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Research Article

Auditory Behavior in Adult-Blinded Mice

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ABSTRACT

Cross-modal plasticity occurs when the function of remaining senses is enhanced following deprivation or loss of a sensory modality. Auditory neural responses are enhanced in the auditory cortex, including increased sensitivity and frequency selectivity, following short-term visual deprivation in adult mice (Petrus et al. Neuron 81:664–673, 2014). Whether or not these visual deprivation–induced neural changes translate into improved auditory perception and performance remains unclear. As an initial investigation of the effects of adult visual deprivation on auditory behaviors, CBA/CaJ mice underwent binocular enucleation at 3–4 weeks old and were tested on a battery of learned behavioral tasks, acoustic startle response (ASR), and prepulse inhibition (PPI) tests beginning at least 2 weeks after the enucleation procedure. Auditory brain stem responses (ABRs) were also measured to screen for potential effects of visual deprivation on non-behavioral hearing function. Control and enucleated mice showed similar tone detection sensitivity and frequency discrimination in a conditioned lick suppression test. Both groups showed normal reactivity to sound as measured by ASR in a quiet background. However, when startle-eliciting stimuli were presented in noise, enucleated mice showed decreased ASR amplitude relative to controls. Control and enucleated mice displayed no significant differences in ASR habituation, PPI tests, or ABR thresholds, or wave morphology. Our findings suggest that while adult-onset visual deprivation induces cross-modal plasticity at the synaptic and circuit levels, it does not substantially influence simple auditory behavioral performance.

Keywords: Cross-modal plasticity, Adult-onset blindness, Psychoacoustics, Acoustic startle reflex, Prepulse inhibition

INTRODUCTION

Sensory deprivation results in reorganization of central pathways by either increasing gain or devoting resources to other intact sensory modalities (Bavelier and Neville 2002; Kupers and Ptito 2014; Merabet and Pascual-Leone 2010). Cortical and subcortical plasticity in the auditory system following auditory deprivation is well established, although much more is known about physiological changes than behavioral outcomes (e.g., Kral 2007; Lauer et al. 2019; Sanes and Woolley 2011). Sensory deprivation–induced cross-modal plasticity is observed as recruitment of the deprived sensory cortex for processing of the remaining senses, referred to as cross-modal recruitment (Kral 2007; Lee and Whitt 2015), and refinement of the sensory processing within the spared sensory cortices, termed compensatory plasticity (Rauschecker 1995). This reorganization

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is often viewed as a way to compensate for the loss of input and optimize the organism's ability to navigate its environment in the face of reduced sensory function. Enhanced auditory capabilities have been reported primarily in early-blind listeners compared to sighted controls (Hugdahl et al. 2004; King and Parsons 1999; Rauschecker and Kniepert 1994; Wan et al. 2010); however, recent experiments in mouse models and adult human subjects have challenged the long-held notion that cross-modal plasticity in sensory cortices is restricted to early life (Lee and Whitt 2015; Merabet et al. 2008; Voss et al. 2004).

At the neural circuit level, visual deprivation in adult mice leads to widespread cortical plasticity both in the deprived primary visual cortex (V1) and in spared primary auditory (A1) and primary somatosensory (S1 barrel) cortices. The specific plasticity in the deprived V1 conforms to cross-modal recruitment, as it involves strengthening of intracortical synapses (Petrus et al. 2015) which may convey multisensory information to V1 (Ewall et al. 2021). In contrast, plasticity in the spared A1 involves potentiation of thalamocortical inputs (Petrus et al. 2014) and refinement of local A1 circuitry (Meng et al. 2015, 2017; Solarana et al. 2019), which correlates with lowered sound detection threshold and narrowing of frequency tuning curves of A1 layer 4 neurons (Petrus et al. 2014).

In light of these intriguing physiological effects, we performed a series of behavioral and evoked potential tests as a first-pass assessment of possible auditory perceptual and processing benefits resulting from this form of plasticity. We tested learned behavioral tasks and a series of acoustic startle reflex (ASR) and prepulse inhibition (PPI) assessments to investigate hearing in mice that were blinded as young adults. This test battery was chosen to evaluate performance on learned tasks in which attention is focused on the stimuli versus unlearned responses that do not require attention by the animal (Lauer et al. 2017). The behavioral tasks were selected to assess both subcortical and cortical activity since auditory cortex feedback pathways can shape coding in subcortical regions (Asilador and Llano 2020; Blackwell et al. 2020; Terreros and Delano 2015). Auditory brain stem response (ABR) evoked potentials were measured to confirm normal physiological sensitivity to sounds and assess the status of responses generated by subcortical pathways in an unconscious state.

METHODS

Subjects

A total of 38 male and female CBA/CaJ mice were obtained from the Jackson Laboratory (stock #000,654). Animals were group-housed and maintained in a quiet, low-traffic vivarium in a 12-h light–dark cycle (lights on 7 a.m.–7 p.m.). Sound levels in this housing room were previously described (Wu et al. 2020). Animals were group-housed in filter top shoebox cages with corncob bedding and nestlets, up to five mice per cage. All cages resided on the same rack. Subjects were randomly assigned to control or enucleated conditions, but the experimenters could not be blinded to the sight condition of each animal due to the obvious enucleation status. All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Johns Hopkins University. All experimental procedures were performed in accordance with the guidelines provided therein.

Binocular Enucleation

Binocular enucleation was performed at 3–4 weeks of age under anesthesia as in our published study (He et al. 2012). In brief, mice were anesthetized using isoflurane (1–2%) until the disappearance of corneal reflex and maintenance of a steady anesthetic plane. Both eyes were removed, and triple antibiotic ointment was applied. Mice were given meloxicam (5 mg/kg, SQ) before recovery, returned to the animal colony afterward, and maintained at a 12-h light–dark cycle.

Conditioned Lick Suppression

Mice were tested on an active listening task that requires the animal to attend to and make decisions about the sounds it is hearing. Primary auditory cortex plasticity has previously been associated with behavioral performance after auditory deprivation in a conditioned avoidance task in mice (Chambers et al. 2016). Tone detection thresholds and frequency difference limens (FDLs) were determined using a conditioned lick suppression (CLS) paradigm that was previously used by our lab and which is briefly described here (Fig. 1A) (Schrode et al. 2018). Nine control and nine enucleated female mice were tested using CLS, and five mice from each group were subsequently used for ASR, PPI, and ABR testing. Only females were used in this experiment because young adult male mice fight when one is removed for testing and then placed back in the home cage. Concerns over the unintended effects of stress from this fighting or the alternative long-term single housing led us to focus on female mice because they show no aggression when placed back in the home cage after testing. Training began at 6 weeks of age. During training and testing, mice were water restricted and allowed unlimited access to food. Animals received liquids during behavioral sessions. On non-testing days (weekends, holidays), each animal was given up to 5 g of hydrating gel as a weight maintenance supplement. Animals were weighed regularly to ensure maintenance within 80–90% of their free water weight, and they were observed to be active and healthy under these conditions.

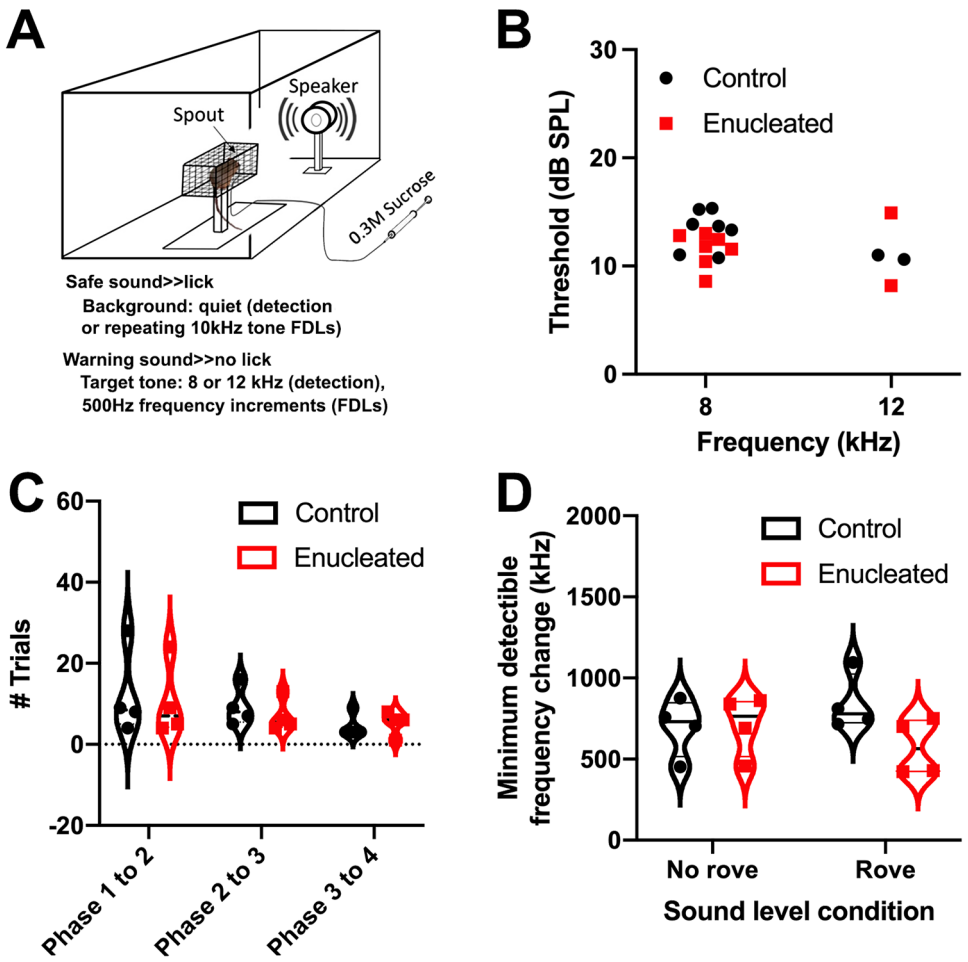

Fig. 1 Tone detection and frequency discrimination performance measured using conditioned lick suppression procedures in control and enucleated mice. **A** Schematic diagram of testing paradigm. **B** Tone detection thresholds for 8- and 12-kHz stimuli ($n = 9$ control, $n = 9$ enucleated). **C** Number of sessions required to advance through successive phases of the frequency discrimination task ($n = 4$ control, $n = 4$ enucleated). **D** Frequency discrimination thresholds for constant and roving level conditions

Behavioral training and testing took place in a small sound-attenuating booth (IAC) lined with 1-in anechoic foam (Sonex). The animal was placed in an elevated mesh wire cage located 15 cm from a speaker (Vifa tweeter XT25TG30-04) positioned directly in front of the cage. A metal lick spout extended into the cage and supplied a 0.3 M sucrose and water solution. Placement of the spout maintained the animal's head in a position facing the speaker during test trials since contact with the spout was required to initiate a trial. Tone stimuli were calibrated using a sound level meter with a 1/2-in microphone placed at the position of the mouse's head (Z-weighting; SoundTrack LxT; Larson Davis).

Behavioral testing was automated via a custom MATLAB program (MathWorks) controlling a multichannel input/output processor (Tucker-Davis Technologies, RX8). Mice initiated trials by licking the spout to receive a liquid reward and were first trained to detect a single frequency tone in quiet (Schrode et al. 2018). Mice licked the spout during "safe" quiet background trials and had to suppress licking to avoid a mild shock presented 40 ms after two presentations of a 240-ms "warning" tone presented with a ~ 25% probability. Warning tones were randomly presented in level increments of 10 dB (10–80 dB) using the method of constant stimuli, which has been shown to yield good sensitivity in mice (Klink et al. 2006; Kobrina et al. 2020; Radziwon et al. 2009; Radziwon and Dent 2014; Schrode et al. 2018). To avoid providing experience with the 10-kHz background stimulus used in the frequency discrimination testing, warning tones of either 8 or 12 kHz were used. Behavioral detection thresholds for these two frequencies are similar in CBA/CaJ mice (Radziwon et al. 2009; Schrode et al. 2018). Licking was monitored in 20-ms bins for 720 ms before and after the warning stimulus and for equivalent periods on safe trials. Suppressing licking in response to warning sounds was considered a hit while suppressing licking on a safe trial was considered a false alarm. These responses

were used to calculate d' measures of sensitivity. The subject's criterion was calculated such that the false alarm rate was approximately 16%. Threshold was defined as the stimulus level yielding $d' = 1.0$, and thresholds collected over the course of five sessions were averaged to determine the final threshold for each subject. Mice ran a total of approximately 300–400 trials per session.

For frequency discrimination testing, 4 mice from each group were subsequently trained to discriminate incremental frequency changes (warning/comparison sounds) from a repeating 10-kHz reference tone (safe/reference sound). Tones were presented at either constant or randomly roving sound levels (± 1.5 dB); the roving condition controls for potential loudness cues that can occur because tones of different frequencies and equal sound pressure levels may not be perceived as equally loud. Training proceeded in phases with the discrimination increasing in difficulty.

In phase 1 of frequency discrimination training, a gradual introduction of a repeating background sound occurred. Each trial consisted of a repeating 10-kHz, 50-dB sound pressure level (SPL) reference 240-ms tone on safe trials (240-ms inter-stimulus interval), interspersed with warning trials with 15-kHz, 70-dB SPL tones. Mice could lick for liquid reward during the repeating reference tone, but had to suppress licking during the warning tone to avoid a mild shock. Once the animal's discrimination performance exceeded 80% correct, the reference tone level was increased to 60 dB SPL and then to 70 dB SPL as performance improved to 80% correct. When discrimination performance reached 80% correct with the reference and comparison tones presented at equal SPLs, the subject moved on to phase 2.

In phase 2, comparison tones were gradually introduced in 1000-Hz frequency increments, again with equal levels of 70 dB SPL (no level rove). When performance reached 80% correct for the two highest frequency comparison tones (14 and 15 kHz) and remained stable for the other comparison frequencies (11, 12, 13 kHz), the subject moved on to phase 3 in which 500-Hz increments were gradually introduced. In cases where the mice were able to detect a change of 500 Hz (comparison tone of 10,500 Hz) with $d' > 1.0$, smaller increments were used to determine threshold. Once data from 5 sessions were obtained in which the frequency discrimination threshold (frequency difference limen) no longer improved and was stable across sessions, the subject moved onto phase 4, in which a ± 1.5-dB random level rove was introduced. Again, the mice were tested until five sessions in which the frequency discrimination thresholds remained stable were obtained. The number of sessions required to pass from each phase to the next were tracked, and thresholds were averaged across the five sessions run in phases 3 and 4 to compute final thresholds for no-rove and rove conditions.

ASR and PPI General Procedures

ASR and PPI tests that involve both cortical and subcortical function were performed on a total of 30 mice between 2 and 4 months of age using previously reported procedures (Clause et al. 2017; Lauer and May 2011; McGuire et al. 2015; Schrode et al. 2018). Most of the animals ($n = 25$) were used in multiple ASR and PPI experiments, but 5 animals were not used in multiple experiments due to death or aging out of the 2–4-month range. Startle reactivity increases slightly around 6 months of age in this strain before decreasing at older ages, and we sought to avoid this temporary increase in responses (Ison et al. 1998). Animals were brought into a quiet testing room 30 min prior to the start of each test session to acclimate them to the experiment room. Care was taken to minimize exposure to unnecessary stressors on test days. Animals were tested one at a time in random order. All startle experiments were conducted between 10 a.m. and 6 p.m., by the same experimenter. Before the start of each session, mice were acclimated to the sound-isolation chamber and the testing cage for 5 min. At the end of testing, animals were returned to their home cage. A minimum 6-day rest period was given between different ASR tests to minimize stress and avoid potential confounding effects of long-term habituation.

ASR and PPI tests were conducted in a tabletop sound-isolation chamber (Controlled Acoustic Environments, Industrial Acoustic Company Inc., Bronx, NY). A window in the door allowed ambient light from the room to enter the chamber, but the overhead chamber light was kept off to reduce excessive heat buildup inside the enclosure and to avoid presenting an extraneous stressor (bright light) to the animals. The interior surface of the sound-isolation chamber was lined with Sonex® acoustic foam (Pinta Acoustics, Minneapolis, MN) to reduce acoustic reflections. Mice were placed inside a small, custom-made, sound-permeable, half-cylindrical testing cage (ID: 7.2 × 3.3 × 2.8 cm) built with Delrin® and wire mesh. The base of the testing cage was mounted onto a piezoelectric accelerometer and placed in the center of the sound chamber. The piezoelectric disk transduced the animal's movement into voltage signals, which were then amplified by a custom-made amplifier. Startle stimuli were generated by an RP2.1 real-time processor (Tucker-Davis Technologies (TDT), Alachua, FL) and a PA5 programmable sound attenuator (TDT), amplified (Crown D 75A, Crown Harman, Elkhart, IN), and delivered through a speaker (RadioShack® Super Tweeter). Sound speakers (RadioShack® Super Tweeter) were placed in front of the animal, 15 cm from the animal's head in the horizontal plane. Speakers were calibrated with a sound level meter (SoundTrack LxT®, Larson Davis, Depew, NY) by Z-weighting.

For all but the ASR habituation test, trials were presented after random inter-trial intervals ranging

from 5 to 15 s. A 5-s quiet activity period followed the inter-trial interval during which the animal's movement was monitored. The startle stimulus was presented only when the animal remained still during the 5-s quiet period. The animal's startle responses were recorded over 120 ms following the startle-eliciting stimulus onset. ASR amplitude was defined as the maximum peak-to-peak voltage during the 120-ms time window after stimulus onset, unless otherwise noted. All ASR test parameters, stimuli, and recordings were controlled with custom MATLAB (MathWorks® Inc., Natick, MA) software interfacing the Tucker-Davis acoustic processing and acquisition platform. Test stimulus configurations are visually depicted in each figure.

ASR Habituation

Habituation or sensitization to startling acoustic stimuli represent non-associative learning processes, in contrast to the conditioned lick suppression task (Pilz and Schnitzler 1996; Plappert and Pilz 2005). To investigate possible differences in responsiveness to repeated presentations of the ASR stimuli, we measured short-term habituation, which primarily involves subcortical pathways, and long-term habituation, which involves both subcortical and cortical mechanisms (Davis and Gendelman 1977; Groves et al. 1974; Leaton et al. 1985; Leaton and Supple 1986; Weber et al. 2002). Short-term and long-term ASR habituation are variable across mouse strains, possibly due to differences in the influence of modulatory pathways (Lauer et al. 2017). CBA/Ca mice obtained from European suppliers show slight short-term habituation within a "traditional" PPI test session with noise-burst prepulses presented in background noise, whereas CBA/CaJ mice only show habituation in about 5% of gap prepulse inhibition test sessions (Charitidi et al. 2012; Lauer et al. 2017; Longenecker et al. 2018). A total of 11 control (5 males, 6 females) and 15 enucleated mice (5 males, 10 females) were used in this experiment. ASR habituation stimuli were short (20 ms) broadband noise bursts presented at 100 dB (Z) SPL in a quiet/ambient background. To assess short-term habituation, the startle stimuli were presented for 15 trials, with a fixed inter-trial interval of 10 s and no period in which "quiet" activity was checked (Fig. 2A). Mice were acclimated to the sound-isolation chamber and the testing cage for 5 min before the start of testing. Each test session lasted for 3–4 min, and mice were returned to their home cage immediately afterward. The tests were repeated 1 day following the first test session to assess long-term habituation. The day 2 data from one control and enucleated mouse were not saved due to technical difficulties with the experimental setup.

ASR in Quiet and Background Noise

ASR is normally enhanced in the presence of moderate background noise relative to an ambient quiet background (Hoffman and Fleshler 1963; Hoffman and Searle 1965, 1968; Ison 2001), presumably due to activation of arousal circuits by the background noise. The facilitating effect of background noise on the ASR is modulated by somewhat unspecified cortical circuits (Davis and Gendelman 1977; Ison and Silverstein 1978). ASR in a quiet background is also temporarily exaggerated after bilateral auditory cortex lesions, suggesting that the auditory cortex can confer an inhibiting effect on the ASR (Hunter and Willott 1993). Thus, cortical plasticity in adult-blinded mice could have facilitating or inhibiting effects on the ASR, and these effects could depend on background acoustic conditions.

A total of 15 control (5 males, 10 females) and 15 enucleated mice (5 males, 10 females) were used in this experiment. Startle stimuli were 20-ms broadband noise bursts with varying stimulus levels (70, 80, 90, 100, 105 dB (Z) SPL) presented either in quiet (Fig. 3A) or in the presence of continuous 65-dB (Z) SPL broadband background noise (Fig. 3B). Only one background condition, either quiet or noise, was tested within a session. Each ASR session consisted of 50 trials, 10 of which included a startle stimulus presented at a given intensity in pseudorandom order. For the ASR in noise, a second speaker (RadioShack® Super Tweeter) was placed next to the startle stimulus speaker to generate the continuous background noise. Each test session lasted for 20–25 min, and mice were returned to the home cage at the end of each session. The data from one enucleated mouse were not saved due to technical difficulties with the experimental setup. For all following ASR-based tests, a startle-eliciting stimulus of 105 dB SPL was used to avoid differences in control ASR amplitude between groups.

ASR Noise Offset Lead Time

To assess auditory processing using a PPI task that does not require the auditory cortex (Bowen et al. 2003), and to provide a check to determine if any potential group differences observed were due to overall sensorimotor gating differences or were specific to particular stimulus parameters, we measured PPI in response to an abrupt noise offset preceding the startle-eliciting stimulus. Procedures were similar to those reported previously (Ison et al. 1998; Ison and Allen 2003; Lauer and May 2011). A total of 13 control (2 males, 11 females) and 14 enucleated mice (5 males, 9 females) were used in this experiment. Startle stimuli were presented after a varying noise offset lead time after the 65-dB (Z) SPL broadband noise (BBN) background was abruptly turned off (Fig. 4A). Control trials consisted

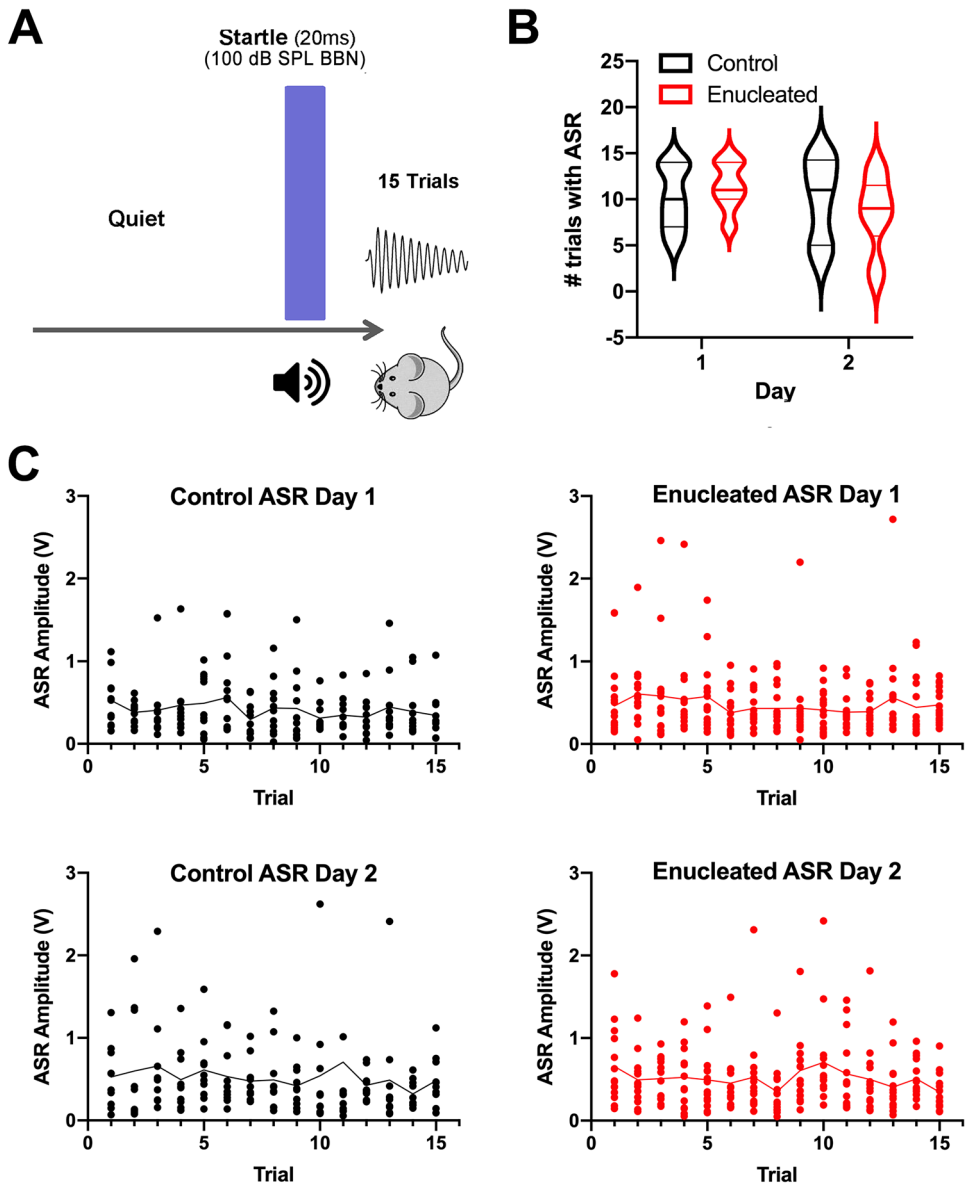

Fig. 2 Habituation of the acoustic startle response (ASR) in control ($n=11$) and enucleated ($n=15$) mice. **A** Schematic diagram depicting the stimulus parameters. A startle-eliciting noise burst was presented every 10 ms for 15 trials on day 1 to assess short-term habituation. The test was repeated on day 2 to assess long-term habituation. **B** Distribution of trials in which a startle response was present on days 1 and 2 of testing. **C** Individual (dots) and average (line) for control and enucleated mice on days 1 and 2 of testing show no habituation of the ASR amplitude to repeated presentation of a startling sound

of presentation of the 20-ms 105-dB (Z) SPL broadband noise startle-eliciting stimulus with no noise offset (0 ms). Test trials consisted of startle-eliciting stimulus presentation with noise offset lead times of 5, 10, 15, 25, or 50 ms. Each session consisted of a total of 11 blocks of 7 trials, each block consisting of 2 control and 5 test trials presented in pseudorandom order. Each test session lasted for 30–40 min, and mice were returned to their home cage at the end of testing. The first block of each session was dropped to reduce variability, and the 10 remaining blocks were used for analysis.

ASR Frequency Difference Limens

To assess responsiveness to changes in the frequency of a background tone, we adopted PPI procedures that were similar to methods described in previous studies (Aizenberg et al. 2015; Clause et al. 2011, 2017). Previous work has shown that inhibitory neurons in the auditory cortex influence performance on a similar behavioral procedure (Aizenberg et al. 2015). A total of 10 control (5 males, 5 females) and 15 enucleated mice (5 males, 10 females) were used in this experiment.

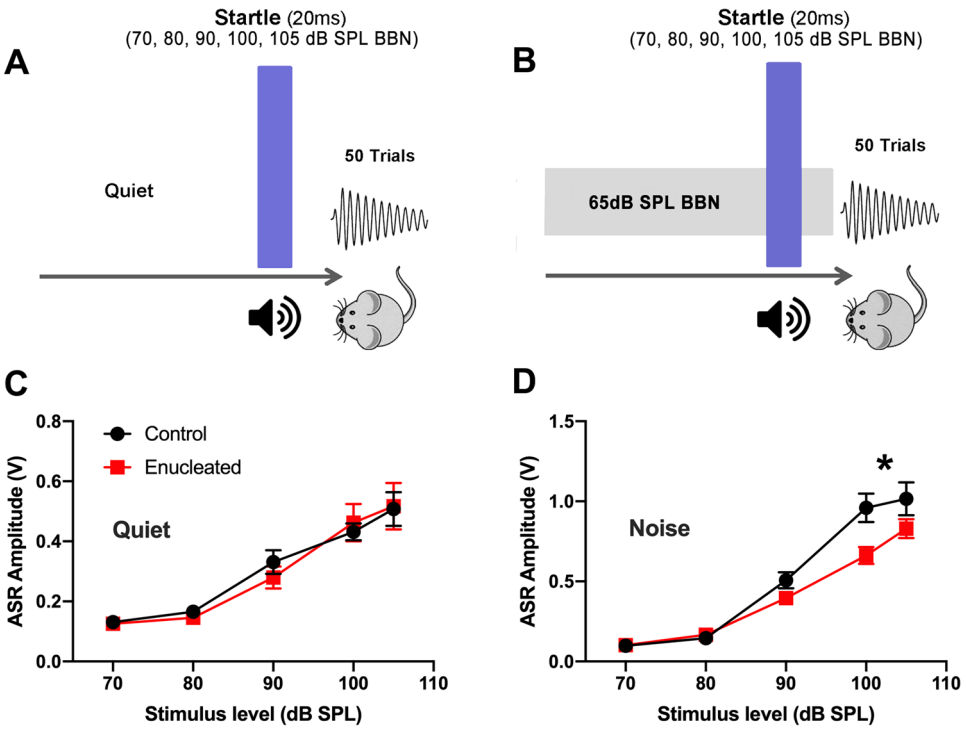

Fig. 3 Acoustic startle response (ASR) in control ($n=15$) and enucleated ($n=15$) mice measured as a function of stimulus level in quiet and 65-dB SPL broadband background noise. **A**, **B** Schematic diagram depicting the stimulus parameters. Startle-eliciting noise bursts were presented at a range of levels in pseudorandom order in quiet or noisy backgrounds. **C**, **D** Average ASR amplitudes in quiet and noisy backgrounds for all trials. *$p<0.05$; error bars indicate SEM

The startle-eliciting stimuli were short 20-ms broadband noise bursts presented at 105 dB (Z) SPL. Startle stimuli were presented immediately after a prepulse, during which the background tone frequency (F2) changed from the 70-dB (Z) SPL, 10-kHz tone background (F1) as shown in Fig. 5A, B. Control trials consisted of a startle-eliciting stimulus with no frequency change ($\Delta F = 0\%$) during an 80-ms prepulse. Test trials consisted of startle-eliciting stimulus presentation immediately after an 80-ms prepulse. For both control and test trials, following startle stimulus presentation, F1 was presented again until the next prepulse of the next trial. Eight frequencies (F2: 7, 8, 9, 9.5, 10.5, 11, 12, 13 kHz) were used as prepulses, which correspond to ΔF of $-30, -20, -10, -5, +5, +10, +20$, and $+30\%$, respectively, and were presented in pseudorandom order. Each session consisted of total of 11 blocks, each consisting of 8 test trials and 1 control trial. The ASR amplitude was defined as the maximum startle voltage within a 100-ms window after the startle stimulus onset. Each test session lasted for 45–50 min, and mice were returned to their home cage at the end of testing. For analysis, the first block was excluded to reduce variability; therefore, only the subsequent 10 blocks were used. Since d'-like estimates of sensitivity do not exceed 1.0 in PPI tests, we did not calculate frequency discrimination thresholds for this experiment (Lauer et al. 2017).

Auditory Brain Stem Response

Despite being a subcortically elicited response, studies have reported that ABRs are modulated by auditory cortex ablation and microstimulation (Aedo et al. 2016; Lamas et al. 2013). As a check on auditory nerve and brain stem function, we measured ABRs in a total of 10 control (4 males, 6 females) and 15 enucleated mice (5 males, 10 females), after learned tasks and startle testing were completed. Mice were between 4 and 7 months of age. Procedures were similar to those previously reported by our lab (Kobrina et al. 2020; Lauer 2017; McGuire et al. 2015; Schrode et al. 2018). Briefly, mice were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine via intraperitoneal injection. Mice were then placed on a heating pad inside of a sound-attenuating chamber (Controlled Acoustic Environments) lined with Sonex® acoustic foam (Pinta Acoustics). A temperature probe was inserted in the rectum or under the abdomen to monitor animal's body temperature during testing. Subdermal platinum needle electrodes (E2, Grass Technologies, West Warwick, RI) were placed behind the ventral edge of the pinna of the left ear (inverting), on the vertex of the skull (non-inverting), and in the hind leg (ground) for differential recording.

ABR stimuli were generated by a custom-made MATLAB program interfacing with TDT System 3 hardware

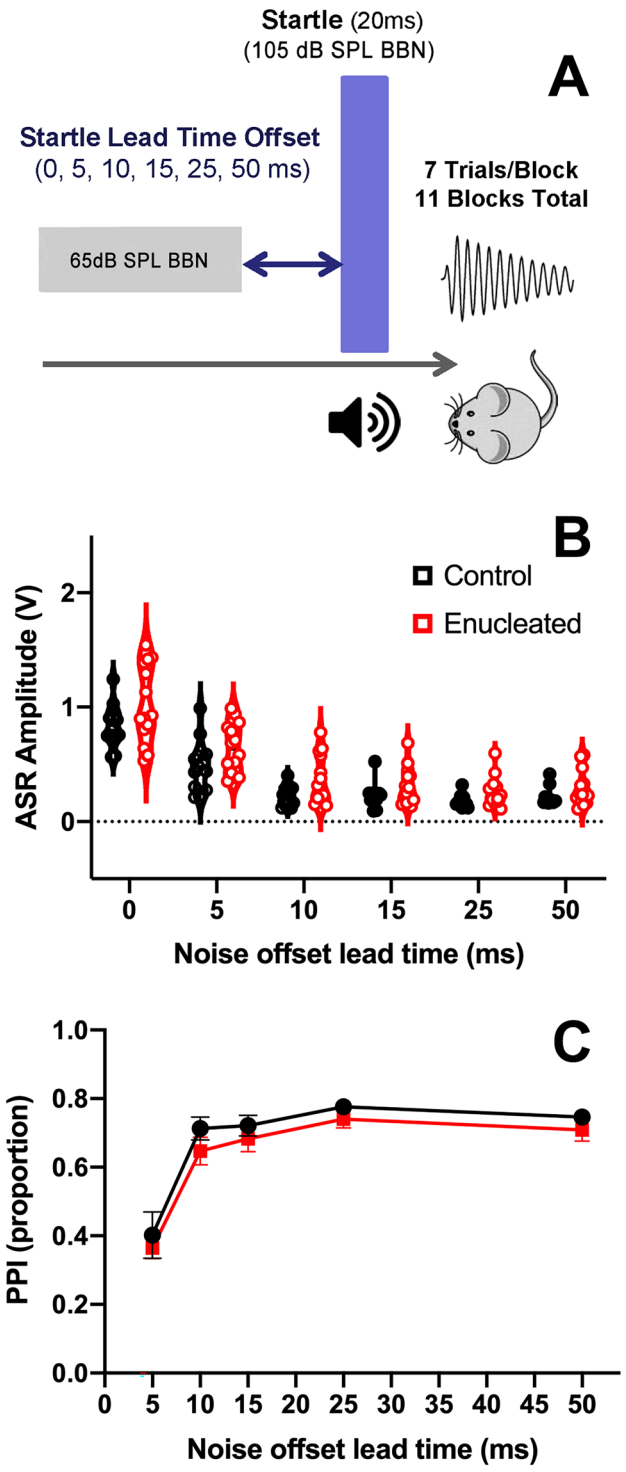

Fig. 4 Acoustic startle response (ASR) in control ($n=13$) and enucleated ($n=14$) mice measured in a noise offset prepulse inhibition (PPI) test. **A** Schematic diagram depicting the abrupt offset of a broadband noise (BBN) background occurring at variable lead times before the startle-eliciting stimulus. **B** Distribution of raw ASR amplitudes for each lead time for control and enucleated mice. Dots indicate individual data points. **C** Average proportion PPI for control and enucleated mice. Error bars indicate SEM

modules. ABR stimuli were amplified (Crown CH1, Crown Audio Inc., Elkhart, IN) and delivered through a free-field dome tweeter (FD28D, Fostex, Tokyo, Japan), placed at 0°, 30 cm away from the animal's head. Speaker output was calibrated with a free-field 1/4″ microphone (type 4939, Brüel & Kjær, Nærum, Denmark) placed at the position of the pinna, and the output of the speaker in response to frequency sweeps was analyzed using a custom MATLAB-based Golay code and software interface designed for the setup.

ABR stimuli consisted of 1-ms clicks (square pulse) and 5-ms tone pips (0.5-ms rise/fall) of varying frequencies (8, 12, 16, 24, 32 kHz) and were presented with alternating polarity at a rate of 20/s. ABR signals were recorded over a 30-ms epoch beginning immediately after stimulus onset. ABR signals from differential recordings were amplified × 300,000 by a preamplifier (ISO-80, Isolated Bio-Amplifier, World Precision Instruments, Sarasota, FL) and a custom-made amplifier, band pass filtered 300–3000 Hz (Krohn-Hite model 3550, Krohn-Hite Corporation, Avon, MA), digitized (RX6 multifunction processor, TDT), and averaged across 300 presentations. ABR signal acquisitions and recordings were controlled through a custom-made MATLAB program. Stimuli were presented in descending sound levels in 5–10-dB increments until a threshold was determined. Thresholds were statistically determined by ABR input/output function as the stimulus level that produced a peak-to-peak ABR signal that was 2 standard deviations (+2 SD) above the average background noise. Peak-to-trough amplitudes and peak latencies of 70-dB peSPL click-evoked ABR waves 1–4 were measured by an observer who was blind to the sight conditions of the animals.

Data Analysis

For PPI tests, the proportion of PPI was calculated as ((mean ASR amplitude control trials − mean ASR amplitude test trials)/mean ASR amplitude control trials) × 100. In other words, the proportion of PPI for each test condition was obtained by normalizing the difference between the mean ASR amplitudes for control trials and the mean ASR amplitudes for test trials for each animal. Statistical analysis was performed using GraphPad Prism (GraphPad Software version 9, Inc., La Jolla, CA) and R (R Core Team, 2014) software for statistics, and MATLAB, Prism, and OriginPro 7.5 (OriginLab Corporation, Northampton, MA) were used to generate figures. Two-way repeated measures analysis of variance (ANOVA) or Student's *t*-tests were used to compare statistical differences between groups. An alpha level of $p<0.05$ was considered to be statistically significant for main effects and interactions, and effect sizes were computed. Where main effects or interactions were statistically significant,

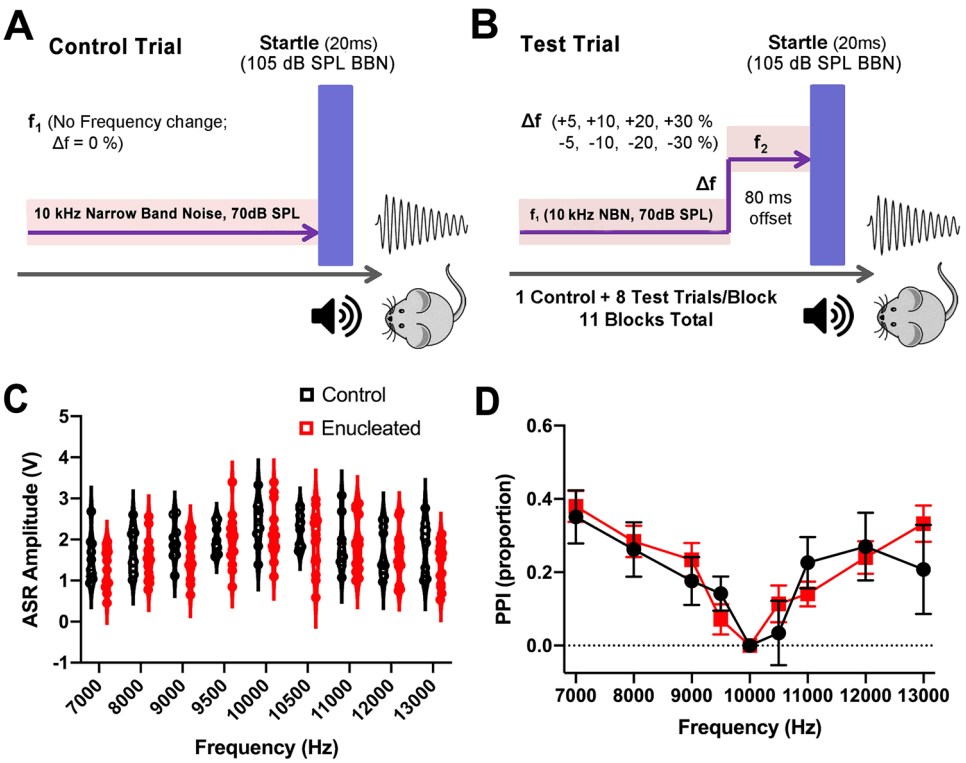

FIG. 5 Acoustic startle response (ASR) in control ($n = 10$) and enucleated ($n = 15$) mice measured in a frequency change prepulse inhibition (PPI) test. **A** Schematic diagram depicting the stimulus parameters for control trials consisting of a startle-eliciting stimulus in the presence of a constant-frequency tone background. **B** Schematic depicting the stimulus parameters for test trials in which an increment or a decrement in the background tone frequency occurs prior to a startle-eliciting stimulus. **C** Distribution of raw ASR amplitudes in response to each frequency condition for control and enucleated mice. **D** Average proportion PPI in response to each frequency condition for control and enucleated mice. Error bars indicate SEM

post hoc tests with Bonferroni or Sidak's adjustments for multiple comparisons were used to further determine statistical differences between specific pairwise comparisons. All data are shown as mean ± standard error of the mean (SEM), unless otherwise noted.

RESULTS

Conditioned Lick Suppression

Tone detection thresholds were measured using a behavioral task that requires the animals to attend to sounds and make decisions about what they heard, in contrast to the reflexive startle-based procedures. All mice from both groups learned to perform the task within 2–3 weeks. Tone detection thresholds were similar between control and enucleated groups for 8- and 12-kHz tones (Fig. 1B). Data were pooled across frequencies, and a two-tailed Student's t test indicated no significant difference between the groups ($t(16) = 1.281$, $p = 0.2186$). After completion of testing on the tone detection task, a subset of mice was trained to perform a frequency discrimination task. Since this task is difficult for mice to learn and requires a multiphase training paradigm, the number of sessions to advance from one phase to the next was tracked to identify any potential differences in learning between the two groups. No difference in the number of sessions required to graduate from one phase to the next was observed between control and enucleated groups for any phase (Fig. 1C). A two-way repeated measures ANOVA indicated that the main effects of sight condition ($F(1, 6) = 0.1088$, $p = 0.7527$, $\eta_p^2 = 0.0179$) and phase ($F(1, 6) = 2.627$, $p = 0.1513$, $\eta_p^2 = 0.3045$) were not significant, and the interaction between these factors was not significant ($F(2, 12) = 0.1606$, $p = 0.8534$, $\eta_p^2 = 0.0261$). Frequency discrimination thresholds were similar across groups for both the no-rove (phase 3) and roving (phase 4) level conditions (Fig. 1D). A two-way repeated measures ANOVA indicated that the main effects of sight condition ($F(1, 6) = 1.398$, $p = 0.2818$, $\eta_p^2 = 0.3742$) and rove ($F(1, 6) = 0.0044$, $p = 0.9490$, $\eta_p^2 = 0.0007$) were not significant, and the interaction between these factors was not significant ($F(1, 6) = 4.418$, $p = 0.0803$, $\eta_p^2 = 0.4241$).

ASR Habituation

To test the animals' ability to habituate to repeated sound stimulation, we tested short-term ASR habituation by exposing the mice to repeated presentation of 100-dB (Z) SPL BBN stimuli (Fig. 2A). Control and enucleated mice showed similar trends across 15 trials, with fluctuating ASR amplitudes across trials. The number of trials in which a startle was observed was not different between groups for either test day, although day 2 included slightly more no-ASR trials for each group (Fig. 2B). Overall, neither group of mice showed habituation to the startle-eliciting stimulus (Fig. 2C). We first compared both groups on days 1 and 2 separately to look for short-term habituation within a session. For day 1, there was not a significant main effect of group ($F(1, 24) = 1.24$, $p = 0.2773$, $\eta_p^2 = 0.0092$) or trial number ($F(14, 336) = 0.76$, $p = 0.6198$, $\eta_p^2 = 0.0308$) on ASR amplitudes. Furthermore, the interaction of group × trial was not significant ($F(14, 308) = 0.51$, $p = 0.9286$, $\eta_p^2 = 0.0207$). The mean and standard deviation of ASR amplitude across trials on day 1 was 0.4101 (± 0.0798) for controls and 0.4739 (± 0.0782) for enucleated mice. For day 2, there was not a significant main effect of group ($F(1, 22) = 0.01$, $p = 0.9216$, $\eta_p^2 = 0.0001$) or trial number ($F(14, 308) = 0.67$, $p = 0.6646$, $\eta_p^2 = 0.0308$) on ASR amplitudes. Furthermore, the interaction of group × trial was not significant ($F(14, 308) = 0.51$, $p = 0.9286$, $\eta_p^2 = 0.0294$). The mean and standard deviation of ASR amplitude across trials on day 2 was 0.5180 (± 0.0977) for controls and 0.5095 (± 0.0998) for enucleated mice.

To determine if there were long-term habituation effects across sessions, we compared ASR trials on day 1 and day 2 for control and enucleated subjects separately. For control mice, there was not a significant main effect of test day ($F(1, 19)) = 1.25$, $p = 0.2775$, $\eta_p^2 = 0.0198$), and the effect of trial was not significant ($F(14, 266) = 0.52$, $p = 0.7148$, $\eta_p^2 = 0.0266$), as expected. The interaction between factors was not significant ($F(14, 266) = 0.46$, $p = 0.9519$, $\eta_p^2 = 0.0237$). For enucleated mice, there was not a significant main effect of test day ($F(1, 27) = 0.49$, $p = 0.4912$, $\eta_p^2 = 0.00025$), and the effect of trial was not significant ($F(14,378) = 0.63$, $p = 0.7572$, $\eta_p^2 = 0.0229$), as expected. The interaction between factors was not significant ($F(14, 378) = 0.95$, $p = 0.5090$, $\eta_p^2 = 0.0338$).

Acoustic Startle Response in Quiet and Background Noise

To examine the animals' behavioral reactivity to sound, we measured ASR in quiet and in the presence of continuous background noise (Fig. 3A,). In the quiet condition, a gradual growth of ASR amplitudes with increasing startle stimulus level was observed in all groups (Fig. 3C). A two-way repeated measures ANOVA indicated a significant main effect of startle stimulus level ($F(4,108) = 48.54$, $p < 0.0001$, $\eta_p^2 = 0.6425$) on ASR amplitudes. The main effect of group was not significant ($F(1,27) = 0.0354$, $p = 0.8521$, $\eta_p^2 = 0.001$), indicating there was no difference in ASR amplitudes between control and enucleated mice. Additionally, no significant interaction of startle stimulus level × group was found ($F(4, 108) = 0.3966$, $p = 0.8521$, $\eta_p^2 = 0.0015$).

ASR amplitudes were larger for controls compared to the enucleated mice when startle stimuli were presented in continuous noise (Fig. 3D). A two-way repeated measures ANOVA showed a significant main effect of startle stimulus level ($F(4, 112) = 130.08$, $p < 0.0001$, $\eta_p^2 = 0.8229$) and a significant main effect of group ($F(1, 28) = 4.950$, $p = 0.0343$, $\eta_p^2 = 0.486$) on ASR amplitudes. There was a significant interaction between startle stimulus level × group ($F(4, 112) = 4.18$, $p = 0.0034$, $\eta_p^2 = 0.13$). A post hoc analysis with Sidak's multiple comparisons test revealed that the difference in ASR amplitude between groups was only statistically significant for the 100-dB (Z) SPL startle stimulus level ($p = 0.0427$).

We also considered the possibility of sex differences in startle reflex modification by non-startle-inducing stimuli, in this case the background noise, contributing to the observed effects. Such effects have been reported in the literature for other species, but rarely in mice (Lauer et al. 2017). We compared amplitudes of ASR in noise for trials with startle responses for the 90-, 100-, and 105-dB SPL stimulus conditions for each group of mice separately. For control mice, there was not a significant main effect of sex ($F(1, 13) = 0.26$, $p = 0.6196$, $\eta_p^2 = 0.0641$), but the main effect of stimulus level was significant, as expected based on the previous analyses ($F(2, 26) = 22.58$, $p < 0.0001$, $\eta_p^2 = 0.6346$). The interaction between factors was not significant ($F(2, 26) = 0.37$, $p = 0.6957$, $\eta_p^2 = 0.0275$). For enucleated mice, there was not a significant main effect of sex ($F(1, 13) = 0.78$, $p = 0.3919$, $\eta_p^2 = 0.0609$), but the main effect of stimulus level was significant, as expected based on the previous analyses ($F(2, 26) = 20.35$, $p < 0.0001$, $\eta_p^2 = 0.6102$). The interaction between factors was not significant ($F(2, 26) = 1.16$, $p = 0.3288$, $\eta_p^2 = 0.082$). It must be noted that our sample sizes may be too small to detect small statistically significant sex effects on startle behavior in CBA/CaJ mice (Ison and Allen 2007; Longenecker et al. 2018). However, the sex differences in ASR amplitude in the present study do not even come close to approaching significance.

For subsequent ASR-based tests, a startle-inducing stimulus level of 105 dB SPL was used to avoid differences in baseline startle between the two groups.

ASR Noise Offset Lead Time

The ASR amplitude is reduced if a short gap precedes a startle-eliciting stimulus, in which the silent gap acts as a prepulse (Ison 1982; Ison et al. 1998). To investigate temporal acuity to sound with a measure that does

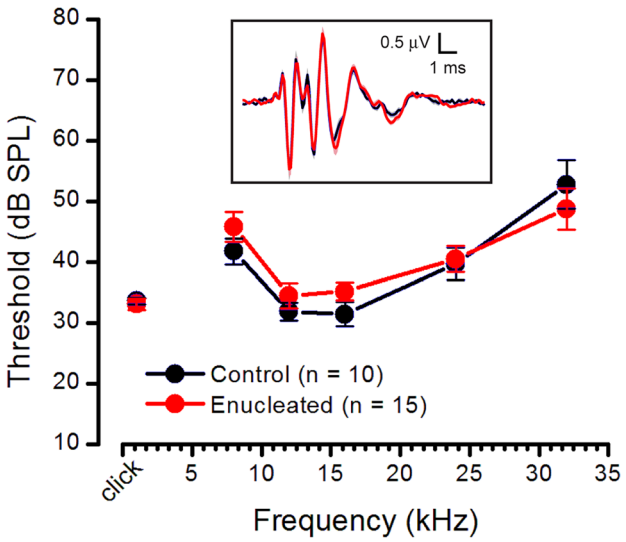

Fig. 6 Auditory brainstem response (ABR) thresholds for tone pips and click stimuli in control (n = 10) and enucleated (n = 15) mice. Inset depicts grand average 70 dB pe SPL click-evoked waveforms for each group

not involve the auditory cortex (Bowen et al. 2003), we tested ASR noise offset lead time, where 65-dB (Z) SPL background noise was abruptly interrupted by noise offset immediately before startle presentation (Fig. 4A) (Bowen et al. 2003; Ison and Allen 2003; Stitt et al. 1974). Control and enucleated groups both showed decrements in raw ASR amplitudes in response to noise offset lead time, although there was slightly more variability in the raw ASR amplitudes observed in the enucleated groups (Fig. 4B). However, the proportion of PPI increased similarly for both groups as the noise offset lead time increased in duration (Fig. 4C). A two-way repeated measures ANOVA revealed a significant main effect of noise offset lead time on the proportion of PPI ($F(5, 115) = 107.21$, $p < 0.0001$, $\eta_p^2 = 0.63$). The main effect of group was not significant ($F(1, 23) = 2.21$, $p = 0.15$, $\eta_p^2 = 0.018$), and no significant interaction of noise offset lead time × group was found ($F(5, 115) = 0.82$, $p = 0.54$, $\eta_p^2 = 0.0049$).

ASR Frequency Difference Limens

To test the animal's frequency discrimination ability, we used frequency changes as an adapted version of a prepulse inhibition test (Aizenberg et al. 2015; Clause et al. 2011; Stitt et al. 1974). ASR is attenuated or inhibited if a relatively weaker stimulus precedes the startle-eliciting stimulus, known as prepulse inhibition (PPI). In the frequency difference limens (FDL) ASR test, changes in background frequency served as a prepulse (Fig. 5A, B). ASR amplitudes were not different between groups (Fig. 5C). A two-way repeated measures ANOVA indicated a significant main effect of frequency (ΔF) ($F(8, 184) = 17.74$, $p < 0.0001$, $\eta_p^2 = 0.4354$) on PPI. The main effect of group was not significant ($F(1, 23) = 0.95$, $p = 0.3387$, $\eta_p^2 = 0.1004$). Furthermore, no significant interaction of frequency × group was found ($F(8, 184) = 1.16$, $p = 0.3252$, $\eta_p^2 = 0.0481$). Control and enucleated mice showed similar PPI to background frequency change (Fig. 5D), where the proportion of PPI increased as the frequency change (ΔF) from background became greater. A two-way repeated measures ANOVA indicated a significant main effect of frequency (ΔF) ($F(8, 184) = 16.27$, $p < 0.0001$, $\eta_p^2 = 0.23$) on PPI. The main effect of group was not significant ($F(1, 23) = 0.05$, $p = 0.82$, $\eta_p^2 < 0.0001$). Furthermore, no significant interaction of frequency × group was found ($F(8, 184) = 1.46$, $p = 0.17$, $\eta_p^2 = 0.0021$).

Auditory Brain Stem Response

To test whether there was a difference in hearing sensitivity between control and enucleated mice under unattended conditions and to verify the health of peripheral and brain stem pathways, we measured ABRs at the completion of behavioral learned tasks and startle tests. Control and enucleated mice showed normal ABR thresholds, with no signs of hearing loss, increased sensitivity, or abnormal wave morphology (Fig. 6). We did not find any significant difference in the ABR thresholds between groups, indicating that both control and enucleated mice maintained normal hearing sensitivity. A two-way repeated measures ANOVA indicated a significant main effect of ABR frequency ($F(5, 115) = 26.34$, $p < 0.0001$, $\eta_p^2 = 0.39$) on ABR thresholds. No significant main effect of group was found ($F(1, 23) = 0.25$, $p = 0.62$, $\eta_p^2 = 0.0027$). Further, no significant interaction of frequency × group was found ($F(5, 115) = 1.19$, $p = 0.32$, $\eta_p^2 = 0.018$).

We also measured amplitudes and latencies of peaks 1–4 of ABRs in response to 70-dB peSPL clicks. A two-way repeated measures ANOVA indicated there was no significant effect of the interaction between group and peak ($F(3, 57) = 0.31$, $p = 0.785$, $\eta_p^2 = 0.01$), nor of the main effect of group ($F(1, 22) = 0.66$, $p = 0.427$, $\eta_p^2 = 0.03$), on amplitudes. Similarly, the group × peak interaction ($F(3, 57) = 0.17$, $p = 0.821$, $\eta_p^2 = 0.01$) and group effect ($F(1, 22) = 2.35$, $p = 0.140$, $\eta_p^2 = 0.10$) were not statistically significant for latencies. Peak did have a significant effect on both amplitudes ($F(3, 57) = 23.79$, $p < 0.001$, $\eta_p^2 = 0.52$) and latencies ($F(3, 57) = 6053.93$, $p < 0.001$, $\eta_p^2 = 0.99$), which is to be expected since the size and location of the neural generators within auditory pathways differ.

DISCUSSION

Adult-onset blindness decreases neural thresholds, enhances frequency selectivity, and increases the reliability of neural firing in the auditory cortex of mice (Meng

et al. 2015; Petrus et al. 2015). This plasticity is triggered by circuit-level changes in the primary auditory cortex that involve potentiation of thalamocortical synapses to layer 4 (Petrus et al. 2014), strengthening of layer 4 to layer 2/3 feedforward connections (Petrus et al. 2015), and refinement of intracortical circuits (Meng et al. 2015, 2017). Despite this remarkable capacity for neural plasticity, we found few effects in a battery of behavioral tests performed in mice that were blinded as young adults. The tests were chosen to assess both cortical and subcortical function as indicated by previous literature. Performance on tone detection and frequency discrimination tasks was similar in blind and control mice in a learned behavioral task. Prepulse inhibition tests, which do not require the subject to attend to a stimulus or learn to produce a specific behavioral response over many successive training days, also did not show substantial differences in performance across groups. Interestingly, the ASR in response to 100-dB SPL startle-eliciting stimuli was smaller in blind mice when the startle-eliciting stimuli were presented in noise, but not in quiet. Evoked potential measurements indicated normal hearing sensitivity and synchronous population responses in the brain stem.

Tone Detection and Frequency Discrimination

The lack of enhanced tone detection and discrimination thresholds observed in blind mice compared to sighted controls in the perceptual experiments are consistent with studies in humans that have shown little auditory perceptual enhancement associated with adult-onset blindness (Wan et al. 2010). It is likely that our subjects were already performing using the best neural information available to them across a large population of neurons to support the detection and discrimination of sounds. Therefore, ceiling effects may have been a factor in our failure to observe superior perceptual performance in blind mice. An additional factor to consider is that the daily training, testing, and attending to predictable sound frequencies inherent to the conditioned lick suppression task may have itself improved the responses of cortical neurons such that group differences were obscured by optimized neural representation of sounds in all behaving subjects. Engagement in trained behavioral listening tasks is known to refine spectrotemporal neural representations of sound (Fritz et al. 2003, 2005, 2007).

A handful of studies performed in human listeners have identified perceptual enhancements in blind adults in some listening situations. Older-blind adults can recognize time-compressed speech better than sighted adults (Gordon-Salant and Friedman 2011). It is possible that behavioral advantages only emerge during performance of more cognitively and perceptually taxing auditory tasks in which the listener must make a more complex perceptual judgement than simply indicating whether or not they detected a sound or a change in some acoustic feature of a sound. To date, procedures have not been developed for testing laboratory mice on such auditory perception tasks (Dent et al. 2018).

Spatial hearing abilities can also be enhanced in cases of adult-onset blindness (Dufour et al. 2005; Fieger et al. 2006; Voss et al. 2004), but these perceptual benefits have typically been attributed to recruitment of the visual cortex by the auditory system (Kolarik et al. 2014; Rao et al. 2007). The role of auditory cortex plasticity and the underlying synaptic mechanisms in facilitating supra-normal spatial hearing are unclear. Studies investigating the plasticity of spatial tuning of auditory cortex neurons of adult-blind animals are necessary to address this question.

Acoustic Startle Response Reactivity and Prepulse Inhibition

ASR-based measures provide a convenient measure of reactivity to sound and sensorimotor gating that can be modified by numerous manipulations to the background and preceding sounds (Ison 2001; Koch and Schnitzler 1997; Lauer et al. 2017). The mechanisms underlying many of these behavioral effects have not been thoroughly elucidated, but there is evidence that some ASR behaviors, including long-term habituation, facilitation by noise, and frequency discrimination, are controlled by cortical mechanisms, whereas other ASR behaviors, such as inhibition by a noise offset, are controlled via brain stem mechanisms (Bowen et al. 2003; Davis and Gendelman 1977; Groves et al. 1974; Leaton et al. 1985; Leaton and Supple 1986; Weber et al. 2002). Habituation to startling stimuli was not impaired in the adult-blind mice compared to controls, but we observed larger ASR in controls compared to enucleated mice for 100-dB SPL startle-eliciting stimuli presented in the presence of continuous background noise.

Facilitation of the ASR in the presence of background noise may be related to increased vigilance, and it has been reported in rats, mice, and humans (Hoffman and Fleshler 1963; Hoffman and Searle 1965, 1968; Ison 2001). The group differences in startle reactivity to sounds presented in background noise observed in the present study are suggestive of differences in central inhibitory circuits in blind versus sighted mice. Increased reactivity to loud sounds such as we observed in control mice compared to blind mice has been demonstrated in rodents with noise-induced and conductive hearing loss, and these conditions have been linked to diminished inhibition in subcortical and cortical structures (Hickox and Liberman 2013; Kotak et al. 2008; Salloum et al. 2014; Schrode et al. 2018; Sun et al. 2011). While the precise mechanism remains unclear, we can speculate that the blind mice may exhibit abnormal processing in auditory cortical inhibitory neurons or that the previously reported changes in cortical neuron sensitivity and tuning result

in top-down effects on subcortical inhibition of the ASR. Our experiments indicated that abnormal modulation of the ASR in enucleated mice may be specific to the startle-in-noise test. No differences between groups occurred for a noise offset temporal processing test or in a frequency change detection test. In aggregate, these findings indicate that adult-onset blindness does not result in overall sensorimotor gating deficits, but that specific auditory behaviors that are sensitive to plasticity in cortical inhibition may be affected. Additional behavioral and physiological experiments are necessary to confirm this hypothesis.

Evoked Potentials

As a check on the status of more peripheral auditory structures, we measured ABRs and found no difference in thresholds, wave amplitudes, or wave latencies between groups. This finding indicates that cochlear and brain stem function remains normal in adult-blind mice. It must be noted that ABR measurements in anesthetized mice may not be sensitive to top-down effects, although there is evidence that training and experience can affect ABR wave 1 and other brain stem–evoked potential measures (Bieszczad 2019; Chandrasekaran et al. 2014; Rotondo and Bieszczad 2020). As with behavior, group differences between control and enucleated mice might emerge under more challenging listening conditions. Additional studies are needed to investigate this possibility.

CONCLUSIONS

In conclusion, our results suggest that cross-modal plasticity observed in adult A1 with visual deprivation is associated with minimal changes in the battery of behavioral tasks examined in this study. While this is counter to what is predicted from the robust functional plasticity observed at the neural circuit level in A1, it suggests that not all auditory behavior is improved with visual deprivation and it is consistent with variable reports of auditory performance in blind individuals, especially related to late-onset blindness (Scheller et al. 2021; Voss et al. 2004; Wan et al. 2010). Based on the finding that auditory experience is necessary to observe plasticity in A1 related to visual deprivation (Petrus et al. 2014), it would be pertinent to explore auditory learning tasks that can better assess such experience-dependent changes to further test how vision loss affects auditory function.

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Data Availability Data will be made available upon request.

Declarations

Ethics Approval All experiments were approved by the Johns Hopkins University Animal Care and Use Committee.

Conflict of Interest The authors declare no competing interests.

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