LETTERS

A synaptic memory trace for cortical receptive field plasticity

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Receptive fields of sensory cortical neurons are plastic, changing in response to alterations of neural activity or sensory experience[1–12]. In this way, cortical representations of the sensory environment can incorporate new information about the world, depending on the relevance or value of particular stimuli[1,6,9]. Neuromodulation is required for cortical plasticity, but it is uncertain how subcortical neuromodulatory systems, such as the cholinergic nucleus basalis, interact with and refine cortical circuits[13–24]. Here we determine the dynamics of synaptic receptive field plasticity in the adult primary auditory cortex (also known as AI) using *in vivo* whole-cell recording. Pairing sensory stimulation with nucleus basalis activation shifted the preferred stimuli of cortical neurons by inducing a rapid reduction of synaptic inhibition within seconds, which was followed by a large increase in excitation, both specific to the paired stimulus. Although nucleus basalis was stimulated only for a few minutes, reorganization of synaptic tuning curves progressed for hours thereafter: inhibition slowly increased in an activity-dependent manner to rebalance the persistent enhancement of excitation, leading to a retuned receptive field with new preference for the paired stimulus. This restricted period of disinhibition may be a fundamental mechanism for receptive field plasticity, and could serve as a memory trace[9,25] for stimuli or episodes that have acquired new behavioural significance.

A major subcortical nucleus critical for receptive field plasticity is nucleus basalis, the main source of cortical acetylcholine[4,9,14–17,19–21]. How are neuromodulators such as acetylcholine involved in plasticity, and what circuit elements do they act on? One possibility is that neuromodulation creates a cellular tag or memory trace for synaptic events that occurred in conjunction with neuromodulator release. However, the effects of acetylcholine on cortical neurons are diverse, including increased excitability[19,22,23] and suppression of synaptic transmission[15,17,23,24], and it is unclear how these effects could produce long-term response enhancement specific for particular stimuli. Extracellular recordings cannot reveal which cellular events are responsible for receptive field plasticity, and studies *in vitro* do not permit investigation of receptive fields or subcortical interactions with cortical networks. Instead, here we use whole-cell recording and nucleus basalis stimulation in the intact brain to determine the synaptic basis of cortical receptive field plasticity.

We made *in vivo* whole-cell recordings from adult rat primary auditory cortex (Fig. 1a). Pure tones of different frequencies were played in pseudo-random sequence to the contralateral ear. Frequency tuning was characterized in voltage-clamp at hyperpolarized (–70 mV) and depolarized (–20 mV) levels to reveal tone-evoked excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs), respectively (Fig. 1b).

Initially, cortical neurons had similar profiles of excitatory and inhibitory frequency tuning, confirming that their levels of excitation and inhibition were balanced[26,27]. Excitatory and inhibitory tuning curves usually had one shared peak at the best frequency. There was a high correlation between the relative amounts of excitation and inhibition across all frequencies (Supplementary Fig. 1).

After 5–15 min of baseline receptive field characterization, a tone was paired repetitively for 2–5 min with electrical stimulation of nucleus basalis ('nucleus basalis pairing', Fig. 1b) to release endogenous acetylcholine within primary auditory cortex (although other substances may also be released[28]). Tetanic stimulation (100 Hz, 250 ms) was performed in a manner similar to natural spiking patterns of nucleus basalis cholinergic cells[20].

After cessation of nucleus basalis pairing, we observed large changes to synaptic inputs evoked by the paired tone: pairing rapidly potentiated tone-evoked EPSCs and depressed IPSCs (Fig. 1c, d). Similar results were obtained when conductance and charge transfer were measured (Supplementary Figs 2 and 3). Synaptic modifications were long lasting and frequency specific (but see below). On average, currents evoked by unpaired tones were not significantly altered, although we consistently observed that responses to the original best frequency were reduced over a longer time course (Fig. 1d). Thus the main effect of nucleus basalis pairing is to break the balance between excitation and inhibition at the paired frequency.

Synaptic modifications required paired nucleus basalis and sensory stimulation. Frequency tuning was not persistently altered when nucleus basalis was stimulated in silence or when a given tone was repeated without nucleus basalis stimulation (Supplementary Fig. 4a, b, d). Surprisingly, this was the case even in current-clamp recordings in which tone presentation reliably evoked postsynaptic spikes (Supplementary Fig. 4c, d), demonstrating that repetitive pairing of tones with postsynaptic spikes does not induce long-term potentiation of EPSPs at these synapses.

How do the synaptic modifications observed here correspond to previously reported changes in excitability[17,22] and spike generation[4,9,19]? To determine the relation between changes in synaptic input and spike output, we made current-clamp recordings from primary auditory cortex neurons to measure tone-evoked spiking responses before and after nucleus basalis pairing. Before pairing, tones generally evoked subthreshold EPSPs or a single spike[26,27,29]. As expected, pairing increased spiking evoked by the paired frequency. This included a dramatic (>7-fold) increase in the probability of firing bursts of 2+ spikes (Supplementary Fig. 5). Consistent with the specific increase in firing rate, pairing had no long-term effect on input resistance (R_i; Supplementary Fig. 5b). Thus, nucleus basalis pairing alters the firing mode of cortical neurons, increasing the output of primary auditory cortex to enhance the salience of particular stimuli such as those with new behavioural relevance[4,9,18], or during periods of increased perceptual demand on attention[15,30].

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Synaptic modifications and enhancement of spiking occurred not only after nucleus basalis pairing, but also during pairing itself. To determine the time course of pairing-induced changes, we examined the responses to the paired tone during the pairing procedure (Fig. 2). Nucleus basalis pairing suppressed IPSCs within twenty seconds, but enhancement of EPSCs took approximately three times as long (Fig. 2a–c). Cortical application of atropine, an acetylcholine receptor antagonist, prevented the effects of nucleus basalis pairing (Fig. 2d)[9,17]. Therefore, despite the existence of multiple transmitter systems in nucleus basalis[28] and the heterogeneity of cholinergic neuromodulation[15,17,19,22–24], the net effects of nucleus basalis pairing are suppression of inhibition followed by enhancement of excitation. These results suggest that a central role for nucleus basalis activation in receptive field plasticity is to trigger spectrotemporally restricted disinhibition, permissive for induction of Hebbian synaptic plasticity[1,8,9,11,17].

We wondered which inputs were modified after nucleus basalis pairing. The decoupling of inhibition from excitation implied that a primary site of synaptic modification was directly within primary auditory cortex. However, it is unclear to what degree extrinsic or intrinsic projections mediate cortical plasticity[7,9,11,15,17,24]. To localize the effects of nucleus basalis pairing, we used an additional pair of stimulation electrodes to concurrently monitor two distinct inputs: one from the ventral division of the thalamic medial geniculate body, and one from primary auditory cortex (Fig. 3a). We ensured that both stimulation electrodes were in co-tuned areas by making extracellular recordings of receptive fields through the electrodes.

We initially recorded electrically evoked synaptic currents by intracortical and thalamic stimulation for 5–10 min in the absence of sensory stimulation. Then, electrical stimulation was stopped, and nucleus basalis stimulation was paired with the best frequency at the sites of thalamic and intracortical stimulation for 2–5 min. Finally, sensory and nucleus basalis stimulation were stopped, and electrical stimulation was resumed (Fig. 3b).

Nucleus basalis pairing persistently modified synaptic currents evoked by intracortical stimulation (Fig. 3c, e) but not thalamic stimulation (Fig. 3d, f). These modifications were similar in sign, magnitude and duration to changes in tone-evoked synaptic responses. Intracortical EPSCs were potentiated, whereas intracortical IPSCs were suppressed (Fig. 3e). Thalamocortical EPSCs were

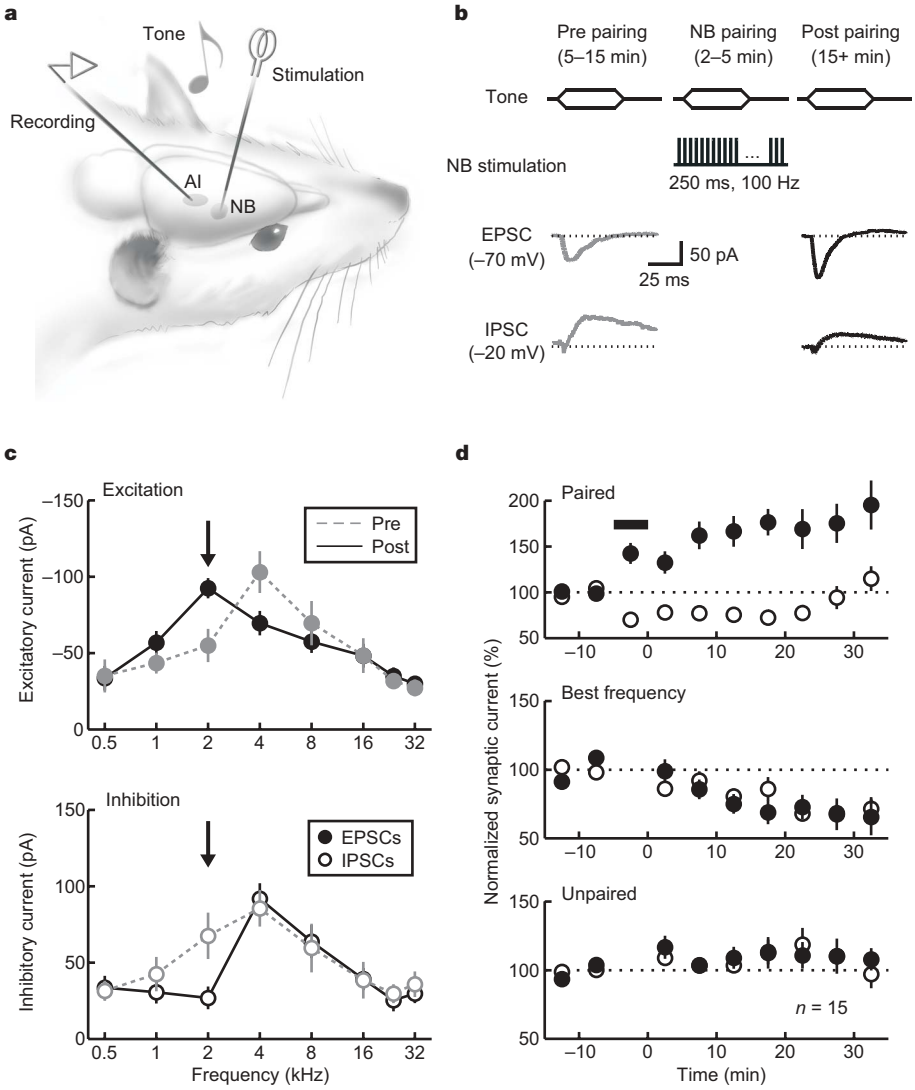

Figure 1 | Synaptic modifications induced by nucleus basalis pairing. **a**, Experimental configuration. **b**, Experimental procedure. **c**, Example of pairing-induced modification of synaptic tuning curves. Upper panel, excitatory tuning. EPSCs at the paired frequency (2 kHz) increased from -54.9 ± 10.9 to -92.4 ± 6.6 pA (68.4%, $P < 0.006$, *t*-test; filled symbols). Lines, tuning before (dashed grey) and ~10 min after (solid black) pairing. Arrow, paired tone. Lower panel, inhibitory tuning. IPSCs at the paired frequency decreased from 67.6 ± 15.2 to 27.0 ± 7.4 pA (-60.1%, $P < 0.03$; open symbols). **d**, Time course. Uppermost panel, paired frequency (excitation: $68.0 \pm 13.9\%$, $n = 15$, $P < 0.0007$; inhibition: $-24.8 \pm 6.0\%$, $P < 0.0002$). Horizontal bar, nucleus basalis pairing. Middle panel, original best frequency. Lowest panel, other unpaired tones. Error bars, s.e.m. NB, nucleus basalis; AI, primary auditory cortex.

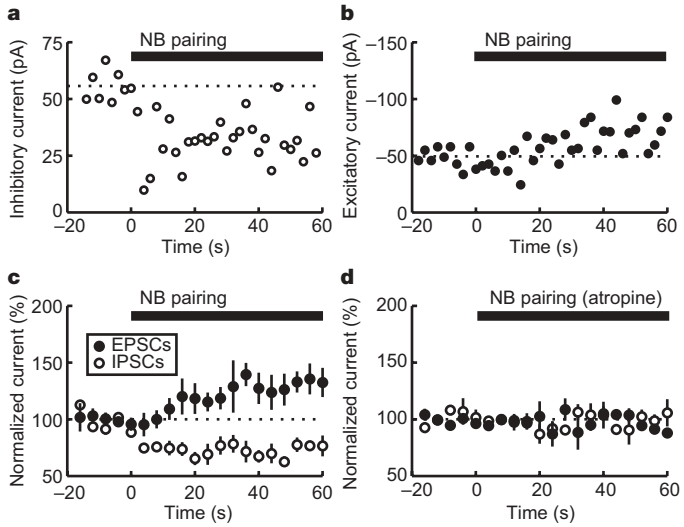

Figure 2 | Rapid suppression of inhibition during nucleus basalis pairing. **a**, IPSCs decreased from 55.7 ± 2 to 32.3 ± 4 pA (–41.9%, $P < 0.002$) 41–60 s after pairing. Solid line, nucleus basalis pairing. Dashed line, mean current before pairing. **b**, EPSCs increased from −49.5 ± 3 to −70.3 ± 5 pA (42.0%, $P < 0.01$). **c**, Suppression of inhibition occurred before enhancement of excitation (inhibition, 20.7 ± 9.3 s; excitation, 52.4 ± 4.1 s, $P < 0.009$). Filled symbols, excitation (1–20 s, 6.5 ± 8.6%, $n = 9$, $P > 0.4$; 41–60 s, 29.8 ± 2.2%, $P < 0.003$); open symbols, inhibition (1–20 s, −24.5 ± 3.8%, $n = 6$, $P < 0.005$; 41–60 s, −28.1 ± 7.0%, $P < 0.02$). **d**, Atropine (1 mM) blocked the effects of nucleus basalis pairing ($n = 4$, $P > 0.5$). Error bars, s.e.m.

unaffected (Fig. 3f), and thalamocortical IPSCs were not observed. These data suggest that nucleus basalis pairing does not induce strengthening of direct thalamocortical input from neurons of the ventral division of the thalamic medial geniculate body that are tuned to the paired frequency, but it does enhance connections from the region of primary auditory cortex initially tuned to that frequency. The decrease in intracortical IPSC amplitude demonstrates that one location of synaptic modification is directly within primary auditory cortex, at the connections between interneurons and excitatory cells. However, these results do not exclude potential for modification of other synapses elsewhere in the auditory pathway, perhaps on a different timescale or with other requirements for induction[7,9].

Finally, we noticed that towards the end of long-term recording sessions (~30 min after pairing; Fig. 1d), IPSCs evoked by tones of the paired frequency seemed to recover back towards their initial amplitudes. This indicated that modification of inhibitory frequency tuning occurred with more complex dynamics than enhancement of excitation. However, as it was difficult to maintain stable recordings for longer than 30+ minutes, we were unable to follow the complete evolution of inhibitory modifications within individual recordings.

To examine the time course of synaptic receptive field plasticity thoroughly, we made consecutive whole-cell recordings from the same location in primary auditory cortex for hours after nucleus basalis pairing in each animal (Fig. 4). To compare synaptic modifications across cells, we took advantage of the consistency of frequency tuning for neurons in a given tonotopic region of primary auditory cortex (Supplementary Fig. 1b), and normalized current amplitudes to their maximum values across frequencies.

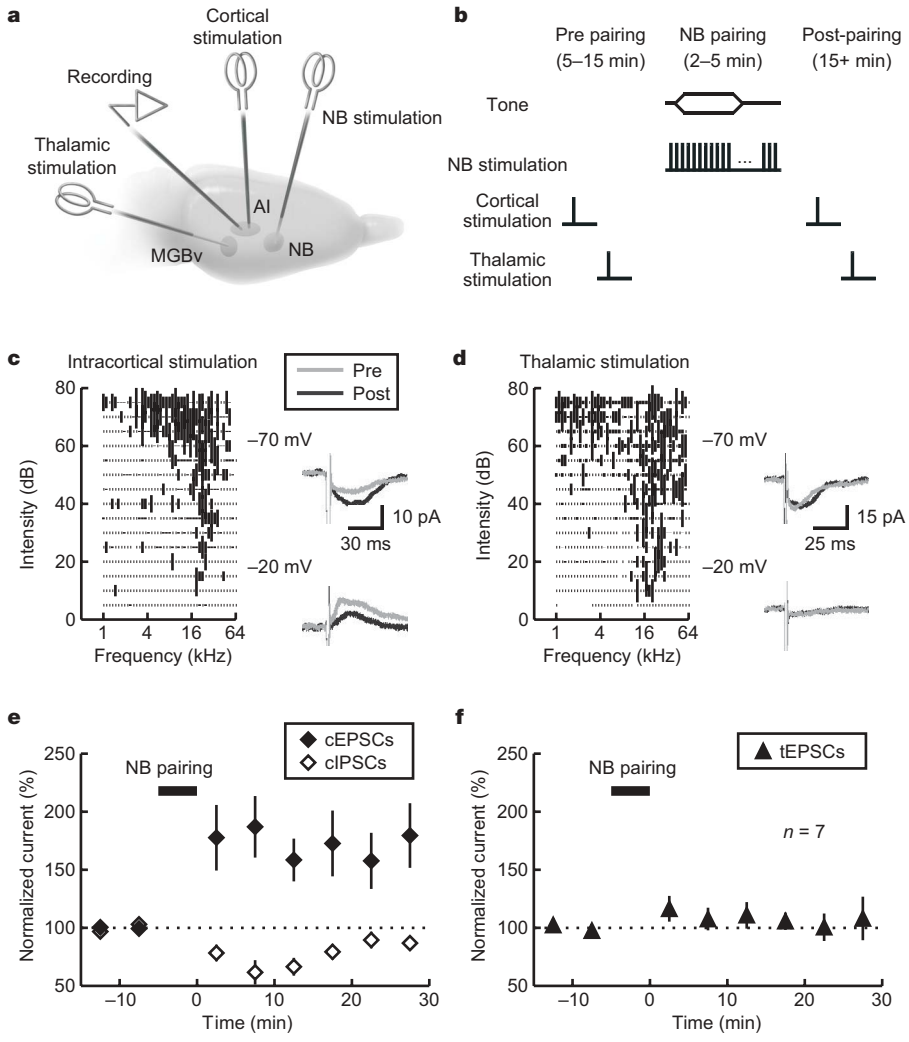

Figure 3 | Nucleus basalis pairing altered intracortical connections. **a**, Experimental configuration. MGBv, ventral division of the thalamic medial geniculate body. **b**, Experimental procedure. **c**, Intracortical stimulation. Left panel, receptive field recorded with stimulation electrode (16 kHz region of AI). Right panel, whole-cell recording from the 6 kHz region of AI, before (grey) and after (black) pairing nucleus basalis stimulation with 16 kHz tones. Intracortical EPSCs (cEPSCs) increased (73.4%; $P < 0.03$) and intracortical IPSCs (cIPSCs) decreased (−47.1%; $P < 0.03$). **d**, Same experiment as **c**, but thalamic stimulation. Thalamocortical EPSCs (tEPSCs) were unchanged ($P > 0.5$). **e**, Nucleus basalis pairing enhanced cEPSCs (72.8 ± 20.5%, $n = 7$, $P < 0.02$; filled) and suppressed cIPSCs (−30.7 ± 3.6%, $P < 0.004$; open). **f**, Same experiments as **e**, but for tEPSCs ($P > 0.4$). Error bars, s.e.m.

For example, the recordings shown in Fig. 4a–d were each made from the 16 kHz region of primary auditory cortex in the same animal. As expected, best frequencies of excitation and inhibition for the first recorded neuron were both initially 16 kHz (Fig. 4a, open arrowhead). After pairing nucleus basalis stimulation with 4 kHz tones, we observed a large increase in the excitation–inhibition ratio (E:I ratio) at the paired frequency (Fig. 4b, arrow). After this recording was finished, we obtained another recording from a second cell in the same location ~100 min after pairing (Fig. 4c). The best frequency of excitation for this cell was 4 kHz, but inhibition was maximal at 8 kHz. Finally, we recorded a third cell, in the same location as the two previous recordings, ~3 h after pairing (Fig. 4d), and found that best frequencies of excitation and inhibition were 4 kHz. Thus potentiation of excitation was maintained for hours after transient nucleus basalis pairing, but after an initial period of suppression, inhibition began to progressively increase until it balanced the enhanced excitation.

Re-establishing a normal E:I ratio required approximately two hours after completion of nucleus basalis pairing (Fig. 4e, squares). This rebalancing reflects the gradual growth of inhibitory strength rather than a decrease in excitation at the paired frequency, was apparent for continuously recorded neurons (Fig. 4e, circles), and was registered as shifts in best frequency (Supplementary Fig. 6). Rebalancing required near-continual tonal stimulation. If auditory stimulation was turned off for 60–90 min following nucleus basalis pairing, suppression of inhibition was maintained and the E:I ratio remained unbalanced (Fig. 4f, 'Quiet'). These data are reminiscent of recent findings showing that the timing of the primary auditory cortex critical period can be altered by exposure to different auditory environments[10,12].

We have described here a differential progression for changes in cortical excitation and inhibition after nucleus basalis pairing that reorganizes primary auditory cortex receptive fields. Although nucleus basalis was stimulated only for a brief period, alteration of excitatory frequency tuning required 30+ minutes to manifest fully, leading to increased preference for paired stimuli. Changes to inhibitory tuning, however, occurred first and continued for hours after nucleus basalis pairing, eventually increasing to balance the changes in excitation. These results provide a mechanism for the function of nucleus basalis in attentional modulation: focal disinhibition may act as a synaptic correlate of heightened attentiveness for novel or meaningful stimuli. Furthermore, the long-lasting break in the E:I balance caused by nucleus basalis pairing maintains the immediate effects of nucleus basalis activation, allowing restricted parts of cortex to operate in hyperexcitable states independent of further neuromodulation. This transient disinhibition therefore acts as a synaptic memory trace for sensory information of increased significance[9,25,30], allowing these stimuli to evoke larger bursts of spikes for a limited time while receptive fields are adjusted to represent the new emphasis for paired inputs.

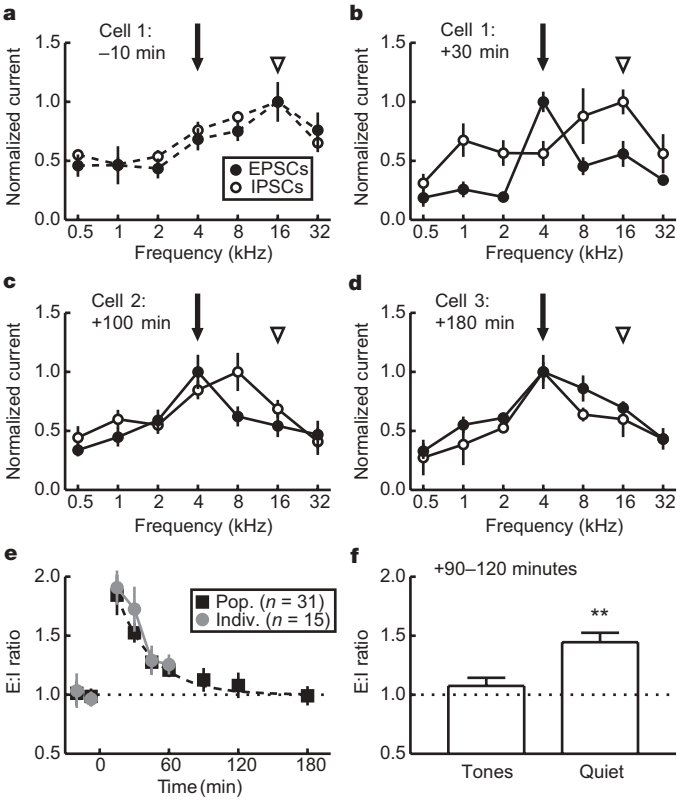

Figure 4 | Temporal dynamics of synaptic receptive field plasticity. **a**, Frequency tuning before pairing. Arrow, paired frequency (4 kHz; E:I ratio, 0.9); arrowhead, original best frequency (16 kHz). **b**, Same cell as **a**, 30 min after pairing (E:I ratio, 1.78). **c**, Second cell, 100 min after pairing (E:I ratio, 1.18). **d**, Third cell, 180 min after pairing (E:I ratio: 1.0). **e**, Rebalance of E:I ratio at paired frequency. Squares, consecutive recordings from cell populations at the same locations (Pop.); dashed line, exponential fit; circles, individual continuous recordings from Fig. 1d (Indiv.). **f**, E:I ratio 90–120 min after pairing (Tones, E:I ratio increase of 7.5 ± 6.9%, $n = 12$; Quiet, E:I ratio increase of 44.6 ± 7.9%, $n = 6$, $P < 0.004$). Double asterisks, $P < 0.01$. Error bars, s.e.m.

METHODS SUMMARY

Experimental procedures were approved under UCSF IACUC protocols. Experiments were carried out in a sound-attenuating chamber. Female Sprague-Dawley rats 3–5 months old were anaesthetized with pentobarbital. A stimulation electrode was implanted in right nucleus basalis[4] and right auditory cortex was exposed. Pure tones (0.5–32 kHz, 50 ms duration, 60–80 dB) in pseudo-random sequence were delivered into the left ear canal by a tube sealed to a calibrated speaker. The location of primary auditory cortex was determined by mapping spike responses using tungsten electrodes[4,12].

In vivo whole-cell recordings were obtained from primary auditory cortex neurons located 400–1,100 μm below the pial surface. Patch pipettes (5–9 MΩ) contained (in mM): 125 Cs-gluconate, 5 TEACl, 4 MgATP, 0.3 GTP, 10 phosphocreatine, 10 HEPES, 0.5 EGTA, 3.5 QX-314, 2 CsCl, pH 7.2 (voltage-clamp); or: 135 K-gluconate, 5 NaCl, 5 MgATP, 0.3 GTP, 10 phosphocreatine, 10 HEPES, 0.5 EGTA, pH 7.3 (current-clamp). Resting potential, −66.0 ± 10 mV (s.d.); R_i, 105.1 ± 54 MΩ.

To make consecutive recordings from the same location of primary auditory cortex, subsequent electrodes were positioned at the same penetrations. Currents were normalized to the largest across frequencies, and the E:I ratio ($EPSC_{paired}/EPSC_{BF}$)/($IPSC_{paired}/IPSC_{BF}$) was calculated (BF, best frequency). Frequency tuning was sampled at 0.5–1 octave intervals, outside of the normal variance in best frequency for a given location in primary auditory cortex.

For microstimulation, stimulation strengths were set at the minimum required (≤20 μA) to reliably evoke small synaptic events. Intracortical stimulation electrodes were placed 400–1,000 μm from recording electrodes. Thalamic stimulation electrodes were implanted in the right ventral division of the thalamic medial geniculate body.

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