been identified as having an effect on PPI are similar across species, further supporting the use of PPI studies in translational comparisons across species (Braff, 2001). Previous studies have found that the PPI mechanism can be deficient in individuals with Schizophrenia, Huntington's disease, Parkinson's disease, seizure disorders, Tourette syndrome, obsessive compulsive disorder, and nocturnal enuresis. The response is also diminished through use of some pharmaceutical agents (Swerdlow, Geyer & Braff, 2001).

Prepulse inhibition is generally recognized as a model of sensorimotor gating. Sensorimotor gating is a physiological process that may occur when an organism's sensory system begins to become overloaded with stimuli. A stimulus that is perceived by the system as being insignificant is filtered out of awareness (Braff & Geyer, 1990). It is believed that the process of sensory motor gating is controlled by an inhibitory mechanism within the central nervous system that can impact the structure and organization of thought processes (Swerdlow, Geyer & Braff, 2001). Sensory motor gating allows for regulation of environmental inputs so that an organism can better navigate the sensory world and is able to selectively assign attentional resources to crucial stimuli. Therefore, activation of the PPI circuit allows the brain to receive potentially important feedback by suppressing the response to the seemingly unimportant sensory input. This protective mechanism shields information contained in a weak, prepulse stimulus so that it may be processed without interference from a potentially disruptive startling stimulus that follows it (Braff, 2001; Graham, 1975; Li et al., 2009; Swerdlow, Geyer & Braff, 1999). Therefore, PPI can be used as a measure of the sensorimotor gating process and to provide quantification of an organism's ability to filter out surplus and/or insignificant stimuli so that the important aspects of the stimulus-filled environment are focused on (Braff & Geyer, 1990).

Table 1 lists a number of neural substrates (including structures, neurotransmitters and neuropeptides) that have been implicated in the regulation of prepulse inhibition in rodents, humans or both over the past 20 years of research in this area. Together, these brain substrates can help to constitute a hypothetical neural 'map' of PPI regulation in these species. The listed substrates range in distribution between the frontal cortex and the pons (Swerdlow, Geyer & Braff, 1999).

Table 1. A list of neural structures, neurotransmitters and neuropeptides that have been implicated in the regulation of PPI in rodents and/or humans (Swerdlow, Geyer & Braff, 1999).

Hippocampus	*Prefontal cortex*
Basolateral amygdala	*Nucleus accumbens*
Striatum	*Ventral tegmental area*
Ventral pallidum	*Globus pallidus*
Substantia nigra reticulata	*Thalamus*
Pedunculopontine nucleus	*Superior colliculus*
Inferior colliculus	

The underlying mechanism for PPI, while still not fully understood, involves higher level neural structures in the central auditory, limbic and motor systems (Basavaraj & Yan, 2012). The behavioral gating processes that are regulated by forebrain neural circuitry are activated by PPI (Braff, 2001). When the ASR is evoked by the startle stimulus, descending projections of the PPI circuits inhibit the startle circuit at the PnC. The inferior colliculus (IC) propagates neural activity evoked by the prepulse stimulus to other components of the circuit (Carlson & Willott, 1996).

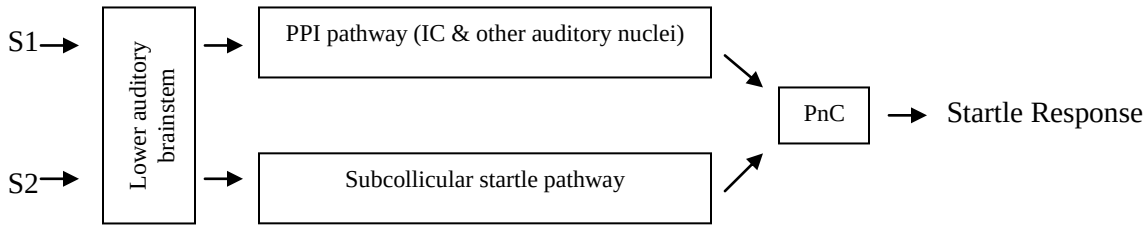

Figure 3. Flow-chart of a simplified model of PPI circuitry depicting a prepulse stimulus (S1) activating the inferior colliculus and other auditory nuclei of the upper brainstem, which causes inhibition of PnC neurons after a startle stimulus (S2) (Carlson, & Willott, 1996).

While there are cross-species similarities in the neural circuitry involved in PPI regulation, there are also differences. For example, current research suggests that similarities exist

in the dopamine and nicotine substrates but not for the glutamate and serotonin substrates (Swerdlow, Geyer & Braff, 1999).

Reflex Modification Audiometry (RMA)

Prepulse inhibition of the acoustic startle reflex is a type of Reflex Modification Audiometry (RMA), in which degree of ASR inhibition is used as a behavioral measure of detection of the prepulse stimulus (Carlson & Willott, 1996; Allen & Ison, 2010). RMA is a fast, reliable and non-invasive method that provides objective behavioral data. It does not require training or food/water deprivation and can be a useful method in the study of auditory detection and processing in genetically modified mice (Allen & Ison, 2010). Consistent measurable modification effects can be obtained with a prepulse stimulus that is at or near the threshold for detection (Hoffman & Ison, 1980). ASR is inhibited for hundreds of ms after the PPI circuit has been activated (Willott et al., 2003).

The Mouse Model

Mice acquire functional hearing as early as postnatal day 10 (Ehret, 1976). As can be seen in Figure 4, mice are known to have poor sensitivity to low frequencies when compared to humans and their range of hearing is approximately 2 kHz – 100 kHz (Heffner & Heffner, 2007). Mouse audiograms obtained using both classical and operant conditioning reveal best thresholds at approximately 20 kHz for many strains of mice (Fay, 1988). However, as depicted in Figure 5, results from studies using behavioral methods have produced varying results (Radziwon, et al, 2009).

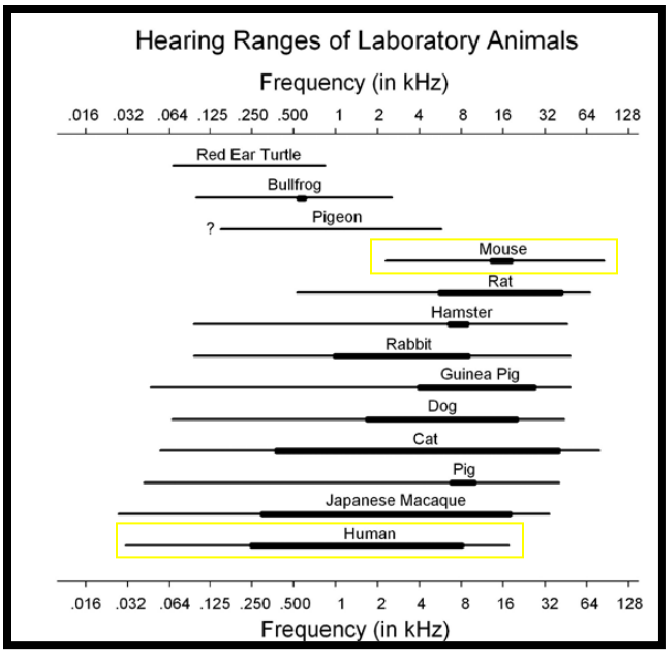

Figure 4. The frequency range of hearing for various species. Of particular interest to this study are the mouse and, for comparative reasons, the human. As can be seen, mice have the ability to hear sounds of approximately 2-100 kHz at 60 dB SPL. Bold lines depict frequencies audible at 10 dB SPL (Heffner & Heffner, 2007)

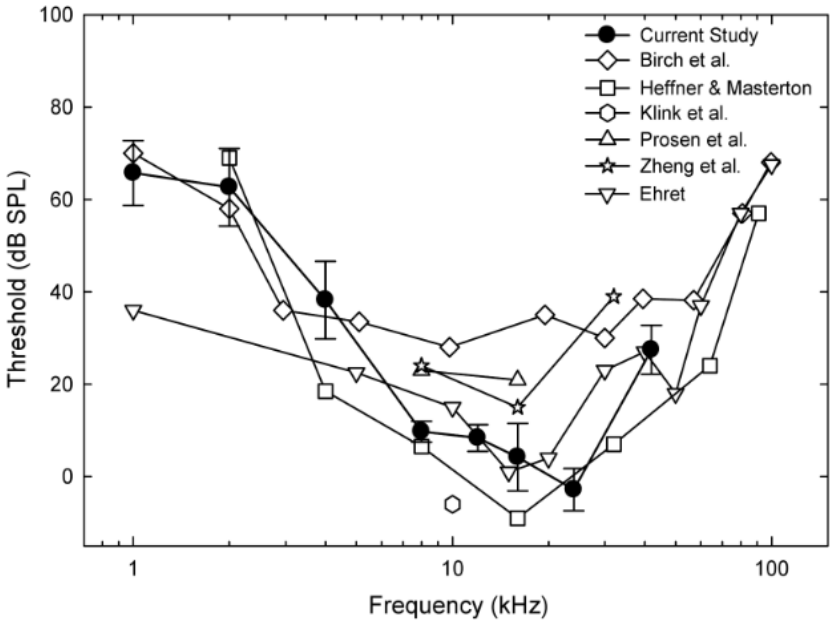

Figure 5. A figure from Radziwon et al. (2009) showing mouse audiograms obtained from various studies compared to a behaviorally obtained method used by Radziwon et al. (2009). All studies shown used behavioral methods, with the exception of Zheng, et al., who used the auditory brainstem response (Radziwon et al., 2009).

In comparison, humans can hear frequencies in the range of 16 Hz – 20 kHz with lowest thresholds obtained at 2-5 kHz (Roeser, 1996). The average distance between the two ears is 22-23 cm (Woodworth, 1938).

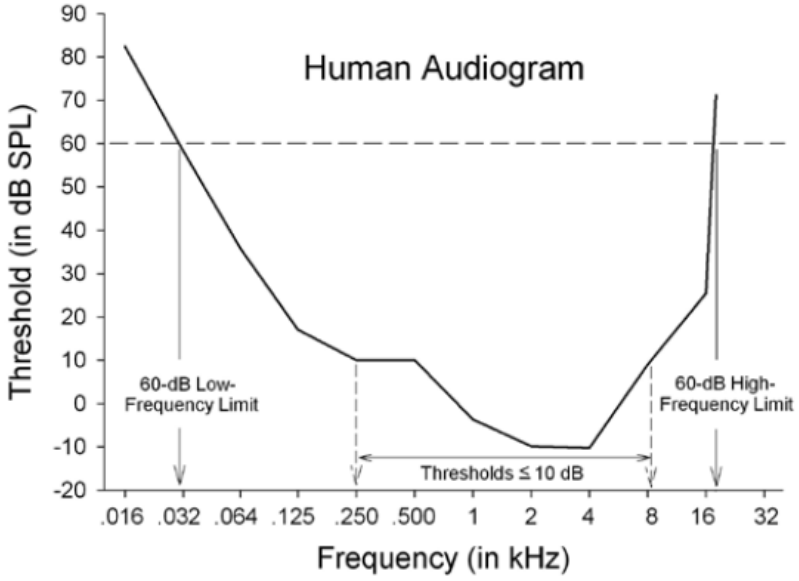

Figure 6. Human Audiogram (Heffner & Heffner, 2007).

Although the hearing ability of humans and mice differs, their auditory systems are strikingly similar; both structurally and functionally. In fact, human and mice have close evolutionary ties. Approximately 99% of mouse and human genomes have evolved from common ancestral genes via the process of speciation (Brown, Hardisty-Hughes & Mburu, 2008).

Mice are efficient research subjects and have been used extensively in testing the peripheral and central auditory systems. In addition, they can be genetically engineered to allow for research into the genetic and cellular bases of hearing loss (Brown, Hardisty-Hughes, & Mburu, 2008). Mutant mice have been used to successfully identify the key loci involved in hearing; which has in turn allowed us to discover many of the genetic bases of human hearing impairment and their underlying mechanisms. The murine model has provided us with a better

understanding of the developmental and physiological mechanisms underlying the auditory process (Brown, Hardisty-Hughes & Mburu, 2008).

However, to fully understand the effects of genetic mutations on hearing, it is important to determine accurate auditory thresholds of mice. This has traditionally been done via electrophysiogical measures and various behavioral methods. A widely used electrophysiological method has been the auditory brainstem response (ABR). Behavioral testing paradigms can be divided into two classes: procedures that involve conditioning and those that rely on unconditioned reflexes to obtain a response to auditory stimuli (Heffner & Heffner, 2001).

The ABR has been a popular research method for assessing hearing in mice because results can be obtained fairly quickly and easily. However, the ABR is not a true test of hearing, but rather a quantification of neural synchrony. Therefore, auditory sensitivity can only be inferred from the results. In addition, the ABR does not assess auditory function beyond the brainstem level (Hall, 2007, p. 13). On the other hand, behavioral methods assess the complete auditory system and they are considered to be a true test of hearing. However, behavioral paradigms that use conditioning procedures involve training the mouse to respond to auditory stimuli; which can be a lengthy process. Behavioral testing methods that use unconditioned reflexes do not involve training and; therefore, results can be obtained more quickly. PPI of the ASR appears to be a promising method for efficiently obtaining absolute auditory thresholds in mice; however, it has not yet been confirmed whether it is as sensitive of a measure as conditioned response procedures and whether or not it can accurately predict sensitivity at a wide range of frequencies. If it is revealed that PPI of the ASR can provide accurate absolute thresholds efficiently, then it could potentially replace conditioned response procedures for hearing assessment in mice (Heffner & Heffner, 2001).

C57BL/6J Mice

C57BL/6J mice are a common strain often used as a "background" for genetic mutations. They are known for age-related progressive hearing loss, beginning at the high frequencies, as early as 60 days of age (Li & Borg, 1991). This hearing loss is due to a recessive gene and is cochlear in nature (Erway, Willott, Archer, & Harrison, 1993). The age-related hearing loss (AHL) seen in C57BL/6J mice, as well as in a number of other mouse strains, is thought to be largely caused by an AHL locus, which can be found on chromosome 10 near the cadherin 23 gene (Brown, Hardisty-Hughes & Mburu, 2008).

Outer hair cell degeneration caused by age-related hearing loss is typically seen in mice by 5-6 months (150-180 days) of age. By 1 year, hearing loss is typically severe at the high frequencies and becoming evident at the mid-frequencies (Henry & Chole, 1980). Physiological changes occur in the upper auditory brainstem and cortex of adult C57BL/6J mice, referred to as hearing-loss induced plasticity (Carlson & Willott, 1996). Specifically, the sensorineural hearing loss experienced by these mice as they age causes changes in the tonotopic organization of the inferior colliculus (IC) in which neurons in the ventral region are re-mapped to better respond to mid-frequency stimuli as opposed to the inaudible high-frequency stimuli (Willott, 1986).

EphA4+/+$_{(WT)}$ Mice

The EphA4+/+$_{(WT)}$ mice are homozygous (+/+) offspring of mice that had a mutation of the ephrin (EphA4) gene. These mice; therefore, are 'wild-type' offspring and are expected to display PPI of ASR responses that are comparable to the C57BL/6J mice. However, it is possible that these mice could potentially have been influenced by any abnormal early rearing effects of their mutant parents.

The Auditory System

The onset of hearing varies from species to species. However, the development of the auditory system can be divided into four phases. In the first phase, electrophysiological and

behavioral reactions to acoustic stimuli are absent. The second phase begins with presence of the cochlear microphonic after acoustic stimulation. This stage covers a period of only about to two to three days. The third phase marks the beginning of neural activity and a behavioral reaction to sound. In this stage, the central auditory pathway has begun to relay signals to motor nuclei. This third phase typically lasts approximately two weeks. It is during this period of time in which auditory sensitivity rapidly increases and the range of frequency response expands. Phases three and four then operate concurrently for a period of several months. Phase four can typically be identified when a rapid change in sensitivity occurs (Kraus, 1981).

The ascending auditory pathway for both mice and humans is very similar. In general, sound is conducted from the cochlea through the eighth cranial nerve to the cochlear nucleus (CN). Next, the signal travels to the superior olivary complex (SOC), where interaural time and intensity differences are processed for sound localization. The signal is then sent via the lateral lemniscus (LL) to the inferior colliculus (IC) and on to the medial geniculate body (MGB), where it is finally sent to the auditory cortex (Martin & Clark, 2000).

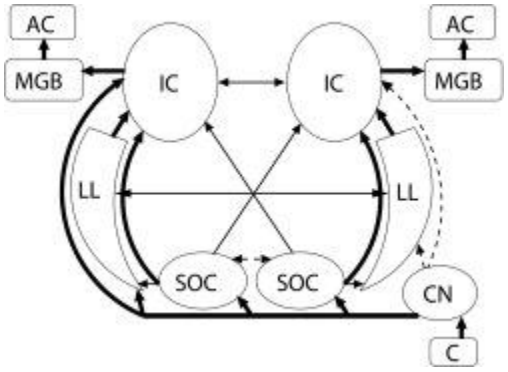

Figure 7. Schematic representation of the ascending auditory pathway of the mouse, obtained through a manganese-enhanced MRI . Thick lines indicate major projections, fine lines point to moderate projections and the dashed line leads to a minor projection (Watanabe, Frahm & Michaelis, 2008).

Importance of Hearing Research

Hearing loss is a common ailment, occurring in approximately 250 million people worldwide, half of which likely have a genetic basis. Of these, approximately 70% are non-syndromic with hearing impairment being the only pathology associated with the genetic component (Gratton, 2003). Presbycusis is especially common. More than 60% of human adults over the age of 70 have a hearing loss greater than 25 dB HL (Brown, Hardisty-Hughes & Mburu, 2008). The human genome has been mapped with close to 130 loci that are known to cause non-syndromic hearing loss; however many of the genes responsible have not yet been identified (Brown, Hardisty-Hughes & Mburu, 2008).

Psychometric Functions

A psychometric function shows how the probability of a response changes for various presentation levels (Gelfand, 2010, p. 148). It can be used to provide a means for interpreting how some parameter of a physical variable affects performance of a psychological variable (Katz et al, 2009). A psychometric function, as plotted on probability coordinates, has two parameters: slope and intercept (Gray, 1991). The slope reveals how quickly responsiveness increases as some stimulus parameter is raised, while the intercept signifies the horizontal position of the function (Gray, 1991). Slope and intercept can be used to quantify the amount of change needed in the stimulus parameter to elicit any percentage of correct responses. In the realm of auditory research, psychometric functions often look at how intensity changes affect stimulus detection. Thus, they can provide a complete picture of how well a subject is able to detect a stimulus: from 0% detection up to 100% detection.

Psychometric functions provide valuable information for experimental design and in defining threshold (Gray, 1991). Threshold, which is the lowest level at which a stimulus is heard, is an often used term to describe the transition between audibility and inaudibility. This simplistic view of threshold fails to acknowledge the fact that this transition does not occur at some obvious point, but rather occurs gradually. Therefore, threshold measurements are often

determined based on some pre-selected arbitrary point along the psychometric function (Green, 1990). It is customary to use the 0.5 probability point on the psychometric function in which the subject is able to detect the stimulus 50% of the time (Gelfand, 2010, p. 148). It can be argued that instead of using some arbitrary point along the function, the point in which the least amount of variability is obtained should be employed. Oftentimes, the stimulus value that minimizes variability of the threshold estimate is not near the middle of the psychometric function and instead corresponds with a high probability of being correct (Green, 1990).

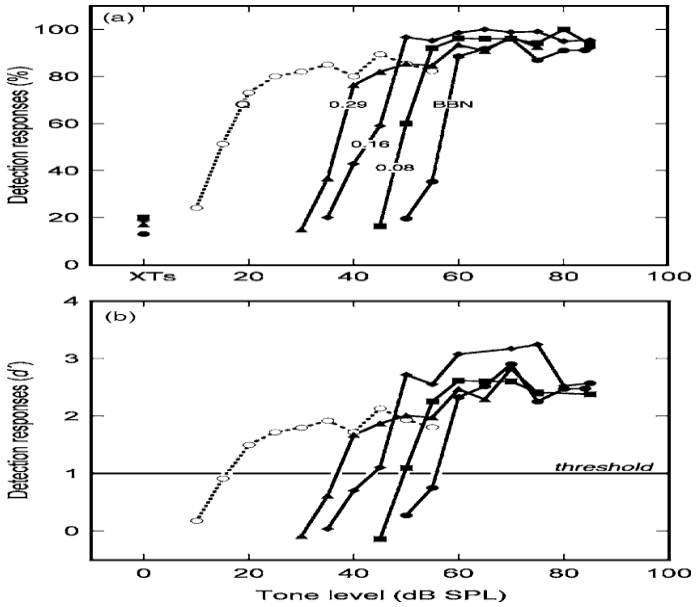

Figure 8. Mouse psychometric function using behavioral assessment in CBA/CaJ mice from a study by May, Kimar and Prosen (2006). Data were obtained using 8 kHz tones in various conditions: quiet, broadband noise, and with three bandwidths of notched noise. Percentage of correct responses at a given level and false alarm rates for catch trials are plotted in the top figure. In the bottom figure, detection scores are plotted as the signal detection statistic *d'* (May, Kimar and Prosen, 2006).

Summary of studies using PPI of ASR to assess hearing in mice.

Allen & Ison (2010) measured PPI of the ASR as the indicator response for stimulus detection in CBA/CaJ mice. CBA/CaJ mice exhibit age-related hearing loss but the mice studied were not yet afflicted. A startle stimulus of 120 dB SPL broad-band noise burst (15ms duration and rectangular-gated with 50 kHz bandwidth) was presented from 15 cm above the mouse

chamber. The prepulse stimulus consisted of a 70 dB SPL broadband noise. Three prepulse conditions were tested: speaker swap, offset, and onset. Each prepulse condition was presented 11 times per block and each block consisted of 16 randomized interstimulus interval (ISI) conditions. The various ISI conditions used were: 1, 2, 5, 10, 20, 30, 40, 50, 60, 100, 150, 200, or 300- ms. Results revealed that PPI was most robust for the offset condition at the mid-range ISI of approximately 50 ms (Allen & Ison, 2010).

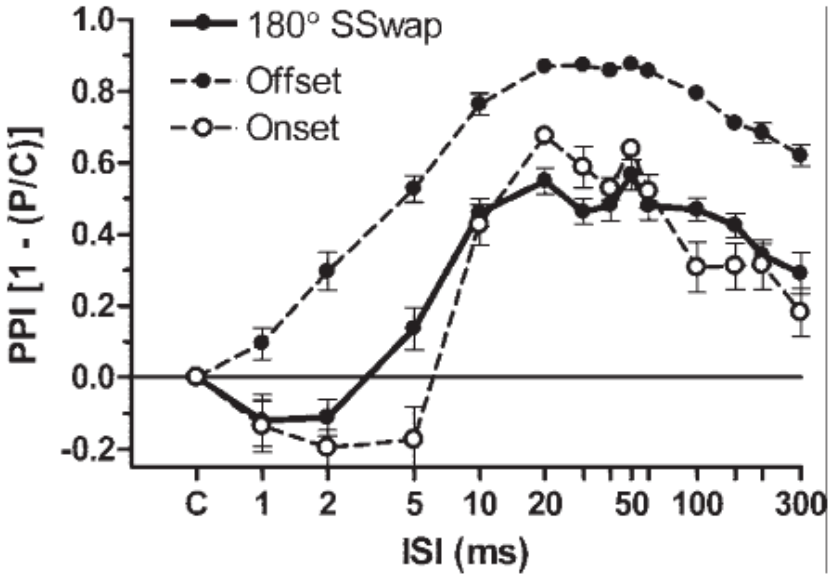

Figure 9. Figure from Allen & Ison (2010) comparing results from three conditions: 180 degree speaker swap, offset of the prepulse and onset of the prepulse stimulus. As can be seen, the offset response produced the most robust prepulse inhibition and peaked at an ISI of approximately 50 ms (Allen & Ison, 2010).

Willott, Carlson & Chen (1994) measured PPI of ASR in C57BL/6J mice to assess auditory changes in the central auditory system associated with age-related hearing loss. The mice in their study ranged from 30-360 days of age. A startle stimulus of 100 dB SPL broadband white noise burst (10 ms in duration with 1 ms rise/fall) was presented. The prepulse stimulus consisted of tone pips (10 ms in duration with 1 ms rise/fall) which were presented 100 ms before the startle stimulus. The prepulse stimulus was presented at a range of randomized frequencies (4, 8, 12, 16, and 24 kHz) and intensities (50, 60, 70, and 80 dB SPL). The interstimulus interval varied

depending upon how long it took the subject to cease grooming or no longer presented other exploratory behaviors between trials, but averaged 20-25 seconds.

The Willott, Carlson & Chen (1994) study found that subject age, prepulse frequency and prepulse intensity each affected the amount in which the startle response was inhibited. At the highest frequency in which they tested (24 kHz), PPI decreased with increasing age and/or required a higher intensity level to be effective. This demonstrates the expected age-related loss of high-frequency hearing sensitivity in this mouse strain. The slightly lower frequencies of 12- and 16-kHz produced more prepulse inhibition from 1- to 5- months of age; however, a decrease in inhibition was then observed for the 16 kHz prepulse when the mice were 12 months of age. This finding further demonstrates a progressive loss of hearing along the frequency range in C57BL/6J mice. PPI remained robust at low to mid frequencies (<12 kHz) even in older age (12 months). This study demonstrates the importance of testing C57BL/6J mice earlier than 5 months of age, before the effects of hearing loss will be imminent (Willott, Carlson & Chen, 1994).

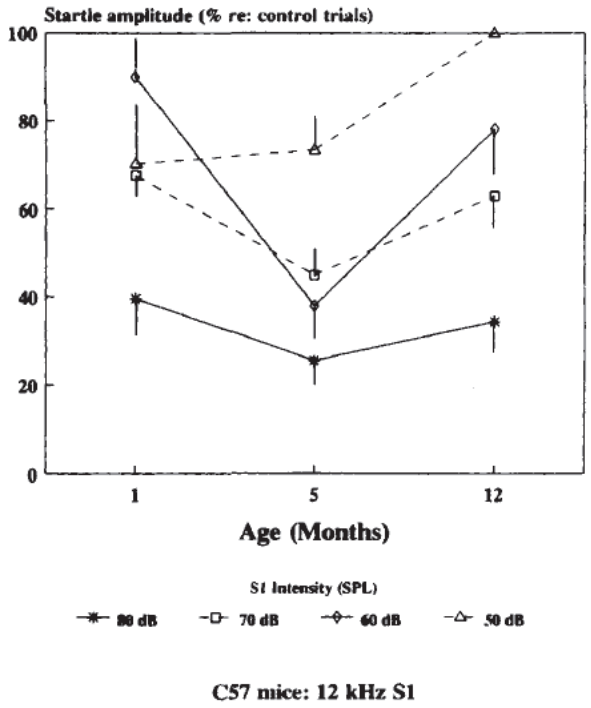

Figure 10. Mean acoustic startle response amplitudes for C57BL/6J mice as a function of age and prepulse intensity with a prepulse frequency of 12 kHz (Willott, Carlson & Chen, 1994).

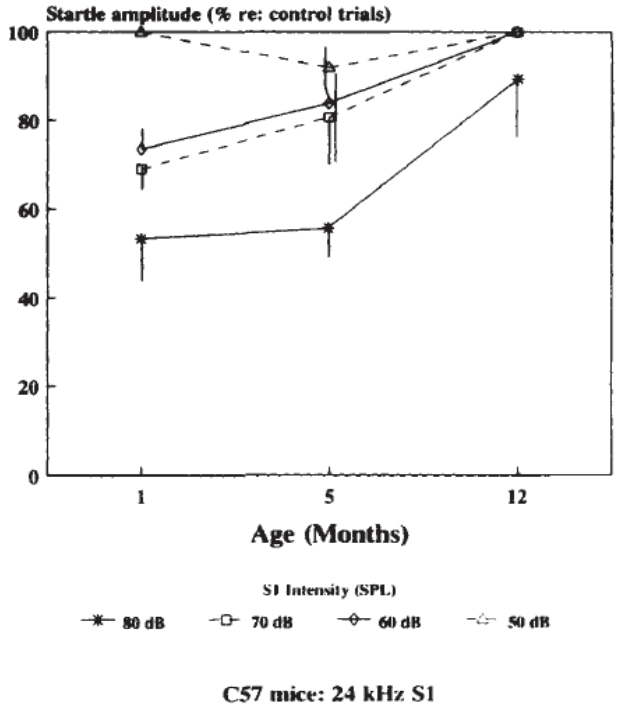

Figure 11. Mean acoustic startle response amplitudes for C57BL/6J mice as a function of age and prepulse intensity with a frequency of 24 kHz (Willott, Carlson & Chen, 1994).

Carlson & Willott (1996) measured PPI of ASR to assess the perceptual consequences of hearing-loss induced plasticity (HLI) in C57BL/6J mice. This study was based on the knowledge that C57BL/6J mice incur an age-related SNHL that causes physiological changes in the upper auditory brainstem and cortex. The occurrence of high frequency sensorineural hearing loss alters tonotopic organization of the inferior colliculus so that neurons in the ventral region respond better to audible mid frequencies. Mid-frequency portions of the IC frequency map expand as high-frequency portions are lost. 1- and 5-month old mice were used. A prepulse stimulus of 70 dB SPL tone pips (10 ms duration, 1 ms rise/fall) of 4, 8, 12, 16, and 24 kHz were presented. The startle stimulus consisted of 100 dB SPL tone pips (10 ms duration, 1 ms rise/fall) at frequencies of 4, 8, 12, 16, or 24 kHz.

Results from Carlson & Willott (1996) revealed that amount of inhibition increased for the 12 and 16 kHz prepulse stimuli in the mice with SNHL. Frequency of the startle did not significantly impact PPI. It was concluded that PPI is mediated centrally and likely involves auditory nuclei of the upper brainstem and /or forebrain. It was also concluded that the changes in

PPI result may be due (completely or partially) to hearing loss induced plasticity in the central auditory system. In assuming that the human auditory system is not fundamentally different than that of the mouse, it could be inferred that hearing loss induced plasticity would also occur in humans (Carlson & Willott, 1996).

Ouagazzal, Reiss & Romand (2006) looked at the consequences of age-related hearing loss on startle reflex and prepulse inhibition for C57BL/6J and hybrid mice. They analyzed mice at various life stages; however, we were most interested in the C57BL/6J mice that were tested before age effects had set in (6 weeks of age) so that the data could be compared to ours. A Plexiglass cylinder was used as the test chamber and a speaker located 28 cm above it produced all auditory stimuli. The startle stimulus consisted of a 40 ms, 120 dB SPL, white noise burst (0 ms rise-fall). The intensity of the prepulse randomly varied between 70, 80, 85, or 90 dB SPL; thus threshold was not obtained. The stimulus contained energy in the 4-14 kHz band with maximal sound pressure being at 10 kHz. The prepulse was an offset response presented 50 ms prior to the startle stimulus. Results showed that the amount of inhibition increased as intensity of the prepulse increased. Age effects were not significant until the mice reached 94 weeks of age (Ouagazzal, Reiss & Romand, 2006).

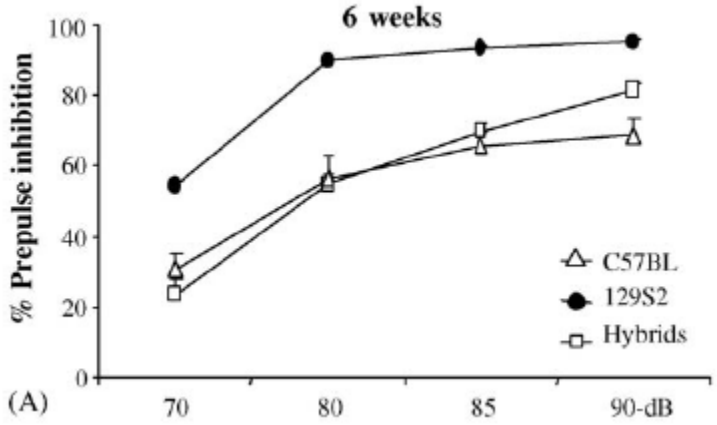

Figure 12. Percent of prepulse inhibition in three strains of mice at 6 weeks of age as a function of prepulse intensity (Ouagazzal, Reiss & Romand, 2006).

Hickox & Liberman (2014) sought to determine if noise-induced cochlear neuropathy results in abnormal auditory behavior in CBA/CaJ mice as seen through PPI of the ASR, for which they obtained a psychometric function. The mice with cochlear neuropathy exhibited enhanced ASR and PPI revealing a hyper-responsivity to sound. While control mice and mice exposed to noise without neuronal loss did not show this enhancement. They also used gap PPI tests, which have been used to assess tinnitus, to assess these same mice.

Results from gap PPI tests showed limited gap detection deficits in the mice with cochlear neuropathy, suggesting that tinnitus is not "filling in the gap" (Hickox & Liberman, 2014). For PPI tests, the startle stimulus consisted of broad band noise bursts at 105 dB SPL. The prepulse was either tone bursts (at 11.3 and 32 kHz) or broadband noise bursts for a duration of 50 ms. The prepulse terminated immediately before startle onset and was presented in both a quiet and noisy background. In a quiet background, results of control mice suggested that a prepulse can inhibit the startle response at levels as low as 25 dB SPL. Other studies, assessing cochlear nerve fiber and behavioral thresholds, obtained similar values of threshold, which suggests that PPI can be used to estimate the behavioral audiogram (Hickox & Liberman, 2014).

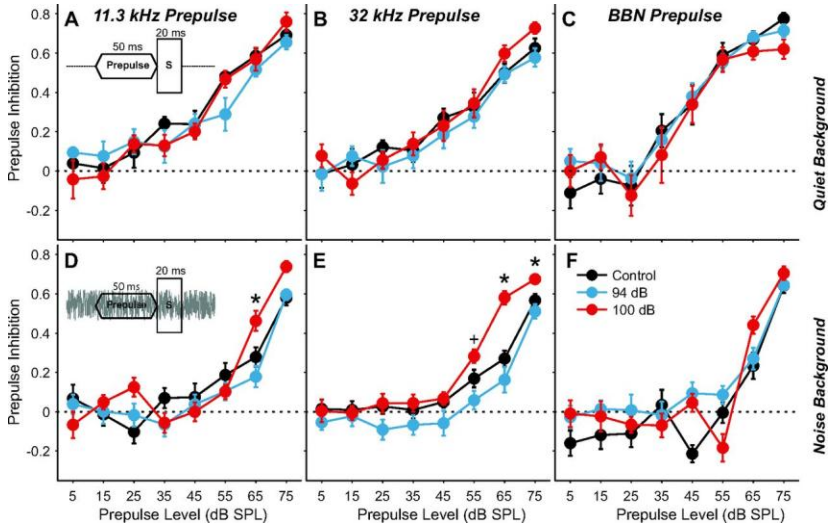

Figure 13. Prepulse inhibition of the acoustic startle reflex in three groups of CBA/CaJ mice: control (no noise exposure), 94 dB (exposure to 2 h of 94 dB SPL octave-band noise at 8-16 kHz) and 100 dB (exposure to 2 h of 100 dB SPL octave-band noise at 8-16 kHz) as a function of prepulse intensity and frequency (Hickox and Liberman, 2014).

General Statement of the Problem

Prepulse inhibition of the acoustic startle reflex has been used in a multitude of studies to date. Previous studies that used PPI of the ASR (several of which are discussed above) were reviewed to determine ideal testing protocols for this experiment. Until the 2014 report by Hickox & Liberman, there had been a lack of data in which PPI of the ASR has been used to obtain a complete psychometric function and therefore a prediction of threshold. At the outset of this experiment, we were unaware of any previous studies in which a complete psychometric function was obtained via PPI of the ASR using mice, and we are not aware of any psychometric function measured with C57BL/6J controls or any genetic mutation. Since the completion of our experiment, the study by Hickox and Liberman (2014) was published, in which a complete psychometric function was obtained for noise-exposed CBA/CaJ mice. The purpose of this study was to obtain a psychometric function and a prediction of auditory threshold in normal-hearing C57BL/6J and EphA4+/+$_{(wt)}$ mice by using prepulse inhibition of the acoustic startle reflex.

Materials & Methods

Subjects

A total of twelve mice (n=12) were used for this experiment. Six of the mice were the control group; which consisted of mice of the C57BL/6J strain. The C57BL/6J mice were obtained from a colony within the James Madison University (JMU) biology department. They were offspring of stock originally from Jackson Laboratory (Bar Harbor, ME). All of the C57BL/6J mice were born and reared on JMU's campus. The rest of the mice were homozygous offspring of mice that had a mutation of the ephrin gene and are therefore labeled as EphA4+/+$_{(WT)}$. The EphA4+/+$_{(WT)}$ mice were expected to be 'normal' except for any early rearing effects from their heterozygous parents. All test sessions took place when mice were between 52 and 77 days of age. The mean age at initial testing was 57 days but ranged from 52-61 days of age. The mean age at final testing was 70 days of age with a range of 66-77 days of age.

The C57BL/6J strain is known for having early onset hearing loss that begins as early as 60 days of age; however, our pilot data showed no significant effect on hearing ability as measured via PPI of the ASR in C57BL/6J mice under 75 days of age. Mice were identified by numbered ear tags which were put in place while the subject was under light anesthesia (3% isofluoroane) and were done at the time that tail samples were taken for genotyping. Each mouse was subjected to testing twice, two weeks apart. Test session duration was approximately 75 minutes and occurred during daylight hours. Presentation of the prepulse stimulus and the recording of the response were both computer-controlled. Mouse behavior during testing was not monitored by the examiner. Approval was obtained by the James Madison University Institutional Animal Care and Use Committee (IACUC) before testing began and the present study complied with the ethical standards set forth in the publication by the National Institutes of Health entitled "Guide for the Care and Use of Laboratory Animals".

Housing

All mice were group-housed in a BioZone MiniSmart Rack System located in the Health and Human Services building at James Madison University. The environment consisted of a controlled climate and a 12 hour light/dark cycle from 6:00 to 6:00 standard time. Food, water, and bedding were readily available at all times except during testing. Each cage was 6.25 inches X 10.5 inches in size and housed a maximum of 6 mice.

Genotyping procedures

The EphA4+/+$_{(WT)}$ colony of mice was established using breeding pairs obtained through the Mutant Mouse Regional Resource Center (MMRRC, NCRR-NIH). DNA was extracted for genotyping using tail samples with the Invitrogen Easy-DNA kit (Carlsbad, CA). The approximately 2 mm tail samples were taken when the mice were under light anesthesia (3% isofluorane). Each sample was digested while rocking on a heat block overnight at 60°C. Easy-DNA kit instructions were followed in the DNA extraction and precipitation processes. PCR amplification (94°C, 30 s; 31 cycles: 94°C, 30 s, 56°C, 30 s, 72°C, 2 min; one final elongation at

72°C, 10 min) was performed using the following primer sequences (Gabriele et al., 2010). "EphA4 primer (EphA4-forward 5' GTTTCCGCTCTGAGCTTATACTGC-3', EphA4-reverse 5' ACAGTGAGTGGACAAAGAGACAGG-3', lacZ 5'-CGCTCTTACCAAAGGGCAAACC-3' ….) were used for PCR amplification [20, 21]. Gel electrophoresis of PCR product resulted in EphA4 WT (639-bp) and/or mutant (800-bp) allele bands (Liuzzo, Gray, Wallace & Gabriele, 2014).

Apparatus

During testing, mice were confined to a 5cm x 12.5cm clear Plexiglas chamber constructed by San Diego Instruments (serial number: CAL 002927). The chamber consisted of seven holes to allow for sound penetration and had removable gates on each end for mouse entry/exit by the examiner. An accelerometer was attached to the bottom of the chamber to measure the amount of ASR response. The test chamber was placed within a 2.13 m x 2.13 m doubled walled, double floored sound attenuating booth manufactured by Industrial Acoustics Company, Inc., which was also lined with sound attenuating foam. The testing chamber was located 45 cm below a Ross Audio systems TW30 tweeter; which served to produce the startle-eliciting stimulus (SES). A second tweeter, this one a Tucker Davis Technology ES1, was located 15 cm to the side of the test chamber and served to produce the pre-pulse stimulus (PPS). The test chamber was cleaned after each test session with a solution of bleach water.

Stimuli

The startle-eliciting stimulus (SES) consisted of a 120 dB SPL broad-band noise for a duration of 15 ms. The SES was high pass filtered at 8 kHz and linear gated with a 10 microsecond rise/fall time. Acoustic calibration showed energy from 8-35 kHz (10 dB down points on the spectrum and energy above 90 dB above 40 kHz). The SES noise was generated using a Tucker Davis Technology RP2 Real-Time Processor, amplified by a Crown XLS202 amplifier, and controlled through Matlab software. The SES from RP2 was fed into a Crown

XLS202 amplifier and then delivered through a Ross Audio systems TW30 compression tweeter placed 45 cm directly above the mouse cage.

The prepulse stimulus (PPS) was presented through a Tucker Davis Technology ES1 tweeter, which was located 15 cm to the side of the test chamber. The PPS was an offset of the continuous background noise and randomly varied between thirteen different intensities of 0, 25, 29.2, 33.3, 37.5, 41.7, 45.8, 50, 54.2, 58.3, 62.5, 66.7, 70.8, 75.0 dB SPL. The 0 dB SPL intensity was presented randomly two times per test block and served as the control. The continuous background noise randomly varied by frequency filter: high-pass (HP) or band-pass (BP). The HP PPS was high-pass filtered at 4 kHz and had a bandwidth of approximately 45 kHz (as defined as a spectrum level above 50 dB SPL) and the BP PPS was a third-octave band centered at 12 kHz with a bandwidth of 9.5 to 15.1 KHz. The prepulse cue was the offset of the 0 to 75 dB SPL either high-pass or band-pass noise, which always occurred 50 ms before the SES.

Protocol

A 3 minute pre-stimulus acclimatization period, in which the test chamber was silent, was provided to each subject at the beginning of each test session. The force of the acoustic startle reflex was measured using an accelerometer that was attached to the bottom of the testing chamber. The accelerometer converted animal movements into voltage signals using piezoelectric transducers. Output was converted from analog to digital signals using the Real-time Processor RP2.1 (TDT). Mouse movement was sampled at a rate of 100 kHz from the electrical onset of the startling stimulus for the next 100 ms. The response signal was amplified 100 times and low-pass filtered at 1 kHz by a Krohn-Hite model 3343 filter and input to the Real-time processor. The RMS voltage in the 100 ms following the startle was the dependent variable measured on each trial. Trials were presented in 11 blocks of 16 trials, totaling 176 trials. Each block contained one each of the 13 ‘real’ trials (prepulses between 25 and 75 dB SPL) as well as two trials with no prepulse to determine the ‘control’ or undiminished startle response (termed ASRc), and one trial

with no sound at all (a measure of baseline activity). The order of these 16 trials was re-randomized within each of the 11 blocks of trials. The inter-trial interval (ITI) was 15 to 25 seconds, randomly determined. The entire test took a little over an hour.

PPI scores were calculated as a ratio of mean startle response amplitude following the prepulse stimulus (ASRp) versus the baseline measure of startle response amplitude in the control condition (ASRc), where the startle eliciting stimulus was present without the prepulse stimulus. The formula used, taken from Allen & Ison (2010), was: PPI = 1 – [ASRp/ASRc]. A value of 0 indicates that the prepulse had no effect on the startle response and it can be inferred that the prepulse stimulus was not heard. A value of 1 demonstrates full inhibition of the startle response while a negative value indicates prepulse facilitation.

Calibration

Calibrations were performed prior to beginning the experiment, mid-way through, and at the end; to ensure speaker and tweeter outputs were of expected values. A custom program was written that presented each of the three critical stimuli; the SES, and the HP and BP PPS's for long enough for a spectrum to be recorded. The Schmitt trigger (timed on/off switch) was adjusted so that the stimulus being measured would be presented for 2500 ms (2.5 s). A condenser microphone was placed through the entry of the testing chamber and held in place with a rubber stopper. The Agilent Spectrum Analyzer performed a Fourier analysis. Output was measured at the loudest levels and the trace was saved on the signal analyzer.

Hypothesis

We expected that the amount of prepulse inhibition would decrease as the intensity of the prepulse stimulus decreased. A wide range of stimulus intensities were tested with the goal of reaching threshold, as exhibited by a PPI of 0 and thereby providing a psychometric function. Since the EphA4+/+$_{(WT)}$ mice are homozygous ($^{+/+}$) offspring of mice that had a mutation of the ephrin (EphA4) gene they are considered 'wild-type' offspring and are expected to display PPI of ASR responses comparable to the C57BL/6J mice. Two frequency filters (high-pass and band-

pass) were used with the prepulse stimulus in an attempt to assess two different frequency regions. The high-pass prepulse contains energy of higher frequency. A stronger response (i.e. more inhibition) is expected with the high-pass filter due to mice having the ability to better hear high frequency sounds.

Results

Baseline Activity

Baseline activity was obtained from accelerometer readings of mouse movement when there was no prepulse or startle stimulus present. Table 2 shows the average baseline activity obtained for each genotype. These measurements were used for a two-way analysis of variance (ANOVA) with a between-subjects design to compare average baseline activity and determine if there were significant differences in baseline activity between genotypes (EphA4+/+$_{(WT)}$ and C57BL/6J) and/or frequency filters (high-pass and band-pass). Results revealed that the baseline activity of the C57BL/6J mice was significantly higher compared to EphA4+/+$_{(WT)}$ mice (see Table 3).

Table 2. Average baseline activity (movement calculated in complete silence with neither the prepulse nor the startle stimuli) for each genotype (EphA4+/+$_{(WT)}$; C57BL/6J).

Dependent Variable: Baseline

Grp	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
EA4	.190	.025	.137	.243
GWT	.276	.029	.216	.336

Table 3. A two-way ANOVA with between-subjects design revealed that baseline activity was significantly different between the two groups of mice. The C57BL/6J mice had significantly higher baseline movement as compared to EphA4+/+$_{(WT)}$ mice.

Tests of Between-Subjects Effects

Dependent Variable: Baseline

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	.050[a]	3	.017	2.049	.141	.244
Intercept	1.225	1	1.225	149.547	.000	.887
Grp	.042	1	.042	5.075	.036	.211
Filter	.006	1	.006	.759	.394	.038
Grp * Filter	.003	1	.003	.372	.549	.019
Error	.156	19	.008			
Total	1.394	23				
Corrected Total	.206	22				

a. R Squared = .244 (Adjusted R Squared = .125)

Acoustic Startle Reflex

Response to the startle stimulus alone (no prepulse) was measured for each mouse and within each test session and labeled as ASR_C. The mean ASR_C value for all test subjects combined was 0.80; whereas the value was 0.83 for the EphA4+/+$_{(WT)}$ mice (standard deviation = 0.33) and 0.76 for the C57BL/6J mice (standard deviation = 0.48). A two-way analysis of variance (ANOVA) with a between-subjects design was used to compare average ASR_C and determine if there were significant differences between genotypes (EphA4+/+$_{(WT)}$ and C57BL/6J) and/or frequency filters (high-pass and band-pass). Results revealed no effect of genotype or frequency filter on ASR_C. This indicates that the magnitude of the startle response to the startle stimulus alone, with no prepulse stimulus present, was not significantly different between the mouse genotypes or with use of the different prepulse frequency filters.

Table 4. Average ASR_C (where startle stimulus was presented without the prepulse) for each genotype (EphA4+/+$_{(WT)}$; C57BL/6J), frequency filter (high-pass; band-pass) and test session (1;2).

Tests of Between-Subjects Effects

Dependent Variable: ASRcontrol

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	.185[a]	3	.062	.364	.779	.054
Intercept	14.492	1	14.492	85.726	.000	.819
Grp	.034	1	.034	.200	.660	.010
Filter	.146	1	.146	.864	.364	.044
Grp * Filter	.003	1	.003	.017	.897	.001
Error	3.212	19	.169			
Total	18.217	23				
Corrected Total	3.397	22				

a. R Squared = .054 (Adjusted R Squared = -.095)

Prepulse Inhibition (co-*written by L.Gray and R. Browne*)

A repeated measures ANOVA was performed to evaluate the effects of PPS intensity (dB SPL in 13 levels), genotype (C57BL/6J or EphA4+/+$_{(WT)}$) and frequency filter (high-pass or band-pass) on amount of PPI. P values less than .050 were considered significant. Intensity (dB SPL)

was the single, within-subjects factor; while both genotype and frequency filter were between-subjects factors. Figures 14 and 15 show the growth of PPI of ASR as a function of intensity with use of the band-pass and high-pass filters, respectively. While there was a strong interaction between PPS intensity and type of frequency filter (which is discussed more in depth below), there was no significant effect of genotype (p=.089) nor an interaction involving genotype (p=.843 for genotype*filter; p=.684 for dB*genotype, and p=.916 for the dB*genotype*filter). Since there were no significant differences between the C57BL/6J and EphA4+/+$_{(WT)}$ mice, the data from the two groups were pooled.

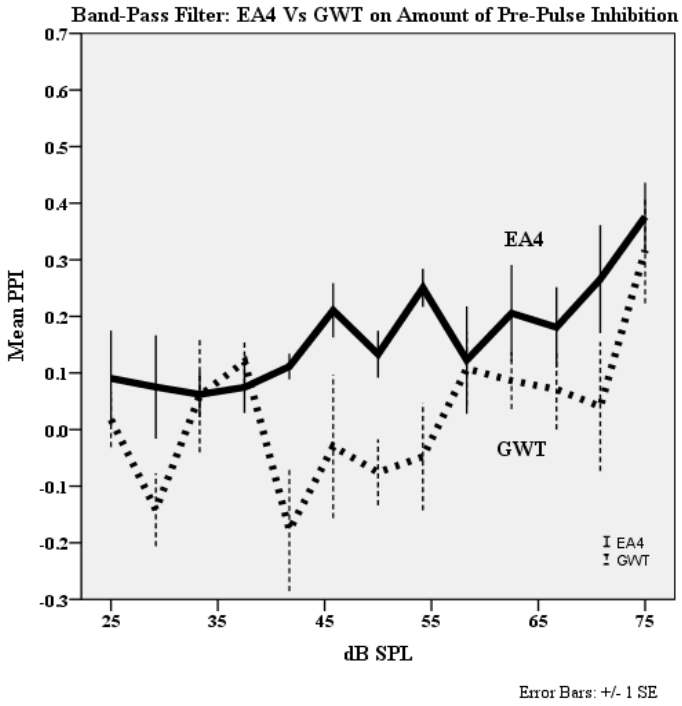

Figure 14. Growth of prepulse inhibition of the acoustic startle reflex as a function of intensity level for C57BL/6J and EphA4+/+$_{(WT)}$ mice (labeled as GWT and EA4, respectively) when a band-pass filter was used. There was no significant difference between the groups.

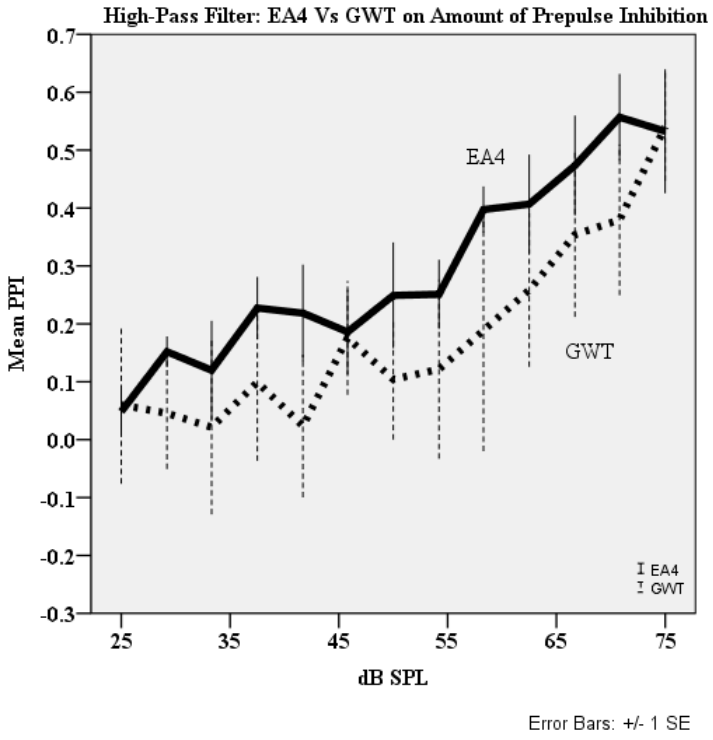

Figure 15. Growth of prepulse inhibition of the acoustic startle reflex as a function of intensity level for C57BL/6J and EphA4+/+$_{(WT)}$ mice (labeled as GWT and EA4, respectively) with use of the high-pass filter. The difference between the genotypes was not significant.

A second repeated-measures ANOVA was employed to further analyze the interaction between intensity and frequency filter. This analysis used intensity (dB SPL) as the within-subjects variable and frequency filter as the between-subjects factor. Results revealed a strong interaction between intensity and frequency filter ($F_{1,21}$ =8.9; p=.007; $_p\eta^2$=.299) with significant main effects of intensity ($F_{1,21}$=72.9; p<.001; $_p\eta^2$=.78) and frequency filter ($F_{1,21}$=4.86; p=.039; $_p\eta^2$=.188). All of these effects are considered 'large' (Cohen, 1988). The significant effect by frequency filter revealed that more pre-pulse inhibition was obtained with the high-pass filter versus the band-pass filter, as can be seen in Figure 16.

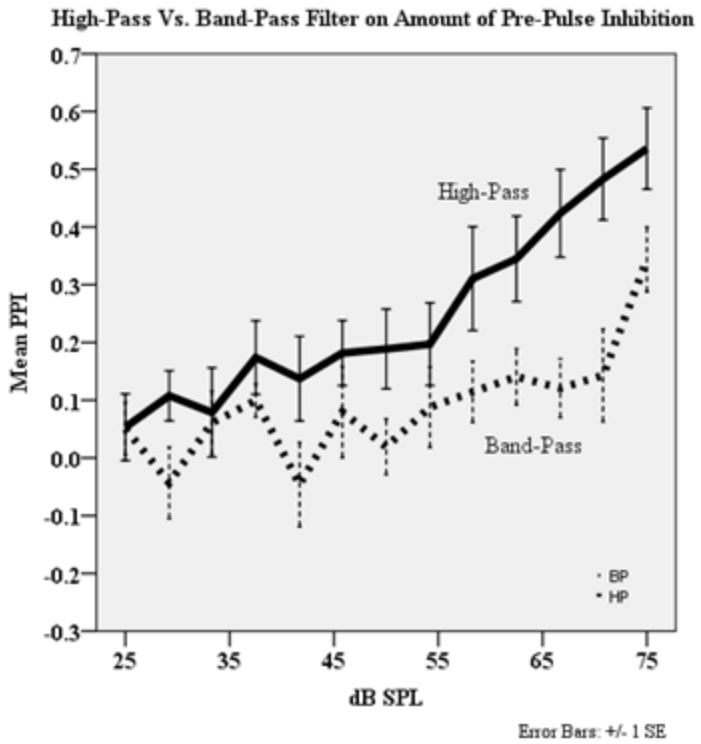

Figure 16. Growth of prepulse inhibition of the acoustic startle reflex as a function of intensity level for the high-pass and band-pass filters. More inhibition was obtained with the high-pass prepulse stimulus.

Defining Threshold *(co-written by L. Gray and R. Browne)*

Challenges arise when attempting to define threshold from these data. We are aware of only one other study that used PPI of ASR to obtain a complete psychometric function in which threshold levels were determined. That study, done by Hickox & Liberman (2014), claimed to have obtained a threshold as low as 25 dB SPL for prepulse tone bursts at 11.3 kHz and 32 kHz. However, they failed to describe the method in which they obtained this value. Looking at their data (See Figure 13, black line within the A and B insets), it appears that threshold was defined as the lowest level at which a PPI of greater than zero was obtained while the function continued to steadily increase as the subsequent higher levels were presented. Since a PPI of 0 represents no change from ASR_C and indicates that the prepulse was not audible, their decision to find threshold in this manner seems logical albeit subjective, which is not always a desired characteristic in

conducting research. When using this same method in the present study, a comparable threshold is obtained because 25 dB SPL produced a PPI that is just above 0 (as can be easily visualized from Figure 16). However, identifying where the curve starts rising beyond 0 is a subjective measure.

It could be argued that threshold should be defined by using a more objective measure. One such method may involve defining threshold as the x-value when y=0. Using this calculation, average threshold for our data is 19 dB SPL. On the other hand, a one-sample t-test could be employed to find the lowest intensity that shows a significant difference in PPI as compared to the PPI obtained for the 0 dB SPL control PPS. Using this calculation, the band-pass stimulus produced a threshold at 58 dB SPL and the high-pass stimulus threshold was at 38 dB SPL. These significances can be seen in Columns 2 and 3 of Table 5. Twenty six different t-tests were completed; one for each of the 13 intensity levels and for each frequency filter (band-pass and high-pass). A Bonferroni correction would dictate that only p-values below .002 (.05/26) would be considered significant. A one-tailed t-test could also be considered if there is no expectation of a negative PPI and would lead to estimations of thresholds of 75 dB SPL for the band-pass stimulus and 63 dB SPL for the high-pass stimuli.

Alternatively, planned contrasts after the repeated measures ANOVA (described above in the results section) could be used. This method would compare the response at each of several intensities to one predetermined level. This is described by 'SPSS Help' as follows: "Simple [contrast] compares the mean of each level to the mean of a specified level. This type of contrast is useful when there is a control group. You can choose the first or last category as the reference." In our case, 25 dB SPL (the lowest intensity prepulse that was presented) could be used as the control group. Columns 5 and 6 of Table 5 reveal the resulting significances from this planned contrast; in which the threshold for the band-pass stimulus is 75 dB SPL and threshold for the high-pass stimulus is 46 dB SPL. However, one should consider that this contrast may be influenced by naturally occurring variability in PPI in response to the 25 dB SPL stimulus.

Yet another attempt was made to estimate threshold using the planned simple contrast in the repeated-measures ANOVA, except this time the lowest three intensity levels (25 dB SPL, 29.2 dB SPL and 33.3 dB SPL) were combined to form the control group. Responsiveness at each of the ten louder levels (37.5 to 75.0 dB SPL) was compared to responsiveness at the three lowest levels. The significance levels of these contrasts are shown in the columns 7 and 8 of Table 5. Threshold is now estimated to be 62.6 dB SPL for the band-pass stimulus and 45.8 for the high-pass stimulus. Using this method, there are more comparisons that are significant because there is more data and; therefore, less variance in this control group.

Sample sizes, of course, can have an effect on statistical significance. For example, lower intensity levels (conceivably at 25 dB SPL or even lower) may become significantly different than zero with an infinitely large sample size. In comparison, effect sizes measure the magnitude of the difference from the control group. SPSS reports partial eta-squared ($_p\eta^2$) as the effect size in ANOVAs. These effect sizes (for the simple comparisons using the pooled three lower intensity levels as the control group) are seen in the final two columns of Table 5 and are graphed in Figure 17. Figure 17 reveals a psychometric function of the data from the stimuli groups (band-pass and high-pass) in which there is a rise from low to high effect sizes in S-shaped functions. The red and green lines in the figure show subjectively determined logistic fits to the data. The same slope, with an upper asymptote of .83 and lower asymptote of 0, was used for both sets of data. As a general rule of thumb; .01, .06 and .14 represent small, medium and large values of $_p\eta^2$, respectively. The level of a 'large effect' (0.14) is shown by the short horizontal blue line in Figure 17. The point at which this line crosses the two ogives provides an estimation of the intensity that would create this effect size. This final threshold estimation attempt, leads to average threshold values of 40 dB SPL and 50 dB SPL for high- and band-pass stimuli, respectively. Levels that elicit 'large effects' are highlighted in yellow in the last two columns of Table 5.

Table 5. Various attempts to objectively estimate threshold from Browne & Gray (2015) PPI of ASR data.

	Filter	BP	HP	BP	HP	BP	HP	BP	HP
	Stat	Two-tailed p values						Effect Size, $_p\eta^2$	
Level	dB	t-test	t-test	Simple contrast in Repeated Measures ANOVA					
1	25	.291	.378	Std	Std	Std	Std	Std	Std
2	29.2	.501	.030	.168	.266				
3	33.3	.295	.329	.823	.660				
4	37.5	.006	.020	.439	.059	.184	.105	.169	.221
5	41.7	.542	.080	.160	.158	.218	.382	.147	.070
6	45.8	.338	.008	.691	.015	.348	.075	.088	.261
7	50	.697	.020	.378	.067	.895	.082	.002	.250
8	54.2	.237	.019	.563	.009	.255	.017	.127	.420
9	58.3	.056	.005	.310	.015	.164	.025	.184	.377
10	62.5	.016	.001	.072	.001	.018	.001	.445	.641
11	66.7	.039	.000	.205	.000	.078	.000	.278	.734
12	70.8	.105	.000	.251	.000	.159	.000	.188	.847
13	75.0	.000	.000	.000	.000	.000	.000	.808	.831

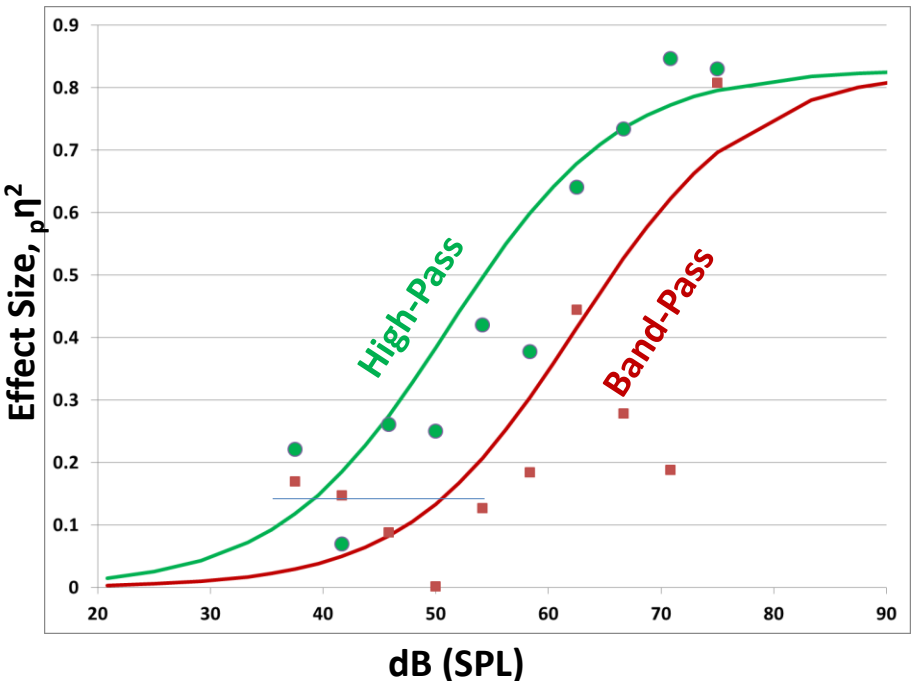

Figure 17. Effect size of the high-pass and band-pass stimuli graphed from data pulled from the final two columns of Table 5; producing a psychometric function with a rise from low to high effect sizes in S-shaped functions. The green and red lines in the figure show subjectively determined logistic fits to the data. The level of a 'large effect' (0.14) is shown by the short horizontal blue line. The point at which this line crosses the two ogives provides an estimation of the intensity that would create this effect size.

Comparative Analysis

Data from the present study was graphed against other studies (reviewed above) that have determined PPI of the ASR at various intensity levels in mice. The studies by Willott, Carlson & Chen (1994) and Ouagazzal, Reiss & Romand (2006) did not seek to define threshold but they did use similar test parameters. We were interested in determining whether or not our PPI results were comparable to other studies so that we could validate our findings. The study by Hickox & Liberman (2014) did include a psychometric function from which a threshold was estimated. To our knowledge, this is the only other study in which this has been done. Therefore, it is important to determine the best methods for estimating threshold with this procedure and whether or not they are creating an accurate estimate when compared with thresholds that have been obtained via many of the traditional behavioral testing paradigms.

Figure 18 provides a comparison between our data and that of Willott, Carlson & Chen (1994). In order to obtain the most accurate comparison, we calculated mean PPI from predicted values of mice aged 2.5 months for both the 12 kHz and 24 kHz prepulse stimuli combined. Data from the present study included mean PPI obtained from the high-pass and band-pass stimuli combined. As can be seen, the two sets of data coincide well.

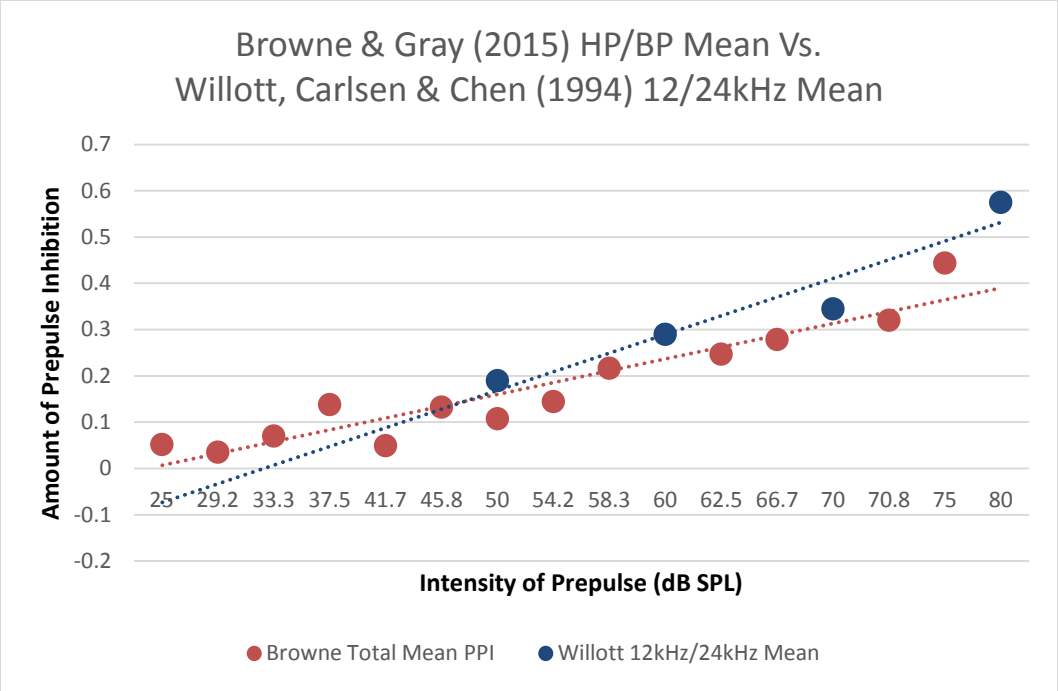

Figure 18. Graph comparing data obtained from Willott, Carlson & Chen (1994) to the present study. The data from Willott, Carlson & Chen are the mean PPI obtained from calculating the predicted values of mice aged 2.5 months for the 12 and 24 kHz prepulses combined. The data pulled from the present study involve the mean PPI obtained from an average of both the high pass and band pass prepulses.

In Figure 19, the mean PPI of the present study is compared to data taken from Ouagazzal, Reiss & Romand (2006). The Ouagazzal, Reiss & Romand (2006) study used several strains of mice up to the age of 94 weeks to analyze effects of age-related hearing loss. Of most interest was the comparison of data from the present study to the C57BL/6J mice before age-related hearing loss effects had set in; therefore, a small subset of the Ouagazzal, Reiss & Romand (2006) data was used to include only that strain of mouse at 6 weeks of age. Ouagazzal, Reiss & Romand (2006) used the offset condition where the prepulse was presented 50 ms prior to a startle stimulus of 120 dB SPL; which is very similar to the methods employed by the present study. It can be subjectively determined by looking at Figure 19 that data from the present study match up quite nicely with the Ouagazzal, Reiss & Romand (2006) study as well.

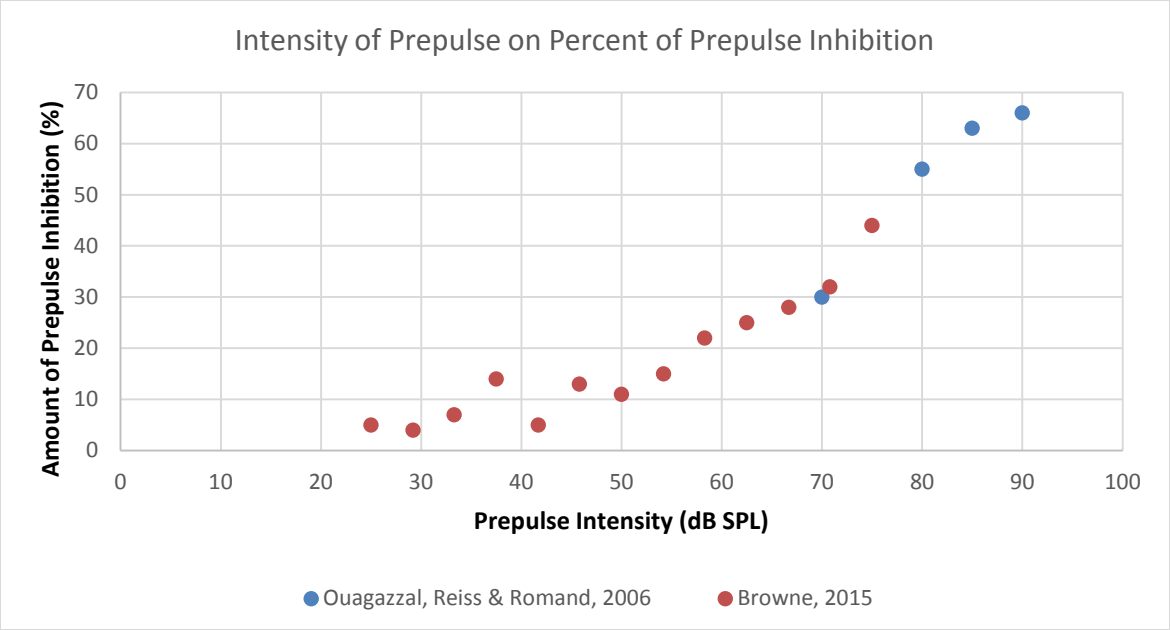

Figure 19. Graph comparing the data obtained by Ouagazzal, Reiss & Romand (2006) to the total mean (high pass and band pass combined) PPI obtained in the present study. The Ouagazzal, Reiss & Romand (2006) data shown is a subset of the actual data to include only C57BL/6J mice at 6 weeks of age. The prepulse stimulus was an offset presented 50 ms prior to a startle stimulus of 120 dB SPL.

Figure 20 plots the high-pass frequency filter data from the present study against data from Hickox & Liberman (2014) and Willott, Carlsen & Chen (1994). The data from Hickox & Liberman contains results obtained from 11.3 kHz and 32 kHz tone burst prepulses, whereas data from Willott, Carlson & Chen results from a 12 kHz tone pip prepulse stimulus. Hickox & Liberman (2014) obtained the most prepulse inhibition with their tone burst stimulus at 11.3 kHz. However, the results from all of these studies are comparable. Put side to side with Hickox & Liberman, data from the present study appears to provide an equivalent threshold estimate and, in following the trend line, it seems as if the data from Willott, Carlson & Chen (1994) would be capable of doing this as well. Figure 20 provides an encouraging outlook on the possibility of using PPI of ASR as a behavioral method for estimating threshold in mice.

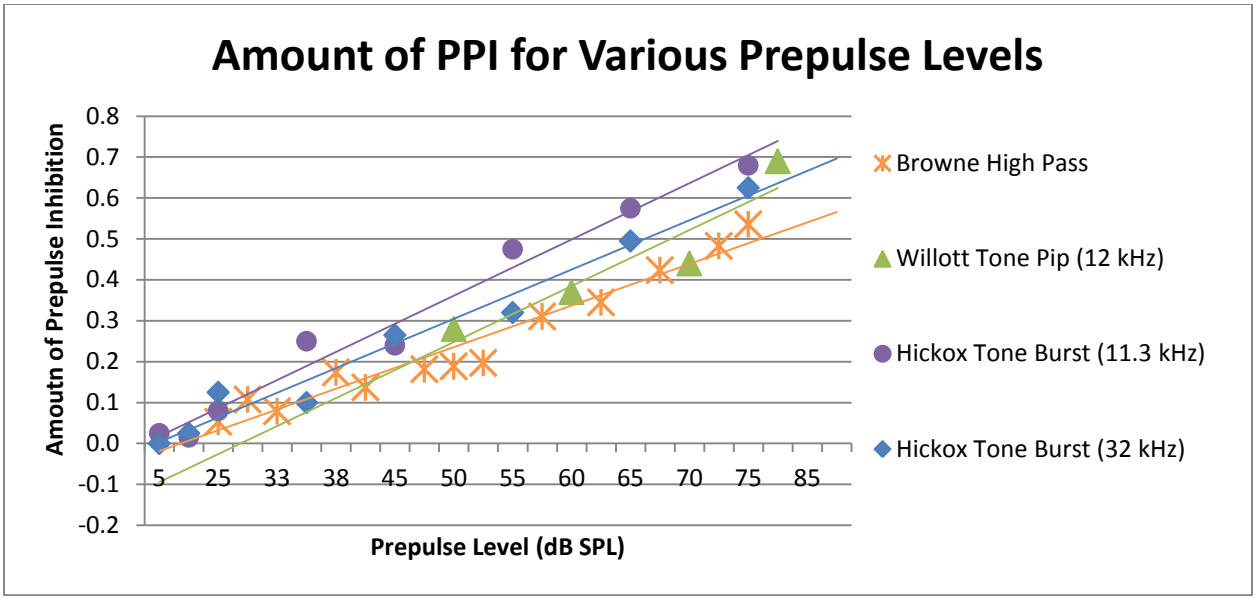

Figure 20. A comparison of data from Hickox & Liberman (2014), Willott, Carlsen & Chen (1994) and the HP results from the present study; revealing that PPI of ASR could potentially be used as an accurate way of behaviorally measuring audiometric thresholds in mice.

Conclusions

A psychometric function was successfully obtained in the present study using PPI of the ASR as a behavioral test of hearing. Amount of PPI increased as the intensity of the PPS increased. A significant effect (P=0.04) was seen by frequency filter wherein the high-pass filter created more PPI than the band-pass filter. No significant difference was found between C57BL/6J and EphA4+/+$_{(WT)}$ mice (P=0.15). The PPI results obtained with use of the high-pass filter provided comparable results to other related studies. Various methods were used to find threshold and the results varied from estimates as low as 19 dB SPL to as high as 75 dB SPL. When plotted against data from other studies that used PPI of ASR, the present study produced reasonably comparable results.

Discussion

Findings from the present study as well as those from Willott, Carlson & Chen (1994) and Hickox & Liberman (2014) provide evidence that auditory sensitivity can be reliably estimated behaviorally in mice using prepulse inhibition of the acoustic startle reflex. However,

more studies will need to be done in order to determine the best methods for obtaining these results. PPI of the ASR provides a simple, efficient and accurate measure of hearing in mice. The method relies on a reflexive response that does not require conditioning or training and it can be completed in a short amount of time (approximately one hour). Mice can be tested multiple times before habituation takes place. Although there have been very few studies that have analyzed the possibility of using PPI of ASR to obtain auditory thresholds comparable to those obtained using behavioral methods with conditioning procedures, the results obtained in the present study and in that of Hickox & Liberman (2014) provide a promising outlook. However, a clear and objective method for defining threshold from the psychometric function has yet to be established.

Suggestions for Future Research

Future studies should expand PPS intensities to increments of 5 from 0 dB SPL to 80 dB SPL. At least one of the methods used to define threshold, indicate that threshold may not have been reached in the present study. In addition, a PPI of 0 was not reached using the high-pass filter and a PPI of 1 was not reached with either of the PPS frequency filters. Using a more comprehensive range of 0-80 dB SPL would provide an even more complete psychometric function.

If using frequency filters, it is recommended that the high-pass filter be used with a higher cut-off frequency. However, it would be interesting to see a study in which tone bursts at various frequencies are employed to create a mouse audiogram. After the best methods for estimating threshold are determined, perhaps PPI of ASR could be used to obtain behavioral audiograms of multiple strains of mice with various genetic mutations. Finally, it would also be interesting to further evaluate the prepulse facilitation (PPI values of less than zero) that was discovered to occur in the C57BL/6J strain of mice.

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