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The effects of Eph-ephrin mutations on pre-pulse inhibition in mice

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Abstract

Eph-ephrin signaling is known to be important in directing topographic projections in the afferent auditory pathway, including connections to various subdivisions of the inferior colliculus (IC). The acoustic startle-response (ASR) is a reliable reflexive behavioral response in mammals elicited by an unexpected intense acoustic startle-eliciting stimulus (ES). It is mediated by a sub-cortical pathway that includes the IC. The ASR amplitude can be measured with an accelerometer under the subject and can be decreased in amplitude by presenting a less intense, non-startling stimulus 5–300 ms before the ES. This reflexive decrement in ASR is called pre-pulse inhibition (PPI) and indicates that the relatively soft pre-pulse was heard. PPI is a general trait among mammals. Mice have been used recently to study this response and to reveal how genetic mutations affect neural circuits and hence the ASR and PPI. In this experiment, we measured the effect of Eph-ephrin mutations using control mice (C57BL/6J), mice with compromised EphA4 signaling (EphA4^{lacZ/+}, EphA4^{lacZ/lacZ}), and knockout ephrin-B3 mice (ephrin-B3^{+/-}, ^{-/-}). Control and EphA4^{lacZ/+} strains showed robust PPI (up to 75% decrement in ASR) to an offset of a 70 dB SPL background noise at 50 ms before the ES. Ephrin-B3 knockout mice and EphA4 homozygous mutants were only marginally significant in PPI (< 25% decrement and <33% decrement, respectively) to the same conditions. This decrement in PPI highlights the importance of ephrin-B3 and EphA4 interactions in ordering auditory behavioral circuits. Thus, different mutations in certain members of the signaling family produce a full range of changes in PPI, from minimal to nearly maximal. This technique can be easily adapted to study other aspects of hearing in a wider range of mutations. Along with ongoing neuroanatomical studies, this allows careful quantification of how the auditory anatomical, physiological and now behavioral phenotype is affected by changes in Eph-ephrin expression and functionality.

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Keywords

Acoustic Startle Response; PPI; Pre-pulse inhibition; Eph/ephrin; EphA4; ephrin-B3

1. Introduction

1.1 Eph-ephrin signaling

The Eph receptors and their ligands, the ephrins, play a strong role in the development of the auditory system in mice by patterning the tonotopic structure from the auditory periphery to the brainstem and up to the auditory cortex [1–3]. Eph-ephrin interactions involve two subfamilies: As and Bs. Binding affinities are strongest within subfamilies; a noteworthy exception is the strong interaction between EphA4 and ephrin-B2, -B3 [4–6]. The mouse organ of Corti and spiral ganglion cells have strong expression of EphA4, EphB1, B2, B3, ephrin-A2, -A5, and ephrin-B1, -B2 [7–9]. Recent findings underscore the importance of Eph-ephrin signaling in the mammalian inferior colliculus (IC) prior to hearing onset [3, 10]. Graded and modular expression patterns of EphA4 and ephrin-B2 correlate with developing projections to various IC subdivisions, suggesting their involvement in guiding tonotopic and patterned arrangements in the mouse midbrain [10]. Furthermore, accurate topographic mapping of terminal fields from the lateral superior olive to the IC is lost in ephrin-B2 mutants with compromised signaling [3]. Given the involvement of these subcortical auditory circuits in the ASR and the influences of Eph-ephrins on their development and organization, we hypothesize that Eph-ephrin mutants will display altered pre-pulse inhibition (PPI) when compared to controls and wild-types (WTs).

1.2 Acoustic Startle Reflex and Pre-pulse Inhibition

The acoustic startle response (ASR) is a motor response elicited by and directly following the presentation of an unexpected intense acoustic stimulus [11, 12]. It is a rapid contraction of skeletal muscles that is considered a defensive response [13]. This behavioral response can be measured with the use of an accelerometer placed directly beneath the subject [14]. The magnitude of this response can be altered by a variety of factors including the addition of a non-startling stimulus (pre-pulse) presented before the startle-eliciting stimulus (ES) [15]. To detect ASR and its attenuation by the addition of a pre-pulse stimulus, reflex modification audiometry (RMA) is rapid and efficient [14, 16].

Pre-pulse inhibition (PPI) can occur when a stimulus softer than the ES, called the pre-pulse, is presented ~ 5–300 ms before the ES. The perception of this pre-pulse stimulus reduces ASR amplitude. The PPI paradigm has been used in various research efforts as it is sensitive to manipulations in many parameters, is reliable across time, is easily quantified, and is controlled by a simple neural circuit that is conserved across mammalian species [13]. It has advantages over operant conditioning paradigms in that it does not require training or reinforcement efforts [17].

Allen & Ison [14] studied the effects of inter-stimulus intervals (ISIs) of the pre-pulse stimulus in CBA/CaJ inbred mice via an offset paradigm, an onset paradigm, and a speaker

swap of 180 degrees azimuth. The most robust PPI was elicited by the offset of a 70 dB SPL broad band noise (filtered from 1 kHz to 50 kHz) with an ISI of 50 ms.

1.3 Critical Auditory Structures

In the mammalian ASR, stimuli are transduced in the cochlea, and subsequently transmitted to the auditory nerve, cochlear nuclei (CN), nuclei of the lateral lemniscus (NLL), nucleus reticularis pontis caudalis (PnC - located at the head of the reticulospinal tract), and ultimately to the spinal motor neurons, which then innervate flexor and extensor muscles of the body [16, 17]. Inhibitory modulation of mouse ASR is influenced by the IC, most notably its lateral and dorsal cortex subdivisions (LCIC and DCIC) [18]. The addition of a pre-pulse stimulus inhibits the ASR by interfering with the neural circuit at the level of the IC where excitatory input is sent to the pedunculopontine tegmental nucleus, which in turn inhibits the PnC of the ASR neural pathway [17].

1.4 Goal of experiment

Experiments addressing the effects of Eph-ephrin mutations on behavioral pre-pulse inhibition in mice are currently lacking. Therefore, the aim of this study is to better understand the behavioral effects of Eph-ephrin signaling by comparing Eph-ephrin mutant mice to controls using Allen & Ison's PPI procedure [14]. The following is a behavioral evaluation of mutations that have been studied genetically and histologically.

2. Materials and Methods

2.1 Subjects

Mice ($n = 20$) of two different Eph-ephrin mutations and a control group were used. The control group consisted of seven C57BL/6J mice and two WT offspring of heterozygous EphA4^{lacZ} parents. Two strains of mutant mice were tested: ephrin-B3^{null} ($n=4$, 2 homozygous, 2 heterozygous) and EphA4^{lacZ} ($n=7$, 4 heterozygous and 3 homozygous). Mice varied between the ages of 31 days and 75 days and were tested twice. The average age at the first test was 37 (± 8.2) days. The average time between the first and second test was 15.5 (± 4.4) days. All mice were tested before the expected onset of age-related hearing loss of 8 months in the C57BL/6J strain [19]. All mice were group-housed (4–6 mice per cage) in a BioZone MiniSmart Rack System in a controlled constant climate. All testing was done during the daylight hours. Food and water were always available except during testing which lasted approximately 60 minutes. The James Madison University Institutional Animal Care and Use Committee (IACUC) approved all procedures prior to experimentation.

2.1.1 Genotyping procedures—Breeding pairs to establish the EphA4 colony were obtained through Mutant Mouse Regional Resource Center (MMRRC, NCRR-NIH). Ephrin-B3 breeding pairs were acquired from Dr. Mark Henkemeyer (UT Southwestern Medical Center). Tail samples of EphA4 and ephrin-B3 mice were processed for genotyping utilizing an Easy-DNA kit (Invitrogen, Carlsbad, CA). EphA4 primer (EphA4-forward 5' GTTTCGCTCTGAGCTTATACTGC-3', EphA4-reverse 5' ACAGTGAGTGGACAAAGAGACAGG-3', lacZ 5'-

CGCTCTTACCAAAGGGCAAACC-3') and ephrin-B3 primer (EB3-forward 5'-GACGGCGGGCCAAGCCTTCGGAGAG -3', EB3-reverse 5'-ATAGCCAGGAGGAGCCAAAGAG-3', lacZ 5'-AGGCGATTAAGTTGGGTAACG-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, and ephrin-B3 WT (401-bp) and/or mutant (142-bp) allele bands.

2.2 Apparatus and stimuli

Mice were tested in a 5 cm inside-diameter by 12.5 cm long San Diego instruments Plexiglas tube attached to an accelerometer taken from the SR-LAB mouse-testing chamber. This tube was placed in the middle of a 7' x 7' (2.13 meters x 2.13 meters) Industrial Acoustic double-walled, double-floored, sound-attenuating booth. The chamber was 18" (45.7 cm) beneath a Ross Audio Systems TW 30 compression tweeter. The pre-pulse stimulus was presented via a Tucker Davis Technology ES1 compression tweeter 15 cm to one side of the testing chamber. Startle eliciting stimuli (ES) were 110 dB, 15 ms broad-band noise, high-pass filtered at 8 kHz, rapidly gated. Calibration showed significant energy up to 50 kHz, 110 dB SPLrms in a 768 Hz to 50 kHz band. The ES noise was generated using a Tucker Davis Technology Real-Time Processor, TDT RP2.1, and was amplified by a Crown XLS202 amplifier. The pre-pulse stimulus was a continuous high-pass noise filtered at 4 kHz (1 kHz to 100 kHz bandwidth = 70 dB SPLrms $\pm$ 1 dB SPLrms). The offset was approximately instantaneous (on to off in one 50 μ s cycle of the DAC). Calibrations of the stimuli were done with an Agilent 35670A Spectrum Analyzer, 1/4" microphone (Bruel & Kjaer 4939) placed in the center of the Plexiglas tube, amplified by a Listen, Inc. Sound Connect amplifier.

The force of the startle reflex was transduced by an accelerometer beneath the testing tube. The voltage from the accelerometer was low-pass filtered at 1 kHz and amplified times 100 (20 dB + 20 dB) by a Krohn-Hite model 3343 filter and input to a TDT-RP2.1. This input was digitized at 200 kHz for 100 ms starting at the same time that the startling stimulus began. Test trials began 2 minutes after the mouse was placed in the testing chamber (2 minute acclimation period), and testing continued for about 60 minutes. All subjects were run with the lights off.

2.3 General Procedures

The pre-pulse in this experiment was an offset of the 70 dB stimulus at 90 degrees azimuth to the mouse. Sixteen different conditions were repeated in 11 different blocks. There were 13 different inter-stimulus intervals (ISI: time between offset of carrier stimulus and presentation of startle-eliciting stimulus). The 13 different ISI conditions were 1, 2, 5, 10, 20, 30, 40, 50, 60, 100, 150, 200 and 300 ms. Each block contained these 13 ISIs plus two no-pre-pulse baseline control trials and a No-ES control trial to measure background activity, for a total of 16 trials per block. The inter-trial interval randomly varied between 15 and 25 seconds. These 16 different trials were presented in 11 different blocks, with the order of trials randomized within each block. RMS voltage from the accelerometer (100 ms from start of the startle stimulus) was calculated for each trial. Pre-pulse inhibition scores were calculated as a ratio of the subject's average response amplitude (mean RMS) in the

pre-pulse stimulus conditions (ASRp) compared to the control baseline measures with only eliciting (startle) stimulus and no pre-pulse (ASRc) using the formula: $PPI = 1 - (ASRp/ASRc)$ as in Allen & Ison, 2010 [14].

The methods above replicated Allen and Ison [14], except that our ES was 10 dB less intense. In addition, 16 no-mouse controls were run exactly as the mice, except instead of a mouse there was water in a mouse-sized glass vial weighing a total of 30g, approximately the weight of our mice, in the chamber.

3. Results

There was a highly significant effect of Eph-ephrin mutations: repeated-measures ANOVA in PPI with 26 within-subjects measures (13 ISIs from 1 to 300 ms at two testing times) from each of the 20 mice in four groups (9 control, 4 ephrin-B3^{null}, 4 EphA4^{lacZ/+} and 3 EphA4^{lacZ/lacZ}) showed a large between-subject effect of genotype ($F_{3,16}=32$, $p<.001$, $\eta_p^2 = 86\%$). Post-hoc pairwise (LSD) comparisons showed the control and EphA4^{lacZ/+} groups to be similar ($p=.63$) and each different from the ephrin-B3^{null} ($p<.001$). The ephrin-B3^{null} and EphA4^{lacZ/lacZ} mutations were not different ($p=0.21$). Among the within-subjects effects there is a strong effect of ISI (Wilk's $\lambda=.02$, $p=.002$), with the strongest planned polynomial contrast being the quadratic ($F_{1,16}=134$, $p<.001$, $\eta_p^2=.89$), indicating the expected curved function of ISI as seen in Fig. 1. There was no effect of time (Wilk's $\lambda=.9$, $p=.24$) nor any interaction with time ($p>=.1$); that is, no effect of the repeated test after an average of 15 days.

Half of the ephrin-B3^{null} mice were homozygous and half heterozygous mutants. There was no difference between these genotypes ($F_{1,2}=.01$; $p=.93$; from a repeated measures ANOVA of only the 4 ephrin-B3^{null} mutant mice; also showing no effect of time).

Because there was no effect of the repeated testing (time), the PPIs at each ISI were averaged over the two tests for each mouse. These means are shown in Fig. 1 along with data from the no-mouse controls. These data were used in a repeated-measures ANOVA with one within-subject factor (13 ISIs) and one between-subject factor (5 groups: C57BL/6J and WT, EphA4^{lacZ/lacZ}, EphA4^{lacZ/+}, ephrin-B3^{null}, and the 30g no-mouse control). Post-hoc tests showed all groups to be different from the no-mouse controls indicating that even the two least responsive groups of mice still showed significant PPI (ephrin-B3^{null} and EphA4^{lacZ/lacZ} at $p=.005$ and $p<.001$ respectively).

Various statistical evaluations also showed no significant differences where none were expected. There was no difference between the groups in their age at testing ($F_{3,19}=2.35$, $p=0.11$). There was no difference between the groups in the inter-test interval ($F_{3,19}=0.4$, $p=0.08$). There was no significant PPI in the 30g no-mouse controls ($F_{1,15}=3.2$, $p=.092$).

Looking only at trials where there was no pre-pulse (startle-alone and no-sound controls in the four groups of mice), repeated measures ANOVA showed no main effect of group or time, but there was a possible group-by-time interaction ($p=.03$ to $.07$ depending on the use of multivariate or repeated-measures approach to analyzing the within-subject factor). The ephrin-B3^{null} mice moved less on the 2nd test: significantly so in the no-sound intervals and

almost significantly so in the startle-alone trials, with RMS voltage about half on the 2nd test compared to the 1st test in this group only.

Figure 2 shows the averaged responses to the startle-alone and no-sound trials. In all groups except the 30g no-mouse control (where $p=0.5$) paired-sample t-tests showed a significant difference between the trials with and without the startle (all $p<.028$) as expected.

A comparison of Figures 1 and 2 shows an interesting interaction between groups (mutations) and responses with and without the pre-pulse. Note that the ephrin-B3^{null} mice startled as much as the WT (in Figure 2), but had the lowest amount of PPI (in Figure 1). The EphA4^{lacZ/lacZ} mice had about 40% of the WT response both with and without the pre-pulse, and the EphA4^{lacZ/+} mice were the same as WT in both conditions.

4. Discussion

The goal of this study was to explore the effects of two Eph-ephrin mutations on hearing as revealed through the acoustic startle reflex and pre-pulse inhibition. We repeated the procedure of Allen & Ison [14] and extended the results to include EphA4 and ephrin-B3 mutant strains. To our knowledge, these are the first data on the effects of Eph-ephrin signaling on behavioral auditory responsiveness in mammals. PPI reveals the full range of effects of Eph-ephrins on hearing from near minimal to near maximal effects. The results of this experiment suggest that Eph-ephrin signaling is essential for behaviorally mediated responses to sound stimuli.

Data from our control group closely replicated the results of Allen and Ison [14]. The slight decrement in the responsiveness of our mice could be due to our 110 dB SPL ES compared to the 120 dB SPL ES of Allen and Ison [14]. We found the responses to be stable over time.

4.1 Pre-pulse inhibition

Inhibition of the ASR elicited by a pre-pulse cue of background noise offset was a strong response in the control group. Controls (C57BL/6J and WT) produced the most robust PPI response with a peak PPI of 0.778 (78% reduction of ASR) at 40 ms. The heterozygous EphA4^{lacZ/+} mutants showed near-normal behavioral responses when compared to the controls with a peak PPI response of 0.746 (75% reduction of ASR) at about 40 ms ISI. Both control and EphA4^{lacZ/+} mice displayed increasing PPI with increasing ISI until about 40 ms where the response saturated. These results are consistent with those of Allen & Ison [14] where an ISI of 50 ms produced the greatest PPI for an offset paradigm with a saturation and subsequent decrease of the PPI response after 50 ms. In comparison, homozygous EphA4 mutants (EphA4^{lacZ/lacZ}) did not produce as strong of a PPI response, with only a 33% reduction of startle amplitude. Additionally, ephrin-B3^{null} mice (both heterozygous and homozygous) were only marginally significant in their responses with PPI measures of less than 21%; but still significantly above the PPI values in the no-mouse control condition. When compared to no-startle trials all groups of mice showed a significant startle response in the ES-alone trials. This indicates that the startle stimulus elicited a reflexive response in all groups of mice.

4.2 Effects of Eph-ephrin signaling

It is thought that various alterations in Eph-ephrin expression will produce varying behavioral responses. This experiment has illustrated that the heterozygous EphA4^{lacZ/+} strain does not show behavioral differences when compared to the controls. Interestingly, homozygous EphA4^{lacZ/lacZ} mice that lack any reverse signaling capability (Eph-to-ephrin) showed decreased PPI as compared to heterozygous EphA4^{lacZ/+} mice. This leads us to believe that EphA4^{lacZ/+} mice have sufficient protein and its signaling function to be spared deleterious auditory circuit development and spared any ensuing behavioral deficits in PPI. The reduction in PPI observed in EphA4^{lacZ/lacZ} mice that have a more significant loss of protein signaling may reflect underlying connectivity aberrations in these animals. Ongoing experiments in the Gabriele lab aim to determine the development of topographic connections between ASR auditory structures in heterozygous and homozygous EphA4 mice. Even more marked than EphA4^{lacZ/lacZ} mice, a complete knockout of the ephrin-B3 protein significantly changes the behavioral results in the PPI paradigm but not in the startle-alone trials, illustrating the importance of ephrin-B3 in the PPI neural circuit. Results from the ephrin-B3^{null} strain were similar for both heterozygous and homozygous mutations; one functional allele did not spare the mice from significantly diminished PPI compared to controls. The results of these experiments show that different members of the Eph-ephrin signaling family may play different roles in the development of the afferent auditory system and thus may exert different influences in PPI as well as the simple startle response. Ephrin-B3 may play an especially important role in mediating PPI.

The complexities in behavioral responses of these mutant strains, revealed through the different patterns in Figures 1 and 2, suggest that documentation of the full psychometric function, that is the growth of responsiveness to both pre-pulses and startling stimuli alone as the intensities of each is varied between the upper to lower asymptotes of responsiveness for each group, may be necessary to fully understand the influence of these signaling proteins on auditory processing. Soon our lab and others will likely have electrophysiological and behavioral threshold estimates for these mutant strains. The present data suggest that the growth of loudness may be different in different mutant strains. Such complex effects on central auditory processing are consistent with reports of complex Eph/ephrin signaling in central auditory nuclei.

4.3 Importance of further research

To further investigate the importance of Eph-ephrin proteins, researchers may want to alter the pre-pulse stimulus and to include other mutations to better understand how Eph-ephrin signaling alters auditory responsiveness. Various psychoacoustical parameters of the pre-pulse could be varied in future studies to test thresholds to onsets, details of the psychometric function, receiver operating characteristics, localization, and gap detection. Data in the present paper suggest that the slope and asymptotes of the psychometric function may be altered by these mutations. Documenting habituation could also reveal changes in the central pathways [11]. Modulation of PPI by background sounds could be changed by alterations in the tonotopic organization in these mutants [10, 22]. Thus, responsiveness to these various PPI stimuli could help researchers understand the behavioral implications of

various functional changes in auditory afferents observed in anatomical studies of various Eph-ephrin mutant mouse strains.

4.4 Conclusions

Eph-ephrin proteins are important in both peripheral and central auditory development in the mammal. Although neuroanatomical research efforts have mapped where these proteins are expressed, there has been little research on the behavioral effects of Eph-ephrin mutations. This experiment lays the groundwork for a better understanding of how Eph-ephrin signaling may alter auditory behaviors. In summary, heterozygous EphA4 mutant mice have near-normal PPI responses to broadband noise offsets, while the ephrin-B3^{null} and homozygous EphA4 mutants show highly altered PPI responses with very little reduction in ASR amplitude to noise offset. It appears that much can be learned about the functional and behavioral effects of Eph-ephrin mutations through PPI in mice.

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References

1. Cramer KS. Eph proteins and the assembly of auditory circuits. *Hear Res.* 2005; 206:42–51. [PubMed: 16080997]
2. Intskirveli I, Metherate R, Cramer KS. Null mutations in EphB receptors decrease sharpness of frequency tuning in primary auditory cortex. *PLoS One.* 2011; 6:e26192. [PubMed: 22022561]
3. Wallace MM, Kavianpour SM, Gabriele ML. Ephrin-B2 reverse signaling is required for topography but not pattern formation of lateral superior olivary inputs to the inferior colliculus. *J Comp Neurol.* 2013; 521:1585–97. [PubMed: 23042409]
4. Gale NW, Holland SJ, Valenzuela DM, Flenniken A, Pan L, Ryan TE, Henkemeyer M, Streibhardt K, Hirai H, Wilkinson DG, Pawson T, Davis S, Yancopoulos GD. Eph receptors and ligands comprise two major specificity subclasses and are reciprocally compartmentalized during embryogenesis. *Neuron.* 1996; 17:9–19. [PubMed: 8755474]
5. Pasquale EB. The eph family of receptors. *Curr Opin Cell Biol.* 1997; 9:608–15. [PubMed: 9330863]
6. Bergemann AD, Zhang L, Chiang MK, Brambilla R, Klein R, Flanagan JG. Ephrin-B3, a ligand for the receptor EphB3, expressed at the midline of the developing neural tube. *Oncogene.* 1998; 16:471–80. [PubMed: 9484836]
7. Pickles JO. Expression of ephs and ephrins in developing mouse inner ear. *Hear Res.* 2003; 178:44–51. [PubMed: 12684176]
8. Cowan CA, Yokoyama N, Bianchi LM, Henkemeyer M, Fritzsche B. EphB2 guides axons at the midline and is necessary for normal vestibular function. *Neuron.* 2000; 26:417–30. [PubMed: 10839360]
9. Zhou CQ, Lee J, Henkemeyer MJ, Lee KH. Disruption of ephrin B/eph B interaction results in abnormal cochlear innervation patterns. *Laryngoscope.* 2011; 121:1541–7. [PubMed: 21647913]
10. Gabriele ML, Brubaker DQ, Chamberlain KA, Kross KM, Simpson NS, Kavianpour SM. EphA4 and ephrin-B2 expression patterns during inferior colliculus projection shaping prior to experience. *Dev Neurobiol.* 2011; 71:182–99. [PubMed: 20886601]
11. Koch M. The neurobiology of startle. *Prog Neurobiol.* 1999; 59:107–28. [PubMed: 10463792]
12. Ison, JR. The acoustic startle response: Reflex elicitation and reflex modification by preliminary stimuli. In: Willott, JF., editor. *Handbook of mouse auditory research: From behavior to molecular biology.* Boca Raton, FL: CRC Press; 2001. p. 59-82.

13. Swerdlow NR, Braff DL, Geyer MA. Animal models of deficient sensorimotor gating: What we know, what we think we know, and what we hope to know soon. *Behav Pharmacol.* 2000; 11:185–204. [PubMed: 11103873]
14. Allen PD, Ison JR. Sensitivity of the mouse to changes in azimuthal sound location: Angular separation, spectral composition, and sound level. *Behav Neurosci.* 2010; 124:265–77. [PubMed: 20364886]
15. Gaese BH, Nowotny M, Pilz PK. Acoustic startle and prepulse inhibition in the mongolian gerbil. *Physiol Behav.* 2009; 98:460–6. [PubMed: 19660482]
16. Ison JR, Agrawal P, Pak J, Vaughn WJ. Changes in temporal acuity with age and with hearing impairment in the mouse: A study of the acoustic startle reflex and its inhibition by brief decrements in noise level. *J Acoust Soc Am.* 1998; 104:1696–704. [PubMed: 9745749]
17. Fitch RH, Threlkeld SW, McClure MM, Peiffer AM. Use of a modified prepulse inhibition paradigm to assess complex auditory discrimination in rodents. *Brain Res Bull.* 2008; 76:1–7. [PubMed: 18395604]
18. Parham K, Willott JF. Effects of inferior colliculus lesions on the acoustic startle response. *Behav Neurosci.* 1990; 104:831–40. [PubMed: 2285482]
19. Ehret G. Critical bands and filter characteristics in the ear of the housemouse. *Biol Cybernetics.* 1976; 24:35–42.
20. Davis C, Yokoyama N, Chumley MJ, Cowan CA, Silvany RE, Shay J, Baker LA, Henkemeyer M. Bidirectional signaling mediated by ephrin-B2 and EphB2 controls urorectal development. *Dev Biol.* 2004; 271:272–90. [PubMed: 15223334]
21. Miko IJ, Nakamura PA, Henkemeyer M, Cramer KS. Auditory brainstem neural activation patterns are altered in EphA4- and ephrin-B2-deficient mice. *J Comp Neurol.* 2007; 505:669–81. [PubMed: 17948875]
22. Carlson, S.; Ison, JR. Modulation of the acoustic startle response by background sound in C57BL/6J mice. In: Willott, JF., editor. *Handbook of mouse auditory research: From behavior to molecular biology.* Boca Raton, FL: CRC Press; 2001. p. 83-90.

Fig. 1.

Eph/ephrin mutations affect behavioral responses of mice to broadband noise. Pre-pulse inhibition elicited by the offset of ongoing 70 dB SPL background noise in varying ISIs before a startle eliciting stimulus (110 dB SPL) is shown for a control group (C57BL/6J and WT), and for EphA4^{lacZ/+}, EphA4^{lacZ/lacZ} and ephrin-B3^{null} mutant strains. Shown also are results of Ison & Allen (2010) and control data from 30g of water in a mouse-size vial in an otherwise empty chamber. All groups are significantly different than the no-mouse control. Error bars are ± 1 SE.

Figure 2.
Averaged responses (V RMS +40 dB) on startle-alone trials and no-sound trials. Error bars are +/-1 SE.