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Bidirectional effects of aversive learning on perceptual acuity are mediated by the sensory cortex

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Although emotional learning affects sensory acuity, little is known about how these changes are facilitated in the brain. We found that auditory fear conditioning in mice elicited either an increase or a decrease in frequency discrimination acuity depending on how specific the learned response was to the conditioned tone. Using reversible pharmacological inactivation, we found that the auditory cortex mediated learning-evoked changes in acuity in both directions.

Traumatic events lead to changes in the emotional response to the environment and to changes in sensory perception[1]. These effects may underlie abnormalities in sensory perception manifested in anxiety[2]. During aversive learning, the subject learns to associate an aversive stimulus with a sensory cue. This model of emotional conditioning has been shown to affect sensory discrimination acuity[3,4]. In a recent study, aversive learning resulted in an increase in sensory discrimination acuity[3], consistent with the notion that sensory resources are consolidated around emotionally salient events. In contrast, aversive learning in a different study resulted in decreased sensory discrimination acuity[4], consistent with the translation of generalization between the emotional and sensory modalities. An important factor that differed between these experiments was the precision of the aversive conditioning task, which affects learning specificity[5]. We propose that changes in sensory discrimination acuity depend on whether the learned response is specific to the conditioned stimulus or generalizes across other stimuli. We tested this hypothesis on the basis of auditory fear conditioning (AFC) in mice, in which subjects are conditioned to associate a shock with a tone at a specific frequency. We predicted that high learning specificity, when the conditioned response is specific to the conditioned tone frequency, would lead to an increase in frequency discrimination acuity, whereas low learning specificity, when the conditioned response is generalized over a range of frequencies, would lead to a decrease in acuity. Furthermore, we examined the role of the auditory cortex, a key area for auditory plasticity, in learning specificity and discrimination acuity.

We designed a set of learning tasks that we expected to result in different levels of learning specificity (**Supplementary Fig. 1**). Classical AFC, in which conditioned tone is paired with foot shock (CS+), is expected to result in low learning specificity and high generalization to other frequencies. In discriminative AFC, in which a tone unpaired with a shock (CS−) is presented along with CS+, learning is expected to be more frequency specific. As the difference between CS+ and CS− in the frequency domain was reduced, learning specificity could further increase. Thus, we expected intermediate specificity for coarse AFC ($CS-_{coarse}$, 1 octave difference between CS+ and $CS-_{coarse}$) and highest specificity for fine AFC ($CS-_{fine}$, 0.23 (15%) octave difference between CS+ and $CS-_{fine}$). To measure learning specificity and perceptual acuity independently of each other, we used two distinct methods (**Supplementary Figs. 1** and **2**). We evaluated learning specificity 24 h after conditioning (**Supplementary Fig. 1a**) as the difference in freezing responses to the conditioned tone and test tones (**Supplementary Fig. 1b**,**c**). Another task, administered in a new context, was used to estimate frequency discrimination acuity. We used a pre-pulse inhibition (PPI) protocol based on measurement of the suppression in the acoustic startle response (ASR) by a warning signal preceding the startle noise. PPI increased with an increase in the saliency of the warning signal, which depended on the frequency shift between the background and the pre-pulse tones (**Supplementary Fig. 2**). Acuity was reported as the frequency discrimination threshold (θ), defined as the frequency shift eliciting 50% of the maximum PPI[6].

We first confirmed that varying learning tasks led to different levels of learning specificity. Learning specificity was lowest for classical AFC, intermediate for coarse AFC and highest for fine AFC (**Fig. 1a**). Notably, these tasks led to different effects on perceptual acuity. Classical and coarse AFC, resulting in low learning specificity, induced an increase in θ. Notably, following fine AFC, learners exhibited decreased θ (learners exhibited at least 10% difference in freezing responses to CS+ and CS−; **Fig. 1b** and **Supplementary Fig. 3**). These results indicate that learning tasks of different precision evoke bidirectional changes in sensory discrimination acuity.

Several controls ensured that the observed changes in θ were the result of the differences between the learning tasks. Changes in θ required the association of CS+ with the aversive stimulus: pseudo-conditioning induced no elevated freezing to tones and no change in θ (**Supplementary Fig. 4**). Decreased θ following AFC did not result from an increase in the number of training sessions as repeating coarse AFC twice preserved, rather than reversed, the increase in θ (**Supplementary Fig. 5**).

Because fine AFC led to a wide range of learning specificity across mice, we quantified the relation between changes in θ with individual levels of learning specificity. Learners exhibited high, and non-learners exhibited low, learning specificity (**Fig. 2a**). The different levels of learning specificity led to different changes in acuity. Learners exhibited a decrease in θ, whereas non-learners exhibited an increase in θ (**Fig. 2b**). Furthermore, across individuals, the level of learning specificity was significantly negatively correlated with θ

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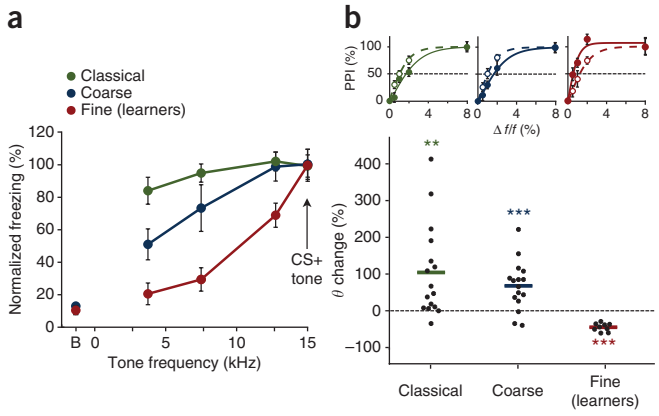

Figure 1 AFC resulting in low and high learning specificity led to an increase and a decrease in frequency discrimination threshold (θ), respectively. (**a**) Classical ($n = 8$, green), coarse ($n = 8$, blue) and fine AFC ($n = 4$ learners, red) resulted in low, medium and high learning specificity (repeated-measures ANOVA, between-subject factor: conditioning type, $F_{2,17} = 28.8$, $P = 0.000003$). Shown are freezing responses to CS+, three test tones and baseline (B). (**b**) θ increased following classical ($n = 15$, $t_{14} = -3.6$, $P = 0.0029$) and coarse AFC ($n = 16$, $t_{15} = -4.1$, $P = 0.0009$), and decreased following fine AFC in learners ($n = 9$, $t_8 = 5.4$, $P = 0.0007$, paired t test). Top, baseline PPI (open circles) and PPI following AFC (closed circles) as a function of frequency shift. Dashed horizontal line indicates 50% of maximum startle suppression used to determine θ. Each data point represents average across subjects. Bottom, θ change following classical, coarse and fine AFC in learners. Here and in subsequent figures, n refers to the number of mice, error bars represent s.e.m., dots in scatter plots represent datum for an individual subject and horizontal bars depict average across subjects; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; n.s., not significant ($P > 0.05$).

(**Fig. 2c**). These results suggest that the effect of learning on sensory acuity is governed not only by the precision of the learning task, but also by the individual level of learning specificity.

The auditory cortex is important for learning-induced plastic changes in acoustic representation[7,8]. To test the role of auditory cortex in the observed changes in learning specificity and perceptual discrimination, we inactivated the auditory cortex by local infusion of fluorescent muscimol during testing following coarse or fine AFC (**Supplementary Fig. 6a**). Muscimol diffusion and its effect on click-evoked local field potentials were restricted to the auditory cortex (**Supplementary Figs. 6b** and **7**). Infusion of muscimol in the auditory cortex did not induce a change in θ without fear conditioning (**Supplementary Fig. 6c**). These results are consistent with classical studies demonstrating that lesions of the auditory cortex do not impair frequency discrimination acuity[9]. However, inactivation of the auditory cortex reversibly abolished the bidirectional effects of coarse and fine AFC on θ (**Fig. 3a** and **Supplementary Fig. 6d**). Notably, specificity of learning was unchanged during inactivation (**Fig. 3b**, **Supplementary Fig. 6e**). The change in θ after fine AFC was no longer correlated with learning specificity across individuals following muscimol injection (**Fig. 3c**). The expected bidirectional changes in θ following coarse and fine AFC, as well as the negative correlation between change in θ and individual learning specificity, recovered 24 h later (**Fig. 3a,c**). The effect of muscimol was not a result of the injection or cannula implantation, as infusing the fluorescent vehicle instead of muscimol preserved the expected bidirectional changes in θ following fine and coarse AFC (**Fig. 3a**) and the negative correlation between change in θ and learning specificity following fine AFC (**Fig. 3c**). Furthermore, the effect of muscimol was not systemic, as infusing muscimol in the somatosensory cortex preserved the expected increase in θ after coarse AFC, as well as the negative correlation between change in θ and learning specificity after fine AFC (**Supplementary Fig. 8**). These results demonstrate that the auditory cortex mediates both the increase and the decrease in sensory acuity evoked by fear conditioning without affecting learning specificity.

We found that the effect of auditory aversive learning on frequency discrimination is governed by how specific learning is to the conditioned tone. Low learning specificity led to decreased acuity, whereas high learning specificity led to increased acuity. Moreover, we identified a previously unknown specific role of the auditory cortex in emotional learning: the auditory cortex controls learning-induced changes in frequency discrimination acuity, but not the specificity of learning. Although previous studies have found that the amygdala is important for the generalization or specificity of learned fear[10], the effects of varying learning specificity levels on auditory perception have not previously been measured. Our results demonstrate a dissociation between the neuronal mechanisms underlying learning specificity and changes in perceptual acuity and suggest a top-down cortical control of learning-evoked changes in sensory acuity.

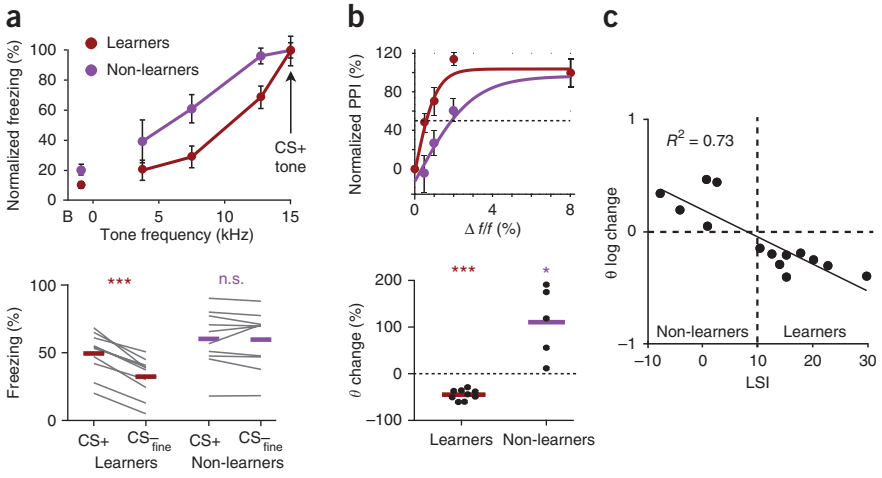

Figure 2 Learning specificity following fine AFC was negatively correlated with the frequency discrimination threshold. (**a**) Learners exhibited higher learning specificity than non-learners by freezing less to three test tones (top) or $CS-_{fine}$ (bottom) than to CS+ (top: each group $n = 4$, repeated-measures ANOVA, between-subject factor: conditioning type, $F_{1,6} = 8.7$, $P = 0.025$; bottom: each group $n = 10$; learners, $t_9 = 4.8$, $P = 0.00096$; non-learners, $t_9 = 0.3$, $P = 0.79$; paired t test). (**b**) Learners exhibited decreased θ ($n = 9$, $t_8 = 5.4$, $P = 0.0007$), whereas non-learners exhibited increased θ ($n = 5$, $t_4 = -3.18$, $P = 0.034$, paired t test). Top, PPI as a function of frequency shift after fine AFC for learners (closed circles) and non-learners (open circles). Bottom, θ change for learners and non-learners. (**c**) θ was negatively correlated with learning specificity index (LSI) for all mice trained on fine AFC (Pearson = –0.86, $P = 0.00009$). LSI was defined as the difference between freezing to CS+ and $CS-_{fine}$. The line represents the linear fit.

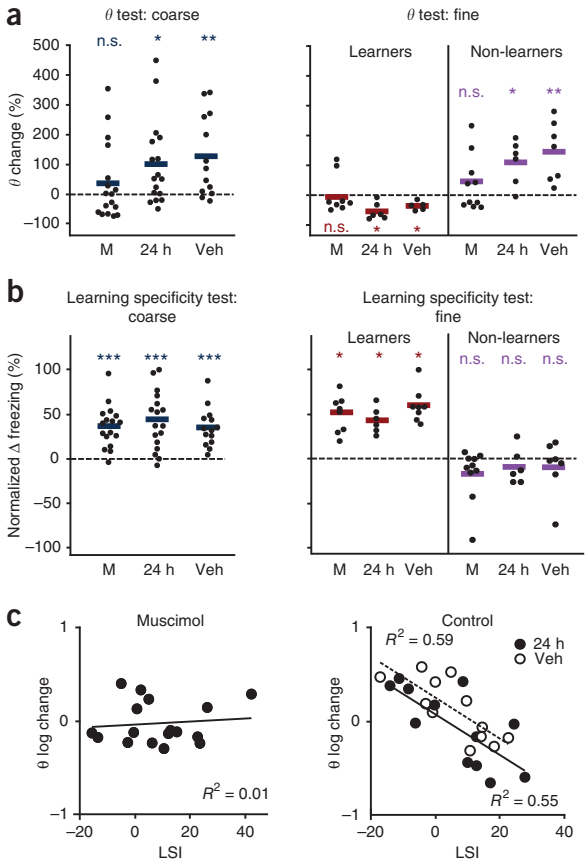

Figure 3 Inactivation of the auditory cortex reversibly canceled the effect of AFC on θ, but did not affect learning specificity. (**a**) Inactivation of the auditory cortex by local infusion of muscimol (M) canceled the effect of AFC on θ (coarse: $n = 17$, $Z = -0.21$, $P = 0.84$; fine learners: $n = 8$, $t_7 = 0.47$, $P = 0.65$; fine non-learners: $n = 10$, $t_9 = -1.1$, $P = 0.62$). This effect was reversed when tested 24 h later (24 h) in the subjects that retained their learning performance (coarse: $n = 17$, $Z = 2.39$, $P = 0.034$, Wilcoxon signed test with Bonferroni correction; fine learners: $n = 6$, $t_5 = 3.9$, $P = 0.022$; fine non-learners: $n = 6$, $t_5 = -3.2$, $P = 0.046$, paired t test with Bonferroni correction). Control vehicle-injected subjects (Veh) tested on days 2 and 6 displayed expected changes in θ (coarse: $n = 13$, $t_{12} = -3.3$), $P = 0.007$; fine learners: $n = 5$, $t_4 = 3.1$, $P = 0.036$; fine non-learners: $n = 7$, $t_6 = -4.0$, $P = 0.0072$; paired t test with Bonferroni correction). (**b**) Inactivation of the auditory cortex before the test session preserved the specificity of learning manifested by differential freezing to CS+ and CS– in coarse (left; $n = 18$, $Z = -3.7$, $P = 0.0002$) and fine AFC (right; learners, $n = 8$ mice, $Z = -2.5$, $P = 0.012$; non-learners, $n = 10$, $Z = -0.36$, $P = 0.72$, Wilcoxon signed test). The differential freezing response to CS+ and CS– was preserved 24 h after muscimol infusion (24 h) after coarse AFC ($n = 18$, $Z = -3.6$, $P = 0.0003$) and after fine AFC (learners: $n = 6$, $Z = -2.2$, $P = 0.028$; non-learners: $n = 6$, $Z = -1.2$, $P = 0.25$). Vehicle-infused control mice also exhibited the same pattern of differential freezing response to CS+ and CS– after coarse AFC ($n = 14$, $Z = -3.3$, $P = 0.001$) and after fine AFC (learners: $n = 7$, $Z = -2.4$, $P = 0.018$; non-learners: $n = 7$, $Z = -0.51$, $P = 0.61$; Wilcoxon signed test). Two-way ANOVA confirmed no effect of muscimol treatment ($F_{2,84} = 0.1$, $P = 0.89$) and a significant reduction of differential freezing in non-learning group ($Z_{2,84} = 36$, $P < 0.000001$). (**c**) Correlation between learning specificity index and change in θ after fine AFC was abolished by muscimol infusion (left; Pearson correlation = 0.11, $P = 0.685$), but recovered 24 h later (bottom, closed circles; Pearson correlation = –0.77, $P = 0.006$), and was spared in vehicle-infused mice (bottom open circles; Pearson correlation = –0.78, $P = 0.004$).

We measured frequency discrimination acuity using a modified PPI of the acoustic startle reflex task. ASR is mediated largely by the caudal pontine reticular nucleus, whereas PPI relies on multiple nuclei in the brainstem[11,12]. The frequency discrimination threshold, which was measured via PPI before emotional conditioning, was therefore likely a reflection of auditory responses of neurons in subcortical areas, such as the inferior colliculus[13]. The subcortical brain areas that are involved in PPI receive strong feedback from the cortex[12]. Neurons in the inferior colliculus in particular alter their auditory response properties depending on feedback from the auditory cortex[14], and cortico-collicular feedback is involved in learning-induced changes in representation of acoustic features, such as sound location[15]. Because neurons in the auditory cortex exhibit learning-induced changes in representation of acoustic features[16,17], the auditory cortex may modulate the behaviorally measured frequency discrimination threshold via its feedback onto the subcortical structures, following emotional learning. These changes may be mediated by the interaction of the inputs from the amygdala and the local cortical circuitry[18]. Alternatively, the auditory cortex may function as a relay nucleus for attention-based modulation from the amygdala. Feedback from the pre-frontal cortex may also be important, controlling the specificity of contextual learning[19]. Although our data indicate the importance of negative emotion, other learning tasks may obey a similar rule[5].

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

M.A. and M.N.G. designed the experiments, analyzed the data, prepared the figures and wrote the manuscript. M.A. carried out the experiments.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animals. All experiments were performed in adult male mice (C57BL/6J, $n = 100$, 12–15 weeks of age, 22–32 g), housed with at most five mice to a cage, at 28 °C on a 12-h light:dark cycle with water and food provided *ad libitum*. All experiments were performed during the animals' dark cycle. All experimental procedures were in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania. Simple randomization was used to assign the subjects to the experimental groups. Blinding was not possible as animals in different groups underwent different experimental protocols and analysis.

Surgery. Mice were anesthetized under isoflurane (1.5–2%, vol/vol). A small craniotomy was performed over the target stereotaxic coordinates relative to bregma, −2.6 mm anterior, ±4 mm lateral, +2 mm ventral. Custom-cut guide cannulas (Plastics One) were lowered in the brain and secured to the skull using dental cement (C&B Metabond) and acrylic (Lang Dental). For post-operative analgesia, Buprenex (0.1 mg per kg of body weight) was injected intraperitonially and lidocaine was applied topically to the surgical site. An antibiotic (0.3% wt/vol gentamicin sulfate) was applied daily (for 7 d) to the surgical site during recovery.

Cannula infusions. Mice were sedated by isoflurane (1%). 0.4 µl of 0.8 mM muscimol conjugated with the Bodipy TMR-X fluorophore or Bodipy TMR-X alone (Vehicle) (Life Technologies)[20] dissolved in phosphate-buffered saline was infused bilaterally via a thin internal cannula inserted in the implanted guide (Plastics One).

Histology. Images of coronal sections (50 µm) of fixed brain tissue were digitally acquired using a fluorescent microscope (Olympus) equipped with Texas Red filters (Chroma). To visualize the spread of muscimol, images from different mice ($N = 18$) were manually aligned along the brain contours, and automatically superimposed by averaging the intensity values of each pixel on the red channel (Matlab).

Experimental setup. During AFC, the mouse was placed in a conditioning cage with a shock floor (Coulbourn) inside a sound attenuation cubicle (Med Associates) housed inside a single-walled acoustic chamber (Industrial acoustics). During learning specificity tests, a custom-made test cage of similar size but different floor and wall pattern and color was used. Auditory stimuli were provided by a free-field magnetic speaker (Tucker-Davis Technologies). Electric shock (0.5 mA, 0.5 s) was delivered by a precision animal shocker (Coulbourn). Freezeframe-3 software (Coulbourn) was used for stimulus control and analysis of animal behavior. During the PPI procedure, the mouse was placed in a custom-made tube on the sensor plate (San Diego Instruments). The speaker, housing, platform and webcam (Logitech) were placed in the sound attenuation cubicle (Med Associates) housed inside a single-walled acoustic chamber. The speaker was positioned above the mouse. The sound delivery apparatus was calibrated using a 1/8-inch condenser microphone (Brüel & Kjær) positioned at the expected location of the mouse's ear, to deliver each stimulus at 70 dB sound pressure level (SPL) relative to 20 µPa. All pure tones presented during training and test sessions were at 70 dB SPL.

Behavioral timeline for non-cannulated mice (Supplementary Fig. 1a). Mice were habituated for three consecutive days to AFC cage for 15–20 min in silence and to the frequency discrimination acuity apparatus for 15–20 min, during which they were exposed to a constant tone at 15 kHz. They underwent the frequency discrimination acuity test on the following day. Following frequency discrimination acuity test, one group of mice was subjected to classical AFC (**Supplementary Fig. 1a**). These mice underwent the learning specificity test 1 d later. Another group of mice was subjected to coarse and fine AFC (**Supplementary Fig. 1a**). Following frequency discrimination acuity test, mice underwent coarse AFC. The mice underwent the learning specificity test and the frequency discrimination acuity test 1 d later. The mice underwent fine AFC 3 d after that, followed by the learning specificity test and the frequency discrimination acuity test 1 d later.

Behavioral timeline for cannulated mice (Supplementary Fig. 6a). Mice were implanted with cannulas and allowed to recover for 7 d. During this time, they were habituated to the AFC cage and frequency discrimination acuity apparatus as described above. The timeline of experiments was the same as for non-cannulated mice, except that the learning specificity and frequency discrimination tests were repeated 1 and 2 d following each AFC session. Muscimol or vehicle was infused in the auditory cortex or muscimol was infused in the somatosensory cortex (**Supplementary Fig. 8**) via the implanted cannula one day after each AFC session, at least 1 h before the first set of tests. Inactivation of the cortex by muscimol was expected to start immediately and to end after 24 h (before the second set of tests)[20,21]. Different groups of mice were used for infusion of muscimol in the auditory cortex, vehicle in the auditory cortex and muscimol in the somatosensory cortex.

Fear conditioning (Supplementary Fig. 1b). During classical AFC, following 5 min of silence, ten tones (15.0 kHz) co-terminated with a foot shock (CS+) were presented at inter-trial intervals that were randomly varied between 2 and 6 min. During coarse discriminative AFC, following 5 min of silence, ten CS+ (15.0 kHz) co-terminated with foot shock, and ten tones at 7.5 kHz, not paired with foot shock ($CS-_{coarse}$), were presented in pseudo-random order with 2-min inter-stimulus intervals (ISIs). During fine AFC, following 5 min of silence, ten CS+ tones and ten $CS-_{fine}$ tones (12.75 kHz) were presented in a pseudo-random order with 2-min ISI. During pseudo-conditioning, the timing of the shock was pseudo-randomized with respect to the timing of the tones.

Learning specificity test (Supplementary Fig. 1c). For one subset of mice, following classical, coarse and fine AFC, the learning specificity test consisted of two tones, CS+ and either $CS-_{coarse}$, $CS-_{coarse}$ or $CS-_{fine}$, respectively, presented sequentially at 5-min ISIs. The LSI was defined by difference in the freezing response to CS+ and CS−. Mice, for which freezing to CS− was lower by more than 10% relative to CS+, were defined as learners. Otherwise, they were defined as non-learners. For another subset of mice, the learning specificity test consisted of CS+ and three test tones (3.75, 7.5 and 12.75 kHz), presented at 3-min ISIs (**Supplementary Fig. 1c**). Learning specificity was assayed as the differential freezing response to CS+ and test tones. To directly compare learning specificity across fine and coarse learning groups, we introduced an LS_{90} index, defined as the estimated frequency at which freezing response to CS− was 10% lower than the response to CS+ (**Supplementary Fig. 9**). The LS_{90} index was strongly correlated with LSI for both coarse and fine AFC. Thus, we used LSI when appropriate to assay learning specificity, which was critical for the pharmacological experiments that required a tight timeframe for behavioral testing following injection of the drug.

During conditioning and test sessions, freezing responses were video recorded and analyzed offline using FreezeFrame software. Freezing responses were judged as complete immobility of the mouse for at least 1 s. All tones were 20.5 s long. Average freezing response during 20 s before the test tones was recorded as baseline, while freezing response during the test tones was recorded as the conditioned response.

Frequency discrimination acuity test (Supplementary Fig. 2). The measurement of frequency discrimination acuity used a modified PPI of the startle reflex protocol as previously described[6]. The test measured the magnitude of the ASR to the startle stimulus as a function of the difference in frequency between the background tone and the pre-pulse tone (PPS), which immediately preceded startle stimulus.

The frequency of the background tone was 15.0 kHz. The background tone was presented continuously between the end of startle stimulus and the start of PPS. The transition between the background tone and PPS included 1-ms ramp to avoid clicks. Five frequencies were used for PPS (13.8, 14.7, 14.85, 14.925 and 15.0 kHz). Thus, PPS differed from the background tone by 0, 0.5, 1, 2 and 8%. PPS was 80 ms long and was presented right before the startle stimulus. The startle stimulus was broad-band noise, presented at 120 dB SPL relative to 20 µPa for 20 ms. The stimuli were calibrated with respect to the frequency sensitivity of the loudspeaker. To verify that perceptual loudness of the tones was similar across the frequency range, PPI of the acoustic startle reflex elicited by the individual tones at each of the five frequencies was measured (**Supplementary Fig. 10**).

Each test session consisted of nine startle-only trials, followed by at least 75 pre-pulse trials, followed by one additional startle-only trial. On startle-only

trials, background tone was followed directly by startle stimulus. On pre-pulse trials, each PPS was presented in pseudo-random order with inter-trial interval varying randomly between 15 and 25 s. Negative frequency changes were used because rodents were previously shown to be more sensitive to downward frequency shifts[6].

The magnitude of ASR was measured using sensor plate (San Diego Instruments) and defined as the maximum vertical force applied in the 500-ms window following startle stimulus minus average baseline activity during the 500-ms period before startle stimulus. In each PPI session, 50% of the strongest ASRs for each frequency were averaged and used to calculate PPI

$$\mathrm{PPI}(\%) = 100 \cdot \frac{\mathrm{ASR}_{\mathrm{noPPS}} - \mathrm{ASR}_{\mathrm{PPS}}}{\mathrm{ASR}_{\mathrm{noPPS}}}$$

where $\mathrm{ASR}_{\mathrm{noPPS}}$ is the response when PPS frequency is equal to the frequency of the background tone (15 kHz) and $\mathrm{ASR}_{\mathrm{PPS}}$ is the response after frequency shift has occurred.

The frequency discrimination threshold (θ) was defined as a frequency shift (Δf) that caused 50% inhibition of the maximum ASR. θ is determined from a parametric fit to a generalized logistic function

$$\mathrm{PPI} = -\frac{a}{2} + \frac{a}{1 + \exp(b + c\Delta f)}$$

In a standard PPI session, 15 repetitions of each PPS were presented (75 trials in total). However, if either θ was out of the range (0.4–8%) or the fit coefficient of the curve (R^2) was below 0.7, the subject underwent ten more repetitions (50 trials). If θ and fit curve failed to meet the above criteria after 175 trials, the subject was excluded from statistical analysis.

Local field potential recordings (Supplementary Fig. 7). Mice (n = 2) were anesthetized with isoflurane (0.6–0.8%) and a craniotomy (2 × 2 mm) was performed over the auditory cortex, followed by a localized durotomy targeted to electrode tips. A silicone multi-electrode probe (four shanks, 200-µm inter-shank distance, two diamonds of four electrodes on each shank, 150-µm vertical inter-diamond distance, impedance ~500 kΩ, Neuronexus) was lowered to 900 µm vertically. The stimulus consisted of six clicks presented at 10 Hz, followed by 500 ms of silence. Local field potentials (LFPs) from 32 electrodes were recorded during presentation of 100 repeats of the stimulus (Neuralynx)[22]. Each waveform was filtered between 1 and 300 Hz. A cannula was lowered targeted to 3.3 mm posterior and 4.3 mm lateral of bregma. This location was 200 µm posterior of the most posterior shank in mouse 1, and 400 µm posterior of the most posterior shank in mouse 2. 0.5 µl of 0.8 mM muscimol conjugated with the Bodipy TMR-X fluorophore was injected via syringe infusion pump (Harvard Apparatus). The LFPs were continuously monitored and saline was applied over the craniotomy as needed. LFPs in response to 100 repeats of the stimulus were recorded 1 h later.

Statistical analysis. Because most experiments consisted of within-subject repetitions, the data (unless otherwise indicated) were analyzed by either two-tailed paired t test or repeated-measures ANOVA using SPSS Statistics (IBM). Samples that did not pass Shapiro-Wilk test for normality were compared using Wilcoxon signed rank test. Whenever independent samples were compared using parametric methods, equality of variances was confirmed by Levene's test. Multiple comparisons were adjusted by Bonferroni correction.

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