Cortical Synaptic Inhibition Declines during Auditory Learning

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Auditory learning is associated with an enhanced representation of acoustic cues in primary auditory cortex, and modulation of inhibitory strength is causally involved in learning. If this inhibitory plasticity is associated with task learning and improvement, its expression should emerge and persist until task proficiency is achieved. We tested this idea by measuring changes to cortical inhibitory synaptic transmission as adult gerbils progressed through the process of associative learning and perceptual improvement. Using either of two procedures, aversive or appetitive conditioning, animals were trained to detect amplitude-modulated noise and then tested daily. Following each training session, a thalamocortical brain slice was generated, and inhibitory synaptic properties were recorded from layer 2/3 pyramidal neurons. Initial associative learning was accompanied by a profound reduction in the amplitude of spontaneous IPSCs (sIPSCs). However, sIPSC amplitude returned to control levels when animals reached asymptotic behavioral performance. In contrast, paired-pulse ratios decreased in trained animals as well as in control animals that experienced unpaired conditioned and unconditioned stimuli. This latter observation suggests that inhibitory release properties are modified during behavioral conditioning, even when an association between the sound and reinforcement cannot occur. These results suggest that associative learning is accompanied by a reduction of postsynaptic inhibitory strength that persists for several days during learning and perceptual improvement.

Key words: associative learning; auditory cortex; inhibition; synaptic plasticity

Introduction

The representation of sound in auditory cortex can be modified by both chronic environmental conditions and learning (for review, see Keuroghlian and Knudsen, 2007; Sanes and Bao, 2009; de Villers-Sidani and Merzenich, 2011; Schreiner and Polley, 2014). For example, learning to detect a spectral cue or discriminate a spectral cue from among other cue causes (1) cortical single-neuron receptive fields to shift rapidly and persistently toward the conditioned frequency, and (2) representation of relevant frequencies to expand (Edeline et al., 1993; Recanzone et al., 1993; Blake et al., 2002; Keeling et al., 2008). In fact, the magnitude of reorganization is correlated with both magnitude of learning, as well as resistance to extinction (Rutkowski and Weinberger, 2005; Bieszczad and Weinberger, 2010). Modification of functional properties is not restricted to sound frequency. Learning can also lead to altered temporal response properties or sound-level tuning to favor the features of salient sounds (Bao et al., 2004; Polley et al., 2004). However, learning-induced alterations of the cortical sensory representation can dissipate as ani-

mals continue to practice a task (Molina-Luna et al., 2008; Yotsumoto et al., 2008; Takahashi et al., 2010; Reed et al., 2011), suggesting that the changes underlying learning can shift to other loci when certain training regimens are used. Thus, auditory learning is accompanied by modifications to the representation of acoustic cues in primary auditory cortex, which may facilitate behavioral sensitivity to the conditioned stimulus.

Disinhibition or the reduction of inhibitory synaptic gain and the associated increase in excitability is one cellular mechanism that may facilitate long-term modification to sensory cortex processing. In fact, the ability to induce or facilitate a neural substrate of learning, long-term potentiation, is known to depend on reducing inhibitory drive (Wigström and Gustafsson, 1986; Steward et al., 1990; Mott and Lewis, 1991; Brucato et al., 1996; Hsu et al., 1999; Ormond and Woodin, 2011). In the auditory system, the experience-dependent plasticity that occurs during classical conditioning is associated with an enhancement of excitation within the auditory cortex (Blake et al., 2006). Consistent with this finding, disinhibition in the auditory cortex is necessary for a simple form of associative learning (Letzkus et al., 2011). We predicted that, if inhibitory plasticity is associated with task learning, then the expression of inhibitory plasticity would emerge and persist until task proficiency was achieved, regardless of the training procedure.

To examine whether adjustments to cortical GABAergic transmission accompany associative learning, we measured spontaneous GABA_A receptor-mediated synaptic currents in auditory cortex brain slices obtained from gerbils undergoing daily auditory training. Animals were trained to detect an amplitude-modulated noise, using either an aversive or an appetitive proce-

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Figure 1. Associative learning and inhibitory synaptic measures during aversive conditioning. **A**, Schematics show the possible behavioral responses to both kinds of trials (left: Go and Nogo trials). Behavioral responses (right) could result in a “hit,” “miss,” “false alarm,” or “correct reject.” **B**, sIPSC amplitude (in picoamperes) as a function of the days that each animal received aversive training. Each data point represents one cell. Cells from naive control animals (black squares) are plotted against cells from aversively trained animals (red circles) and those from animals with unpaired aversive training (light blue squares). Inset, Example traces of sIPSCs taken from a naive animal (black, left), from a trained animal during the initial phase of associative learning (red, middle; day 2), and from a trained animal during the later phase of associative learning (red, right; day 12). **C**, sIPSC amplitudes are averaged by training period. Average sIPSC amplitude from naive control animals are compared with the average sIPSC amplitude from all animals with unpaired aversive conditioning, those animals with aversive conditioning and training for 1–9 d and those animals with aversive conditioning for 10–14 d. **D**, **E**, PPRs for the above named animals as a function of days of training (**D**) as well as averaged by animal group (**E**). Error bars indicate SEM; * $p < 0.05$.

procedure. Auditory cortex brain slices were obtained on sequential days, immediately following each training session. Both presynaptic and postsynaptic measures of inhibitory function were recorded to determine whether synaptic adjustments were associated with learning, and whether they were specific to a particular training procedure. The results showed a decrease in IPSC amplitude that coincided with the onset of associative learning in animals with auditory training, but not in control animals. In contrast, the paired-pulse ratio (PPR) decreased both in animals with auditory training and in controls. These results suggest that a transient decrease in inhibitory gain permit the modification of stimulus representation that accompanies learning.

Materials and Methods

Gerbil (*Meriones unguiculatus*) pups were weaned from commercial breeding pairs (Charles River). Males and females were caged separately and maintained in a 12 h light/dark cycle. Animals were trained on one of two auditory tasks: either a conditioned-avoidance task or appetitive task (both described in detail below). All procedures related to the maintenance and uses of animals were in accordance with the *Institutional Animal & Use Committee Handbook* and approved by the University Animal Welfare Committee at New York University.

Behavior: aversive conditioning

Animals were trained on a Go-Nogo conditioned-avoidance procedure to detect amplitude modulation (Heffner and Heffner, 1995; Heffner et al., 2006; Kelly et al., 2006; Sarro and Sanes, 2010, 2011). Since we were

interested in the physiological changes that may occur in the same animals over the course of associative learning, we recorded cells from animals that had an increasingly greater number of training days.

Apparatus. Gerbils were placed in a small plastic cage in a sound-isolation booth (Gretch-Ken Industries) and observed from a separate room via a closed-circuit monitor. When the animal contacted both the plate and spout, a circuit was completed that initiated water delivery via a syringe pump (New Era Pump Systems). A personal computer, connected to a digital input/output interface (TDT RZ6, Tucker-Davis Technologies) controlled the timing of the acoustic stimuli, water delivery (0.3 ml/min), and a small current delivered through a metal lick spout. Auditory stimuli were generated by a Tucker-Davis Technologies system and delivered via a calibrated tweeter (KEF Electronics) positioned 1 m in front of the test cage at 0° elevation. Sound level was measured with a spectrum analyzer (3550, Brüel & Kjær) via a one-quarter inch free-field condenser microphone positioned at the head location when in contact with the lick spout. The metal lick spout was similar in appearance to that within the home cage (Fig. 1A).

Procedural training. Animals were placed on controlled water access and learned to obtain water from the lick spout in the presence of a continuous unmodulated noise stimulus, which played continuously during each behavioral session. Animals were then trained to suppress their drinking behavior and to withdraw from the lick spout when the continuous noise signal was amplitude modulated at 5 Hz (warning signal). To train the withdrawal response, a low AC current (0.5–1.0 mA, 0.3 s; Lafayette Instruments) was delivered through the lick spout immediately after the warning signal. Since animals display large between-

subject variability in pain sensitivity (Mogil, 1999; Wasner and Brock, 2008; Nielsen et al., 2009), we adjusted the shock level for each animal to reliably produce withdrawal from the spout, but not so great as to dissuade an animal from approaching the spout on subsequent trials. The animals' behavior was monitored constantly to ensure that the level was set correctly. To initially train animals on the task, warning trials (100% modulation depth) were presented until performance reached a criterion of $\geq 70\%$ correct over 10 consecutive trials. Behavioral trials were cued by the animal's contact with the waterspout. Thus, animals had to be in contact with the waterspout for a specific amount of time before the modulation of sound initiated. Once they reached criterion on the conditioned-avoidance procedure, animals received training that consisted of the presentation of 5 amplitude-modulated depths ≥ 5 times in descending order, bracketing each animal's detection threshold from the previous day's performance. This type of training session continued daily until brain-slice assessments were made. It is possible that animals could have learned to perform the task by attending to an acoustic cue other than amplitude modulation (e.g., short-term change in average level), but the experimental design and interpretations depend on learning per se, rather than the learning of a specific cue.

Behavioral trial structure and auditory stimuli. A series of warning trials were interleaved with a variable number of safe trials. Each warning or safe trial was 2.5 s long. The sound level remained constant (45 dB equivalent SPL) throughout all intervals to exclude the use of an energy cue. The noise stimulus was broadband (measured as an rms), with a 25 dB roll-off at 3.5 kHz and a 25 dB roll-off at 20 kHz. Each warning trial contained 1.2 s of unmodulated noise during the pretrial interval, and the spout was monitored for contact during the final 0.5 s of this interval. The trial proceeded only if the animal remained in contact with the spout for >0.25 ms during this interval. The warning stimulus was 1.0 s of sinusoidally amplitude-modulated noise at a modulation frequency of 5 Hz, and the depth of amplitude modulation was varied. Warning stimuli were followed immediately by an aversive unconditioned stimulus (0.3 s electrical current delivered via the lick spout). To determine whether the animal detected the warning stimulus, contact with the spout was monitored during the final 0.1 s of the warning stimulus. A contact time of <50 ms was scored as a hit and a contact time of >50 ms was scored as a miss. For safe trials, a contact time of <50 ms was scored as a false alarm and a contact time of >50 ms was scored as a correct rejection (Fig. 1A). Warning trials were interleaved with 2–4 safe trials, randomized to avoid temporal conditioning. We also ran a control group that received random presentation of the amplitude-modulated stimulus interspersed with shock delivery such that animals were unable to form a temporal association between the auditory stimulus with the unconditioned stimulus (i.e., shock).

Data analysis. A performance metric was calculated by performing a z -transform of both false-alarm and hit values: $d' = z_{\text{false alarm}} - z_{\text{hit}}$. The final value was obtained for z scores that corresponded to the right-tail p values, or the positive difference between the two respective z scores (Swets, 1973; Yanz, 1984), and was calculated for each amplitude-modulated depth. Thresholds were defined as the amplitude-modulated depth at which performance reached a $d' = 1$. Only sessions in which an animal performed ≥ 25 trials were included in the analysis.

Experimental groups. The trained animals and cells were as follows: postnatal day (P) 25–P35: $n = 13$ animals; $n = 31$ cells; adults: $n = 9$ animals; $n = 19$ cells; when pooled: $n = 22$ animals; $n = 50$ cells. Animals were trained as described above on the conditioned-avoidance task. Brain slices were obtained within 3 h of a training session, and animals that were recorded later in the protocol had more cumulative training sessions. The study was initially designed to determine whether there was an effect of age. We were motivated, in part, by previous behavioral results indicating a difference between juvenile and adult learning (Sarro and Sanes, 2010, 2011). However, we found no significant difference in spontaneous IPSC (sIPSC) amplitude or PPR value as a function of age. Thus, sIPSC amplitude at days 1–9 of training was nearly identical for adult-trained animals (21.4 ± 2.1 pA) and juvenile-trained animals (21.5 ± 1.9 pA; ANOVA: $F_{(1,36)} = 0.004$, $p = 1.0$). Similarly, PPR value did not differ significantly (ANOVA: $F_{(1,33)} = 1.6$, $p = 0.2$). Nor was there a difference between juvenile-trained and adult-trained animals

during the later phase of training, from days 10–14 (sIPSC amplitude: $F_{(1,9)} = 0.002$, $p = 1.0$; PPR value: $F_{(1,10)} = 1.7$, $p = 0.2$). Thus, juvenile and adult animals were combined for all subsequent analyses.

Animals categorized as unpaired control (adults, $n = 4$ animals, $n = 24$ cells) were exposed to the training environment as described above but presentations of both auditory stimuli and shock were delivered randomly.

Animals categorized as naive control (adults; $n = 9$ animals; $n = 28$ cells for PPR data; $n = 4$ animals, $n = 16$ cells for sIPSC amplitude measurements) were not exposed to the training environment or auditory stimuli before obtaining brain slices.

Behavior: appetitive conditioning

Separate groups of animals were trained using a Go-Nogo procedure to detect modulations in amplitude using a positive-reinforcement procedure (Buran et al., 2014). Briefly, each animal was trained to initiate a trial upon presentation of a Go stimulus (amplitude-modulated noise) by placing its nose in a nose port and approach a water spout. They were also trained to reprobe upon presentation of a Nogo stimulus (unmodulated noise; Fig. 2A).

Apparatus. All aspects of the testing apparatus were the same as with the aversive-conditioning task with the following differences. A small nose port was present in the testing cage on the side opposite the water spout. Animals were rewarded with 25 μ l of water when they approached the water spout on a Go trial. Auditory stimuli were delivered from a speaker 1 m above the test cage.

Procedural training. Animals were placed on controlled water access and learned to obtain water from a spout upon introduction to the test cage. Initially, when animals received water, the Go stimulus (amplitude-modulated noise) was delivered. Animals were then trained to respond to the Go stimulus by approaching the spout when the Go signal was presented. Once all animals responded strongly to Go presentation, the nose port was placed in the testing cage. In one training session, animals were able to discover and reliably initiate Go trials independently by placing their nose in the port. On the initial day of nose-port training, only Go trials (100% modulation depth) were presented. Once behavioral trials were reliably cued by the animal maintaining position in the nose port, Nogo trials were introduced. On each training session following the introduction of the Nogo stimulus, brain-slice assessments were made within 30–60 min following training.

Behavioral trial structure and auditory stimuli. The same noise stimulus used in the aversive task was used in the appetitive task. Go trials employed sinusoidally amplitude-modulated noise at a modulation frequency of 5 Hz, and the depth of amplitude modulation was kept at 100%. False-alarm trials (contact with spout when Nogo stimulus was presented) were not rewarded. To determine whether the animal detected the Go stimulus, contact with the spout was monitored. For Nogo trials, contact with the water spout constituted a false alarm and a reprobe or no response with a 5 s window was scored as a correct rejection (Fig. 2A). Presentation of Go and Nogo trials were randomized to avoid animals developing possible predictive strategies.

Experimental groups. Animals in the trained category were as follows: P50–P95 (average P72), $n = 39$ cells, $n = 9$ animals. These animals were trained as described above. Brain slices were obtained from animals during the course of the training period.

Brain-slice recordings

Preparation and recordings were done in a manner previously reported to compare our findings with many of our previous studies (Kotak et al., 2008; Takesian et al., 2012). The brain was sectioned perihorizontally at 15–25° to preserve the ventral medial geniculate (MGv) and its ascending pathways to the auditory cortex (ACx). The slices were incubated in ACSF at 32°C for 30–45 min, kept at room temperature for ~ 45 min, and transferred to a recording chamber continuously superfused (~ 3 ml/min) with ACSF at $32 \pm 1^\circ\text{C}$. The ACSF contained the following (in mM): 125 NaCl, 4 KCl, 1.2 KH_2PO_4 , 1.3 MgSO_4 , 24 NaHCO_3 , 15 glucose, 2.4 CaCl_2 , and 0.4 L-ascorbic acid, pH 7.3 when bubbled with 95% O_2 /5% CO_2 . Whole-cell recording were obtained from the thalamorecipient ACx previously validated by extracellular field activity recorded in re-

Figure 2. Associative learning and inhibitory synaptic measures during appetitive conditioning. **A**, Schematics show the possible behavioral responses to both kinds of trials (left: Go and Nogo trials). Behavioral responses (right) could result in a “hit,” “miss,” “false alarm,” or “correct reject.” **B**, sIPSC amplitude (in picoamperes) as a function of the days that each animal received appetitive training. Each data point represents one cell. Cells from naive control animals (black squares) are plotted against cells from trained animals (red circles). **C**, sIPSC amplitudes are averaged by training period. Average sIPSC amplitude from naive control animals are compared with the average sIPSC amplitude from those animals with training for 2–5 d and those animals with training for 9 d. **D**, **E**, PPRs for the above named animals as a function of days of training (**D**) as well as averaged by animal group (**E**). Error bars indicate SEM; * $p < 0.05$.

sponse to MGv stimulation. Ionotropic glutamate receptor blockers, 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 μ M) and 2-amino-5-phosphonopentanoate (AP-5, 50 μ M), were continuously bath applied with ACSF to isolate inhibitory currents.

Recording electrodes were made from borosilicate glass microcapillaries (outer diameter, 1.5 mm) with a puller (P-97, Sutter Instruments). The internal recording solution contained the following (in mM): 100 KCl, 40 K-gluconate, 8 NaCl, 10 HEPES, 2 MgCl₂, 0.1 EGTA, 2 ATP, 0.3 GTP, and 5 lidocaine derivative QX-314, pH 7.2 with KOH. The internal recording solution contained a relatively high chloride concentration, resulting in inward inhibitory currents at a holding potential of -60 mV. The resistance of these electrodes filled with the internal solution was 5–12 M Ω . Access resistances were 15–40 M Ω and were compensated by $\sim 70\%$. Layer 2/3 pyramidal cells were identified by their characteristically shaped cell bodies under infrared differential interface contrast optics at $400\times$. After breaking the ≥ 1 G Ω membrane patch, it was possible to immediately record a resting potential (<50 mV) and an overshooting action potential before spikes were blocked to validate that cells were healthy. Recordings were then made using voltage clamp ($V_{\text{HOLD}} = -60$ mV) after 5 min to allow steady-state blockade of sodium channels as well as diffusion of chloride. sIPSCs were recorded in the presence of ionotropic glutamate receptor blockers, which were added to the oxygenated superfusing ACSF 10 min after the continuous application of the DNQX (20 μ M; Sigma-Aldrich) and AP-5 (50 μ M; Tocris Cookson).

To obtain a PPR, two stimulus pulses (100 μ s) delivered via a stimulus isolation unit (model BSI-9501, Dagan) to a bipolar stimulating electrode placed on L4 were used. The stimulating electrode was fabricated from 0.004-inch-diameter Teflon-coated platinum wires (A-M Systems) inserted into a 2-inch-long double-barrel glass electrode. The exposed tip was 50.8 μ m in diameter. To determine the stimulus magnitude, incremental stimulus intensities were delivered at 0.05 Hz until an evoked IPSC was discernible from failures (Kotak et al., 2008). A stimulus magnitude of 20% above this threshold was chosen to avoid failures. At least

10 evoked IPSC sweeps were acquired at 0.01 Hz. Here, PPR is defined as the ratio between the average IPSC amplitude evoked by the second stimulus pulse divided by the average IPSC amplitude evoked by the first stimulus pulse (IPSC2/IPSC1). A ratio of >1 represents facilitation. A ratio of <1 represents depression. Thus, comparisons of PPR in controls versus behaviorally trained animals would thus reveal whether training altered GABA release. Cells from all animals presented in this study were included in the statistical analysis. We acknowledge that the PPR provides an indirect measure of presynaptic release (Manabe et al., 1993; Dobrunz and Stevens, 1997).

Data were filtered at 5 kHz and acquired at a sampling rate of 10 kHz by a custom-designed IGOR (version 4.08, WaveMetrics) macro on a Macintosh platform called Slice (Apple). A second IGOR macro (Slice analysis) was used for off-line analysis. To identify events, this macro used a semiautomated procedure that used a threshold value based on the SD of the acquired trace. A minimum threshold of -10 pA was used to detect sIPSCs. All IPSC data are reported as mean pA $\pm$ SEM. Cells from all animals presented in this study were included in the statistical analysis. Details on the acquisition and analysis of sIPSC amplitude and frequency, minimum-evoked IPSC amplitude, and PPR have been described previously (Takesian et al., 2010).

Results

To assess whether auditory cortex inhibitory synaptic properties were altered during the acquisition of an auditory task, gerbils were either trained to detect amplitude-modulated noise by pairing the amplitude-modulated stimulus with a mild shock (aversive conditioning) or with water reward (appetitive conditioning). Inhibitory synaptic properties were recorded from brain slices of animals immediately following daily training sessions. The first set of recordings were obtained within a few hours after completion of the first training session, during which animals failed to

learn the task. Subsequent recordings were obtained daily and always within 1–3 h of the training session. Thus, brain slices obtained on the second day came from animals that were showing improvement on the task and had experienced two training sessions; slices obtained on the third day came from animals that had experienced three training sessions; and so forth. We also obtained these measures from a control group of animals in which amplitude-modulated stimuli and unconditioned stimuli (shock) were delivered randomly. For this control group, the total number of stimuli was chosen to match that experienced by the trained animals. The animals that did not experience a pairing of amplitude modulation and shock displayed no learning. Recordings were also compared with sIPSCs obtained from naive age-matched control gerbils.

Learning to perform the task was associated with a reduction in sIPSC amplitude. Figure 1*B,C* plots the reduction in sIPSC amplitude as a function of day of training. Neurons that were recorded from animals trained with the aversive paradigm displayed a reduction in sIPSC amplitude during the first week of training, compared with naive control animals [Fig. 1*C*; ANOVA (all groups included): $F_{(1,3)} = 11.4$, $p < 0.0001$; naive control (34.6 ± 3.7 pA) vs aversive-trained days 1–9 (19.7 ± 2.0 pA); t test: $t_{(1,46)} = 4.4$, $p < 0.0001$]. However, additional training did not induce a further reduction in sIPSC amplitude (naive control vs aversive-trained days 10–14 (31.2 ± 3.8 pA); t test: $t_{(1,25)} = 0.68$, $p = 0.5$). Furthermore, no change in sIPSC amplitude was observed in neurons recorded from animals exposed to randomly presented amplitude modulation and shock [naive control vs random presentation control (34.6 ± 2.9 pA); t test: $t_{(1,38)} = 0.005$, $p = 1.0$; a *post hoc* Tukey's test revealed that sIPSC amplitude differed only within those animals during the first week of training at $p < 0.05$]. The reduction in sIPSC amplitude occurred ≥ 1 d after the associative conditioning began, as cells from animals that were obtained immediately after the first conditioning session were not significantly different from those of control animals (27.8 ± 8.5 pA; t test: $t_{(1,17)} = 0.73$, $p = 0.47$). The decrease in inhibitory strength was not associated with an average reduction in holding current, which would have indicated an increased leak conductance (naive control: $n = 16$, -39 ± 30 pA; aversive-trained days 1–9: $n = 31$, 64 ± 22 pA; aversive-trained days 10–14: $n = 11$, 88 ± 36 pA; random presentation: $n = 24$, 35 ± 25 pA; ANOVA: $F_{(3,78)} = 0.62$, $p = 0.6$).

To determine whether aversive training influenced presynaptic release, paired-pulse protocol (80 ms interstimulus interval; Takesian et al., 2010) was obtained for each recorded neuron. A main effect of treatment group was observed (ANOVA: $F_{(1,3)} = 27.5$, $p < 0.0001$), and a *post hoc* Tukey's test revealed significant differences ($p < 0.05$) between control cells and those from animals with unpaired conditioning, as well as those from cells in later stages of training (days 10–14). Notably, cells recorded from control animals with no explicit pairing of amplitude modulation and shock also displayed significantly diminished PPR values, compared with naive animals (0.41 ± 0.04 ; t test: $t_{(1,46)} = 13.2$, $p < 0.0001$). Furthermore, PPR was reduced primarily during a later stage of training (Fig. 1*D,E*). Thus, PPR values obtained from trained animals during the initial part of training were lower but not significantly different from those obtained from naive animals (naive control, 1.03 ± 0.05 , vs aversive-trained days 1–9, 0.89 ± 0.05 ; t test: $t_{(1,55)} = 1.93$, $p = 0.06$). Neurons recorded from animals with longer durations of training displayed significantly diminished PPR values (0.65 ± 0.05 ; t test: $t_{(1,38)} = 6.82$, $p < 0.0001$). Thus, modifications to presynaptic release seemed

to be dependent on being placed in the learning context, even when no learning occurred.

To determine whether alterations to inhibitory synaptic strength were specific to the aversive training paradigm, an appetitive procedure was used to train a separate group of animals to detect amplitude-modulated stimuli (see Materials and Methods). Neurons recorded from brain slices obtained within 1 hour of appetitive training displayed a decrease in sIPSC amplitudes, similar to that observed for the aversive procedure (Fig. 2*B*). The reduction in sIPSC amplitude occurred during the initial period of training, and further training did not induce a change [Fig. 2*C*; ANOVA: $F_{(1,2)} = 3.9$, $p < 0.05$; a *post hoc* Tukey's test revealed significant differences ($p < 0.05$) between control cells and those from animals in early stages of training; naive control (34.6 ± 3.7 pA) vs appetitive-trained days 2–5 (25.5 ± 2.6 pA); t test: $t_{(1,41)} = 2.75$, $p < 0.01$; naive control vs appetitive-trained for 9 d (31.4 ± 5.7 pA); t test: $t_{(1,21)} = 0.47$, $p = 0.6$]. The reduction in sIPSC amplitude occurred ≥ 1 d after the appetitive training began, as cells recorded from animals immediately after the first session did not differ significantly from cells recorded from naive controls (40.2 ± 7.6 pA; t test: $t_{(1,18)} = 0.65$, $p = 0.5$).

PPR was altered during the course of appetitive training, similar to that observed during aversive training [Fig. 2*D,E*; ANOVA: $F_{(1,2)} = 28.3$, $p < 0.0001$; a *post hoc* Tukey's test revealed significant differences ($p < 0.05$) between PPRs of control cells and those from animals in later stages of training]. However, PPR values declined during an early phase of conditioning, and continued within the later phase. Specifically, PPR values obtained from trained animals during the first week of training were lower than those of naive controls [naive control (1.03 ± 0.05) vs appetitive-trained days 2–5 (0.64 ± 0.05); t test: $t_{(1,52)} = 5.99$, $p < 0.0001$], and lower still for neurons recorded from animals with longer training (0.34 ± 0.07 ; t test: $t_{(1,31)} = 9.55$, $p < 0.0001$). Therefore, there was a significant shift toward paired-pulse depression, indicative of increased release probability.

To determine whether changes to sIPSC amplitude correlated with an improvement in behavioral performance, we examined two phases of the training period: the initial associative learning phase, during which animals continue to improve on the task procedure, and a latter phase, during which animals continued to train and learned to detect more-difficult stimuli. During the initial associative learning phase (Fig. 3*A*, left side), a measure of performance (d') was correlated with sIPSC amplitude ($r^2 = 0.19$, $p < 0.05$). However, once animals reached asymptotic performance levels (i.e., when no further behavioral improvement was observed at the training stimulus, 100% amplitude-modulated depth), sIPSC amplitudes no longer correlated to behavioral performance (Fig. 3*A*, right side; $r^2 = 0.13$, $p = 0.2$). A similar relationship between behavior and sIPSC amplitude was observed for neurons from appetitively trained animals (initial: $r^2 = 0.33$, $p < 0.01$; asymptotic: $r^2 = 0.11$, $p = 0.28$). We calculated sIPSC amplitude as a function of performance for each training regimen (Fig. 3*B*) and found that it was significantly smaller during the early stage of training for animals with a $d' > 1.5$. Figure 3*C* shows that PPR values do not differ as a function of performance.

Discussion

This study examined the premise that changes in cortical inhibitory synaptic function accompany the process of associative learning and improvement on an auditory detection task, independent of the training procedure. The primary finding was that sIPSC amplitudes became significantly smaller during the initial

Figure 3. Initial associative learning is correlated with sIPSC amplitude. **A**, d' (d' prime) for the trained stimulus of 100% amplitude-modulated depth is plotted as a function of days of training to show an initial sharp rise in the performance measure as animals form the initial association. This is followed by a period of asymptotic performance where performance on the easiest trained amplitude-modulated depth stimulus reaches asymptotic values. Vertical line illustrates this transition. Inset illustrates the sIPSC amplitudes obtained from two animals with similar amounts of training and widely different d' values (open circle, good learner; shaded circle, poor learner). **B**, sIPSC amplitude is plotted as a function of training period and performance left to right: early $d' < 1$ (early in training when d' is < 1); Early $d' > 1.5$ (early in training when d' is > 1.5); late $d' > 1.5$ (late in training when d' is > 1.5). This is plotted for both the aversive task (left) and the appetitive task (right). **C**, PPR is plotted as a function of training period and performance as displayed in **B**. Error bars indicate SEM; $*p < 0.05$.

phase of the associative learning for both aversive or appetitive training paradigms (Figs. 1B, 2B). Moreover, during the initial learning processes of the associative learning, the magnitude of sIPSC degradation was correlated with the performance on the task (Figs. 3). Furthermore, after ~ 10 d of training, the diminished inhibitory currents were gradually restored to control values. In contrast, the PPR was reduced both in trained and random presentation control animals. Together, these findings suggest that a transient decrease in inhibitory gain supports the alterations in stimulus processing that accompany learning.

Although there is no direct evidence linking spontaneous inhibitory events to learning, we have found that average sIPSC amplitude is a reliable proxy for evoked IPSC amplitude, as re-

corded in auditory cortex pyramidal neurons (Kotak et al., 2008; Takesian et al., 2012; Mowery et al., 2014). Specifically, hearing loss-induced reduction in sIPSCs is mirrored by a reduction in fast-spiking (FS) interneuron-evoked IPSCs (Takesian et al., 2010). Therefore, our interpretation of the data is that the associative learning observed here coincides with a reduction in auditory cortex FS-evoked IPSCs. Our findings are consistent with those of earlier studies demonstrating that disinhibition is necessary and sufficient for simple auditory fear conditioning to occur. Fear conditioning is associated with the inhibition of parvalbumin-positive layer 2/3 interneurons (Letzkus et al., 2011) and interneurons within the amygdala (Wolff et al., 2014). Likewise, critical work in the primate auditory system demonstrates a general enhancement of excitability in cortex during the initial learning phases of a classical conditioning task, implying excitability as an outcome of the disinhibition (Blake et al., 2006). However, disinhibition is not unique to fear conditioning, having also been linked to motor learning (Smyth et al., 2010; Baarbé et al., 2014; Coxon et al., 2014). Our findings support the idea that reduced inhibition is a general cortical mechanism involved in many forms of associative learning.

While our findings do not demonstrate a causal relationship between changes to sIPSCs and auditory learning, they are consistent with a literature that links inhibitory strength to experience-dependent events or learning. For example, sIPSC frequency increases and tonic inhibitory currents decrease in somatosensory cortex following the pairing of vibrissae stimulation and tail shock (Tokarski et al., 2007; Urban-Ciecko et al., 2010). In the present study, by following the process of learning across several days of training, we demonstrated that diminished inhibitory strength occurred only in animals that also displayed auditory learning (i.e., poor learners and unpaired controls did not show reduced inhibition). The long-term impact of learning on cortical plasticity is not addressed by our findings since inhibitory strength returned to control levels during the course of training. In contrast, well trained animals display increased or decreased sound-driven responses during task engagement, depending on whether the training protocol is appetitive or aversive, respectively (David et al., 2012). Thus, the learning mechanisms that permit an initial sensory association to form may occur via a general transient reduction in inhibitory drive, but additional long-term changes in cortex function are associated with perceptual learning.

Possible mechanisms of diminished inhibition during learning

The reduction of sIPSC amplitude that accompanies the early stages of associative learning (Figs. 1B, 2B) may reflect a postsynaptic change of GABA_A receptor number or conductance. Conductance changes have been demonstrated to result from modification of subunit composition or phosphorylation state of the receptors, as well as change in the number of receptors at the postsynaptic membrane (Morishita and Sastry, 1996; Möhler, 2006). Reduction in GABA_A-mediated sIPSC amplitudes could also be reflective of a removal of GABA_A receptors from the membrane at the onset of associative learning, as has been shown to occur at the onset of fear conditioning in the basolateral amygdala (Lin et al., 2009, 2011). Our results are also consistent with reduced sIPSC amplitudes displayed in a $\beta 3$ subunit knockout mouse model (Huntsman et al., 1994).

Reduction in the PPR in trained animals and controls exposed to random conditioned and unconditioned stimuli (Figs. 1C, 2C) suggest a $>50\%$ increase in release probability. Therefore, pre-

synaptic changes were associated with exposure to an environment in which animals presumably attempted to determine a contingency between conditioned and unconditioned stimuli, independent of associative learning. Short-term inhibitory depression can be attributed to several factors, including depletion of readily releasable vesicles, or activation of presynaptic GABA_B receptors (Davies and Collingridge, 1993; Murthy et al., 2001; Zucker and Regehr, 2002; Takesian et al., 2010), and these mechanisms can display use-dependent changes (Mott et al., 1993; Murthy et al., 2001).

The suppression of inhibition may involve a disinhibitory circuit, including the activation of L1 GABAergic interneurons that suppress other inhibitory neurons (Letzkus et al., 2011; Pi et al., 2013). The learning-associated decrease in inhibitory gain is likely to involve cholinergic activity during training. When paired with sensory stimulation, stimulation of cholinergic nucleus basalis afferents to cortex induces long-lasting modifications to cortical response properties (Tremblay et al., 1990; Metherate and Ashe, 1992, 1993; Edeline et al., 1994; Kilgard and Merzenich, 1998) and is sufficient for memory formation and improved sensory perception (Miasnikov et al., 2008; Bieszczad et al., 2013; Froemke et al., 2013). In the present study, the greatest change in inhibitory synaptic function occurred during procedural learning of the auditory task (Figs. 1*B*, 2*B*), suggesting that nucleus basalis activity could plausibly be involved in the long-term suppression of inhibition. Furthermore, the correlation between sIPSC amplitude and performance (Fig. 3) is consistent with the finding that the magnitude of nucleus basalis activation is correlated to performance (Weinberger et al., 2006).

A transient reduction of inhibition may permit the reorganization of receptive field properties necessary for learning the preferred amplitude-modulated stimulus in both the aversive and appetitive tasks. It is possible that decreased cortical inhibition may lead to heightened excitability, which would in turn contribute to learning-based plasticity. Finally, the return of baseline inhibitory function several days after initial training may restore the balance of cortical excitatory and inhibitory synapse function to the prelearning state, ending the period of heightened plasticity.

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