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Prepulse Inhibition and the Acoustic Startle Response in Nine Inbred Mouse Strains

By

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A Professional Research Project submitted to the Faculty of the Department of Communication Sciences and Disorders University of South Florida in partial fulfillment of the requirements for the degree of Doctor of Audiology

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(ABSTRACT)

This study examined the effects of genetic background on the acoustic startle response (ASR) and its modulation by prepulse inhibition (PPI) by comparing nine inbred strains of mice. The ASR, a jerk-like motor reflex, is elicited by bursts of noise or tones with sound pressure levels of 80-90 dB and greater. PPI is a type of modulation of the ASR, requires no training, and results in observable response in both mice and humans.

Data were obtained from nine inbred mouse strains, sixteen per strain, which were shipped at approximately 3-5 weeks old from The Jackson Laboratory. In general, ASRs were generally smaller when the startle stimulus was less intense. PPI was relatively weak for the 4 kHz prepulse, and stronger with prepulses of 12 kHz and 20 kHz. However, means varied widely across strains for both ASR and PPI, suggesting a strong influence of genetic background on these behaviors. In addition to genetic influences, peripheral hearing loss and central auditory processing factors must be taken into consideration.
Introduction

The genetics of hearing, central auditory processing, and hearing loss are poorly understood. One problem has been the difficulty in studying genetics in humans because it is difficult to separate the effects of genes from numerous possible other factors. For example, irrespective of genes that might cause hearing loss, people are typically exposed to multiple other factors that may affect hearing such as noise exposure, disease, drug therapy, and trauma.

The present study used highly inbred strains of mice to study auditory behavior. Because members of inbred strains are as genetically homozygous as monozygotic twins, differences among individuals of an inbred strain are due to non-genetic variables. On the other hand, if environmental variance is minimized, reliable differences between strains should largely represent differences in genetic makeup of those strains. Animal models show us what kinds of anatomic and physiologic phenomena can and do occur in mammalian auditory systems that are, in most respects, similar to our own (Willott, 1996). Mice provide good models with which to study genetic types of hearing loss as they are genetically well defined and there is a large literature on many aspects of mouse hearing (Willott, 2001).

Behavioral responses to auditory stimuli can be studied in mice using the acoustic startle response (ASR) and prepulse inhibition (PPI). Both are fundamental human/murine auditory behaviors, occurring widely in mammals and other vertebrates (Davis, 1984; Ison, 2001). While the focus of the present project is to examine ASR and PPI in mouse models, these behaviors have been well studied with human subjects (Dawson, Schell, & Bohmelt, 1999; Reiter & Ison, 1979). The ASR, a jerk-like motor
reflex, is elicited by bursts of noise or tones with sound pressure levels of 80-90 dB SPL and greater in the mouse. At the onset of the noise or tone burst, mice will startle, or jump, in response to the stimulus. The reflex is easily measured using movement-sensitive devices, and ASR amplitude can be obtained (Willott, 2001). The mouse’s movement produces a spike-like voltage change in the startle-measuring device. Startle amplitude is defined according to the largest peak-to-peak voltage deflection. Since the ASR occurs only to high intensity sounds, it cannot be used to determine thresholds of auditory sensitivity.

PPI is a type of modulation of the ASR. PPI requires no training and results in observable responses in both mice and humans. A nonstartling stimulus (S1) is presented 10-100 ms before an intense stimulus (S2) used to elicit the acoustic startle response. S1 may be a tone burst, a gap in the background noise, or any other sensory change. S1 activates the PPI circuit that inhibits the startle pathway for a period of several hundred seconds resulting in reduced, or inhibited ASR amplitude. PPI can be expressed by a ratio of ASR amplitude with presentation of a prepulse (S1-S2) to ASR amplitude without the prepulse (S2 only). The degree to which a tone produces PPI indicates the behavioral salience of the tone, providing a test of auditory behavior (e.g. Hoffman & Ison, 1980; Ison, 2001; Willott & Turner, 2000).

Anatomical pathways mediating the ASR and PPI are well established. These pathways are among the best understood of any vertebrate behaviors. The primary neural pathway for the ASR resides in the lower brainstem, with auditory neurons in the cochlear nucleus projecting to startle-triggering neurons of the reticular formation (Davis et al., 1982; Huffman & Henson, 1990; Kandler & Herbert, 1991; Koch, 1999; Leitner &
Cohen, 1985); Lingenhohl & Friauf, 1994; Yeomans & Frankland, 1996). In PPI, the S1 is first processed by the central auditory system (minimally to the midbrain level). Neural output from the auditory system then activates other “post-auditory” components of the PPI neural circuitry, including pathways that ultimately descend to the reticular formation and inhibit the neurons that trigger the startle reflex (Carlson & Willott, 1998; Davis, 1984; Koch, 1999; Ison, 2001; Hoffman & Ison, 1980; Li, Priebe, & Yeomans, 1998; Willott, Carlson, & Chen, 1994). The neural circuits provide numerous potential targets for gene actions that can contribute to behavioral differences among individuals.

The overall goal of this project was to examine the effects of genetic background on the ASR and its modulation by PPI by comparing nine inbred strains of mice. To do so, ASR amplitude was measured in response to a “standard” 100 dB SPL broadband noise startle stimulus, intensity functions were obtained using 90, 80, and 70 dB SPL startle stimuli, and PPI was evaluated using a non-startling stimulus of 70 dB SPL at frequencies of 4, 12, and 20 kHz. Test reliability was also evaluated by immediately re-testing all mouse strains.

Methods

Inbred mice were obtained from The Jackson Laboratory (TJL), Bar Harbor, ME. Mice were tested from the following strains: C57BL/6J, C3H/HeJ, BALB/cJ, A/J, 129/SV1mJ, CBA/J, FVB/N3, CAST/Ei, and DBA/2J. DBA/2J mice show rapid inner ear degeneration, which becomes evident as early as two to three weeks after birth; the other strains have normal hearing at the ages used here. Mice were shipped from TJL to the University of South Florida (USF), where they were housed in a vivarium within the USF medical facility. Sixteen mice per strain were shipped approximately once a month.
from TJL to USF for testing. Eight male and eight female mice, age ranged from 3-5
weeks old, were shipped each time. Testing was conducted when mice were
approximately 4-5 weeks old, after a 48-hour acclimatization period. Mice were housed
in cages with four same-sex members in each cage and were given free access to food
and water. Room temperature and humidity were optimally maintained under a 12-hour
light/dark schedule. This study complied with the National Institute of Health (NIH)
guidelines for the care and use of animals and was approved by the Institutional Animal
Care and Use Committees of the University of South Florida.

Apparatus

Disposable plastic cups (32 oz) were used to hold the mice during testing. The
cup was placed in a Med Associates, Inc. (Georgia, Vermont) sound attenuated test
chamber, where it rested on a movement-sensitive load cell. This load cell was sensitive
to any movements the mouse made, and, in turn, produced a voltage.

Acoustic stimuli were generated by a Radio Shack Supertweeter that fit snugly
above the rim of the plastic cup, inside the sound attenuated test chamber. The system
was initially calibrated using a 1/8 inch Brue & Kjaer condenser microphone placed
through a hole in a plastic cup, where SPL values were measured in various locations.
Mean attenuation values producing the highest sound pressure levels at each frequency
were determined and set. The standard startle stimulus (S2) consisted of a 100 dB SPL
(sound pressure level re: 20 µPa ) broad-band noise (10 msec duration, “0 msec” rise/fall
time based on dial setting). To generate amplitude-intensity functions, S2 stimuli were
also presented at 90, 80, and 70 dB SPL. Prepulse stimuli (S1) consisted of 70 dB SPL
tones (3 msec rise/fall, 10 msec duration) at frequencies of 4, 12, and 20k Hz. The S1-S2
interval was 100 msec. Testing was conducted in a quiet research laboratory, with no continuous background masking noise.

**Procedure**

Testing began after the mouse had been in the plastic cup for at least one minute. The first 40 trials of each test were used to establish baseline startle amplitude in response to the 100 dB SPL S2 and to measure PPI for the three S1 tones. For PPI trials, the S1 tone prepulse preceded the S2 startle stimulus. Stimuli were presented at a variable interval every 3-8 seconds and consisted of sixteen S2-only and eight S1-S2 pairings for each of the three S1 frequencies. Each ten trials contained an equal number of each stimulus in a variable sequence.

After these 40 trials, an additional 15 unmodified startle responses (S2-only) were obtained using stimuli of 90, 80, and 70 dB SPL to determined startle intensity function. Each test procedure required approximately six minutes, and included a total of 55 trials. As an indication of reliability, each mouse was immediately retested. Upon test completion, mice were returned to their original cages in the vivarium.

The ASR appears as an abrupt, spike-like voltage change, with a peak occurring 20-30 msec after the onset of the stimulus. ASR amplitude is defined by the peak voltage deflection. For each session, a mean startle amplitude was computed in response to the S2 alone and in response to S1-S2 pairings (S1 = 4, 12, and 20 kHz). The degree of PPI for each frequency prepulse is expressed as a ratio of the amplitude of the modified startle response (S1-S2) with respect to the amplitude produced by the S2 alone. A lower percent or proportional value is indicative of greater PPI.

**Data Analysis**
The data used for analysis in each session were modified by eliminating the highest and lowest ASR amplitudes in each frequency series (4, 12, 20 kHz) and the highest and lowest two values for startle only. This was done due to the occasional occurrence of very large or small ASRs that might distort the mean. Preliminary analyses were performed for test-retest reliability for each strain using a repeated measure ANOVA. For PPI, S1 frequency (4, 12, and 20 kHz) was a repeated measure. For ASR amplitude, S2 intensity (70, 80, 90, and 100 dB SPL) was a repeated measure. Tukey tests were used when significant ANOVAs were found. For test-retest reliability of PPI and ASR, two-way repeated ANOVAs were used with test session and S1 frequency (PPI) or S2 intensity (ASR amplitude) as variables.

Results

Acoustic Startle Response

Figure 1 presents a summary of the acoustic startle response for the nine strains, at intensities of 70, 80, 90, and 100 dB SPL. ASRs were generally smaller when the startle stimulus (S2) was less intense. The 70 dB SPL S2s produced virtually no evoked ASRs. These values were considered non-ASR movement, and were not computed in the statistical analysis of specific S2 intensities. Means varied widely across strains. For example, ASRs were extraordinarily large in A/J mice, but were small at all S2 intensities in C3H/HeJ, DBA/2J, and CAST/Ei strains.

The statistical analysis showed these observations to be reliable. An ANOVA was computed for ASR at 80, 90, and 100 dB SPL. The main effect of strain (F(8,179)=20.7; p<0.0001) and the main effect of S2-intensity (F(2,358)=243.6; p<0.001) were both statistically significant.
The interaction between strain and S2-intensity (F(16,358)=11.87; p<0.001) was also statistically significant. Due to these findings, an additional one-way ANOVA was run at each intensity. Findings were significant at 80 dB (F(8,179)=10.01, p<0.0001), 90 dB (F(8,179)=17.77, p<0.0001), and 100 dB (F(8,179)=20.64, p<0.0001). As expected, ASRs were generally smaller when the S2 stimulus was less intense. Table 1 presents Tukey test results (*= p<0.05; **= p<0.01) for the ASR at 80, 90, and 100 dB SPL. As S2 intensity increased, more strains demonstrated significant differences.

![Acoustic Startle Response](image)

**Figure 1.** Acoustic startle response for nine strains
Table 1. Tukey test results for ASR at 80, 90, and 100 dB SPL

Prepulse Inhibition

Figure 2 presents a summary of the PPI effect on the ASR for the nine strains.

Scores represent the S1 frequency of 4, 12, and 20 kHz, respectively. In general, the
effect of PPI is relatively weak for the 4 kHz S1 and stronger with S1s of 12 kHz and 20
kHz. Like the non-PPI ASR data, means varied widely across strains. For example, the
mice of the 129/SV1mJ strain had rather strong PPI whereas C3H/HeJ, DBA/2J, and
CAST/Ei had weak PPI. These observations are supported by the statistical analysis with
the main effect of strain (F (8,179)=7.54; p<0.0001) and the main effect of S1-frequency
(F (2,358)=16.7; p<0.001) both statistically significant. The interaction between strain
and S1-frequency (F (16,358)=4.34; p<0.001) was also statistically significant. One way
ANOVAs were computed for each S1 frequency. The 4 kHz (F(8, 179) = 4.05, p = 0.002), 12 kHz (F(8, 179) = 6.81, p = 0.0001), and 20 kHz (F(8, 179) = 8.63, p<0.0001) were each statistically significant.

Table 2 presents Tukey test results (*= p<0.05; **= p<0.01) for PPI at 4, 12, and 20 kHz.

Figure 2. Mean PPI scores for nine strains
Table 2. Tukey test results for PPI at 4, 12, and 20 kHz

Reliability

Mice were immediately retested after the initial run of 55 trials. No significant differences were found between first and second test scores for either ASR or PPI for the nine strains.

Heritability

Heritability is defined as the amount of variance that can be attributed to genetic factors. A basic example of this can be seen in eye and hair color, in contrast to language spoken. Genetics contribute fully to natural hair and eye color, whereas environment dictates the native language that a person acquires. Using this example, eye and hair
color would have a heritability value of 1.0, while language would have a value of 0.00. The closer the number is to 1.0, the more genetic factors contribute to the behavior.

If we assume that the within-strain variance is primarily environmental and the between-strain variance is primarily genetic, then we can roughly estimate the heritability as between-strain variance divided by (within-strain variance + between-strain variance). For 12 kHz PPI, standard deviation among strain means (N=9) = 0.13. Heritability values for each strain are as follows, with a range of 0.30 in CBA/J to 0.46 in C57. Strain 129 (0.31), A/J (0.37), BALB/c (0.42), C3H (0.39), C57 (0.46), CAST (0.32), CBA/J (0.30), DBA/2J (0.38), and FBV (0.34).

For the 100 dB ASR, mean values were 0.55, ranging from 0.41 in A/J to 0.80 in C3H. Strain 129 (0.45), A/J (0.41), BALB/c (0.42), C3H (0.80), C57 (0.61), CAST (0.64), CBA/J (0.46), DBA/2J (0.61), and FVB (0.51).

Discussion

A goal of the present study was to examine the effects of genetic background on the ASR and its modulation by PPI. This was accomplished by comparing nine inbred strains of mice. Data were collected and analyzed using ASR amplitude in response to a “standard” 100 dB SPL broadband noise startle stimulus at three discrete intensities. In addition, the PPI effect was evaluated using a non-startling stimulus of 70 dB at frequencies of 4, 12, and 20 kHz.

Data obtained from this study show clearly that inbred strains of mice vary greatly on both the ASR and PPI. Relatively high heritability indicates a strong genetic component to the between-strain differences in these behaviors. This conclusion is in agreement with previous studies of ASR in rats (Glowa & Hansen, 1994) and ASR and
PPI in inbred strains of mice (Bullock, Slobe, Vazquez, & Collins, 1997; Paylor & Crawley, 1997). The present data are particularly impressive because the earlier studies did not include females in testing, implemented a continuous 70 dB SPL background noise during testing, used longer inter-stimulus intervals, and used a broadband noise for the S1s, rather than tones. The latter variables could have contributed to strain differences irrespective of genetic differences.

In addition to genetic influences that may affect S1 or S2 responses, peripheral hearing loss of inbred mouse strains must be taken into consideration. Mice of some strains exhibit genetically determined hearing loss. In hearing impaired mice, the ability of the S1 and/or S2 could be compromised. This would result in weak ASRs or ASR-PPI amplitudes. Relative differences among animals in the strength of PPI appear to be due, in part, to changes in peripheral auditory sensitivity (Willott & Turner, 2000). This likely contributes to the poor performance in DBA/2J mice, which exhibit early hearing loss (Erway et al., 2001). However, the other strains hear normally when young, so PPI and ASR differences may be due to central auditory processing. Prepulse inhibition is generally viewed as a measure of central auditory processing because the inferior colliculus and other higher order structures comprise the pathway(s) by which the prepulse modulates the startle response (Willott & Turner, 1999). Thus, the data suggest that central auditory processing is strongly influenced by genetic background. This finding may have clinical implications: even with a normal peripheral auditory system, central auditory processes can contribute to performance on auditory tests.
The use of inbred strains of mice holds great promise for understanding the genetic basis of the ASR and PPI. Future research may use some of the mouse models shown here to elucidate genetic influences on ASR and ASR-PPI.
References


