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Long-term modification of cortical synapses improves sensory perception

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Synapses and receptive fields of the cerebral cortex are plastic. However, changes to specific inputs must be coordinated within neural networks to ensure that excitability and feature selectivity are appropriately configured for perception of the sensory environment. We induced long-lasting enhancements and decrements to excitatory synaptic strength in rat primary auditory cortex by pairing acoustic stimuli with activation of the nucleus basalis neuromodulatory system. Here we report that these synaptic modifications were approximately balanced across individual receptive fields, conserving mean excitation while reducing overall response variability. Decreased response variability should increase detection and recognition of near-threshold or previously imperceptible stimuli. We confirmed both of these hypotheses in behaving animals. Thus, modification of cortical inputs leads to wide-scale synaptic changes, which are related to improved sensory perception and enhanced behavioral performance.

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Receptive fields of sensory cortical neurons are highly structured. The anatomical arrangement and strength of synaptic inputs contribute to the functional organization of receptive fields, which in turn underlie the perception of the external world[1–4]. Cortical receptive fields are plastic, meaning that the feature selectivity of individual neurons and cell assemblies can be modified in a manner that depends on the patterns of electrical activity[5–8], sensory experience[9–18] and engagement of neuromodulatory systems such as the cholinergic nucleus basalis[19–24]. Furthermore, various forms of behavioral conditioning and learning are often, though not always, correlated with changes in cortical organization, synaptic strength and response properties. Receptive field plasticity allows cortical neurons to act as dynamic filters, adjusting tuning curves and response properties depending on novelty or behavioral significance of certain inputs[25,26]. These changes are believed to be adaptive in that they may underlie perceptual learning, facilitating the identification and discrimination of relevant environmental features and sensory objects. However, there is little experimental evidence in support of this hypothesis[27,28]. Behavioral training can improve some perceptual abilities without obvious changes in cortical responses[24,29], and studies that have directly examined cortical receptive field plasticity in a behavioral context have variously found enhancements[24,30,31], reductions[32] or no corresponding effect on perceptual abilities[6,30].

Given the precision of receptive field organization in the mature nervous system, persistent modifications of synaptic strength *in vivo* must be carefully orchestrated and coordinated within the overall cortical network to emphasize certain features while preserving the relative structure and selectivity of cortical tuning. These changes are often studied in the context of behavioral conditioning or repetitive exposure to sensory stimuli. In these cases, specific responses to paired or exposed stimuli are generally enhanced[13–18]. One of the main mechanisms thought to underlie this enhancement is long-term potentiation (LTP) of intracortical excitatory inputs[7,8,17,22]. However, theoretical studies have shown that forms of competitive synaptic modifications such as LTP or long-term depression (LTD) are, by themselves, destabilizing influences on network activity. LTP and LTD are positive-feedback processes that drive neural networks into hyper- or hypo-excitable states, respectively, and seem to be insufficient for behaviorally meaningful memory storage[33,34]. Although various activity-dependent and activity-independent mechanisms have been proposed to counteract these problems and help normalize receptive fields—including homeostatic control of neurotransmitter receptor and ion channel expression[35], heterosynaptic plasticity[36], anti-Hebbian plasticity[37] and metaplastic modification of synaptic learning rules[11]—it is unknown how the synaptic drive onto cortical neurons is monitored and calibrated in the intact brain to allow changes in sensory representation to positively influence perception and behavior.

RESULTS

Synaptic modifications conserve net excitation

We investigated the coordination of synaptic receptive field plasticity across multiple inputs and stimulus parameters by making whole-cell

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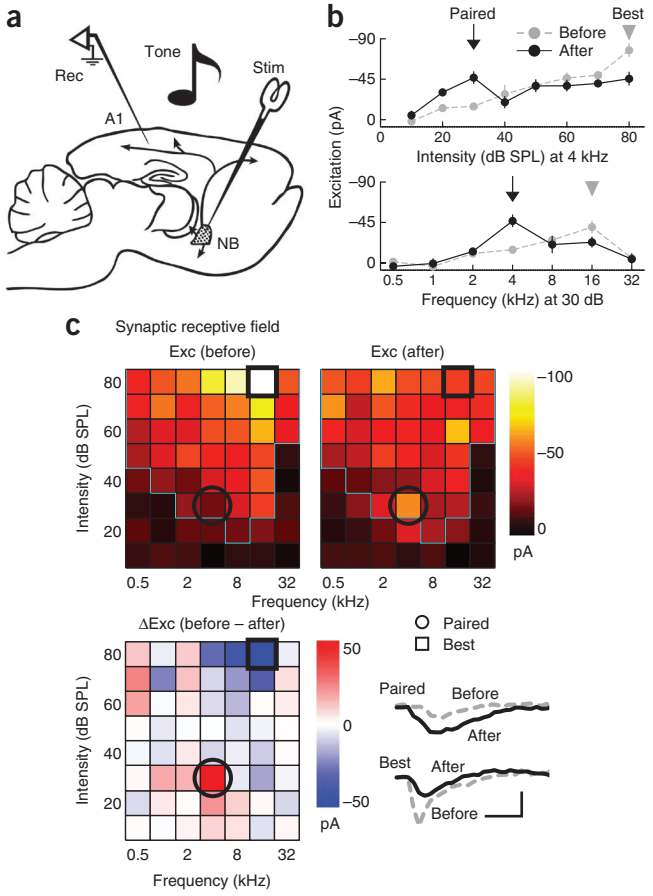

Figure 1 Example of A1 synaptic receptive field modification induced by nucleus basalis pairing. (**a**) Experimental preparation. Rec, recording; Stim, stimulation; NB, nucleus basalis. (**b**) Example of synaptic tuning curve modification induced by nucleus basalis pairing. Top, intensity sensitivity at 4 kHz. Bottom, frequency tuning at 30 dB SPL. Responses to paired stimulus (30 dB SPL, 4 kHz; arrows) are enhanced, whereas responses to peak level and best frequency (arrowheads) are reduced. (**c**) Frequency-intensity synaptic receptive field for same cell as in **b**. Top, before (left) and after (right) pairing. Color, EPSC amplitude. Blue lines, threshold. Bottom, change in EPSCs (post-pairing – pre-pairing). Excitation (exc) at paired tone (circle) increased from –14.8 ± 3.6 pA to –46.8 ± 6.6 pA ($P < 0.01$, Student's paired two-tailed *t*-test); excitation at original best stimulus (80 dB SPL, 16 kHz; square) decreased from –98.6 ± 15.4 pA to –43.3 ± 8.1 pA ($P < 0.01$). Net excitation across stimuli was similar before and after pairing (before, –1.68 nA; after, –1.51 nA; $P > 0.4$). Scale bars: 50 pA, 40 ms. Error bars show s.e.m.

recordings from 29 neurons of adult rat primary auditory cortex (A1) *in vivo*[17,20,23,38]. To rapidly and reliably reorganize synaptic receptive fields of A1 neurons, we combined recordings with electrical stimulation of the cholinergic nucleus basalis[20–24] (**Fig. 1a**), mimicking the activation of this neuromodulatory system during directed attention or arousing behavioral episodes[39,40]. Excitatory synaptic receptive fields were measured in voltage-clamp by playing pseudorandom sequences of pure tones, varying in intensity from 10 to 80 dB sound pressure level (SPL) and frequency from 0.5 to 32 kHz. After characterizing baseline responses for 5–15 min, we induced modifications of A1 synaptic receptive fields by repetitively pairing nucleus basalis stimulation with a tone of a specific intensity and frequency for 1–5 min ('nucleus basalis pairing'), using optimized parameters for pairing first identified with extracellular recordings (**Supplementary Fig. 1**) and confirmed with intracellular recordings (**Supplementary Fig. 2**). After pairing ended, we monitored receptive fields for as long as recording quality remained stable (see Online Methods).

Nucleus basalis pairing induced a set of highly organized changes across the entire frequency-intensity synaptic receptive field (**Fig. 1b,c** and **Supplementary Fig. 3**). In particular, increases in excitation at the paired inputs were matched closely by corresponding decreases at the original best stimuli (that which initially evoked the largest response), leading to a conservation of excitatory input received by A1 neurons.

In the example recording shown in **Figure 1**, 4-kHz tones of 30 dB SPL were paired with nucleus basalis stimulation for 3 min. Before pairing, the peak intensity was 80 dB SPL and the best frequency was 16 kHz. After pairing, excitation increased markedly at the paired stimulus (**Fig. 1b,c**). Additionally, although only 30 dB SPL, 4-kHz tones were presented during nucleus basalis stimulation, excitation at the original best stimulus (80 dB SPL, 16 kHz) decreased (**Fig. 1b,c**; for three other examples, see **Supplementary Fig. 3**). Although changes to some individual unpaired tones could be observed, responses to unpaired stimuli on average were not significantly different after pairing ($P > 0.05$). The net result of these modifications was to shift the preferred sound level and frequency tuning of this neuron while preserving the total strength of excitatory input across all stimuli (**Fig. 1c**).

For 29 recordings, these long-term changes in synaptic strength were on average specific to particular stimuli across dimensions of both intensity (**Fig. 2a**) and frequency (**Fig. 2b**). For intensity sensitivity, we observed maximum enhancements at the paired value, although increases often spread to lower intensities as well (**Fig. 2a**, top, and **Supplementary Figs. 2a,b**). Increased excitation at paired stimuli was matched by decreased excitation at original best stimuli (**Fig. 2a,b**, bottom), albeit with a somewhat slower time course (~10–20 min; **Fig. 2c**). These pairing-induced changes to synaptic strength cooperated to shift intensity sensitivity profiles of A1 neurons, making them less monotonic with regard to sound intensity (**Supplementary Fig. 4**), and conserved the net excitatory drive received by A1 neurons, such that the relative magnitudes of individual enhancements were approximately balanced by an equivalent amount of reduction (**Fig. 2d**).

These changes seemed to be specific to cortical neurons, as indicated by three sets of experiments. First, we made seven multiunit recordings from the ventral division of the medial geniculate body (MGB), the main auditory thalamus. We did not observe long-term changes of MGB responses after nucleus basalis pairing (**Supplementary Fig. 1c,d**), suggesting that thalamic spiking output is not persistently affected by pairing. However, under different conditions, it is possible that thalamic responses are modified, and it remains possible that pairing can induce plasticity in other parts of the MGB, such as the medial division.

Second, pairing also induced long-term changes in tone-evoked inhibitory responses of A1 neurons (**Supplementary Fig. 5a,c**), such that inhibitory responses at the paired stimulus and original best stimulus were reduced. In particular, in the example shown in **Supplementary Figure 5a**, reductions in tone-evoked inhibition were observed at both the paired and original best frequency. Over the population of 29 recordings, however, inhibition at the paired input began to recover (**Supplementary Fig. 5c**) and, as previously reported, eventually recovered to match and rebalance the strength of excitation[22]. As inhibitory inputs to A1 neurons are intracortical, this provides more evidence, together with local

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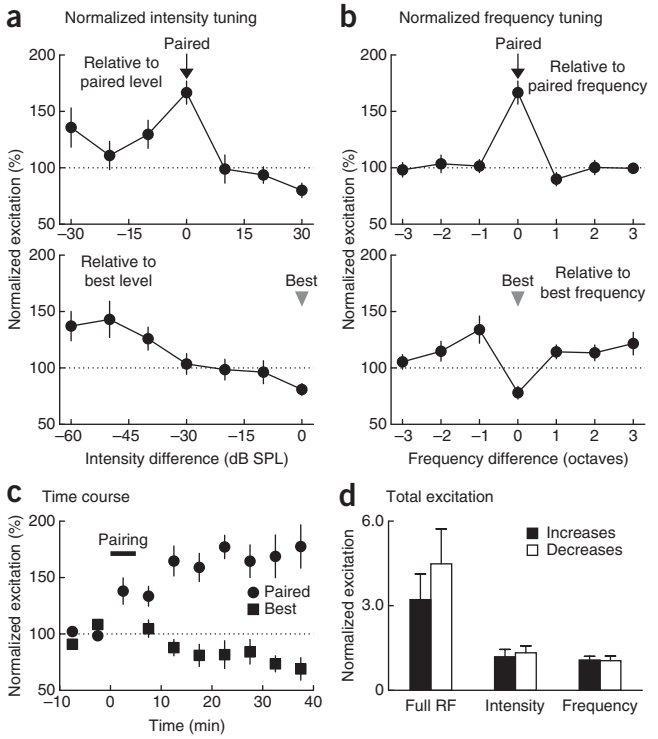

Figure 2 Conservation of total excitation after pairing. (**a**) Intensity-specific changes. Top, summary of changes relative to paired level over all recordings (arrow; increase of 66.7% ± 10.3%, $n = 29$ neurons, $P < 10^{-6}$, Student's paired two-tailed t-test). Significant potentiation also occurred to responses evoked by stimuli of the paired frequency and 10 dB lower in intensity (29.7% ± 12.4%, $P < 0.04$). Bottom, changes to original peak level (arrowhead; decrease of –19.0% ± 5.2%, $P < 10^{-4}$). (**b**) Frequency-specific changes. Top, changes relative to paired frequency. Bottom, changes to original best frequency (–21.9% ± 5.7%, $P < 0.001$). Same recordings as in **a**. (**c**) Time course of changes to paired (circles) and original best (squares) stimuli. Horizontal bar, pairing. Same recordings as in **a**. (**d**) Conservation of total excitation after pairing. Before and after pairing, relative amounts of increases (black) and decreases (white) in synaptic strength were similar across the entire frequency-intensity synaptic receptive field (RF) (excitation increased by a factor of 3.2 ± 0.9 and decreased by –4.5 ± 1.2; $n = 29$ neurons, $P > 0.6$, Mann-Whitney), across intensity at paired frequency (increase, 1.2 ± 0.3; decrease, –1.3 ± 0.2; $P > 0.6$) and across frequency at paired intensity (increase, 1.1 ± 0.1; decrease, –1.1 ± 0.2; $P > 0.5$). Same recordings as **a**. Error bars show s.e.m.

microstimulation experiments of thalamic and intracortical inputs[22], for a cortical locus of synaptic modification.

Third, we found that topical application of cholinergic or NMDA receptor antagonists to A1 prevented modifications of excitatory and inhibitory tuning curves by nucleus basalis pairing. Application of the muscarinic receptor antagonist atropine (1 mM; **Supplementary Fig. 6a**) blocked short- and long-term changes, suggesting that, despite GABAergic and peptidergic projections from nucleus basalis[41,42], muscarinic receptor activation is required for both the immediate and the enduring effects of nucleus basalis pairing. Example excitatory and inhibitory tuning curves from the same neuron are shown in **Supplementary Figure 6a**, before and 10 min after pairing; results from five experiments are summarized in **Supplementary Figure 6a**, bottom. Similarly, cortical application of the NMDA receptor blocker (2*R*)-amino-5-phosphonovaleric acid (AP5, 1 mM; **Supplementary Fig. 6b**) also prevented the long-term (but not the immediate) effects of pairing on excitation and inhibition in six neurons, demonstrating that excitatory and inhibitory modifications are consolidated downstream of NMDA receptor activation.

Therefore, as with frequency tuning[21,22], the peak sound levels and overall intensity sensitivity profiles of A1 synaptic responses are plastic and can be regulated conjointly. These changes act together to locally enhance paired stimuli while globally normalizing excitability across frequency-intensity synaptic receptive fields. Such synaptic modifications are longer-term consequences of complex processes engaged by neuromodulation and nucleus basalis pairing, similar to what might hypothetically occur during episodes of directed attention to salient or behaviorally meaningful stimuli[39–42]. Selective attention might, however, engage other mechanisms also important for processing sensory information, such as decorrelating activity patterns across different neurons or cell assemblies[23,40,43–45].

Best stimuli are dynamically determined

How are neurons or local networks able to sense and selectively modify responses to their original best stimuli? Previous studies of cortical receptive field plasticity in the visual system have examined the influence of homeostatic modifications of synaptic transmission and excitability on receptive field remodeling, especially after prolonged periods of monocular deprivation[11,33,35]. However, here, such homeostatic mechanisms may be too protracted and nonspecific to account for the reduction at the original best stimulus, which decreases over 10–20 min. Given that the best stimulus itself is an empirically determined local maximum, we next asked whether this suppression of synaptic strength was an activity-dependent process sensitive to the recent stimulus history.

To assess to what degree the reduction of synaptic strength at the original best stimulus was experience dependent, we played a restricted stimulus set (generally 10–60 dB SPL tones at 0.5–32 kHz) for approximately 10 min after pairing, excluding the original best stimulus (usually at 70–80 dB SPL). We then played the full stimulus set for the remainder of the recording, to recharacterize the synaptic receptive field and determine whether post-pairing presentation of the original best stimulus was necessary for the observed reduction in excitation evoked by those tones.

The absolute best stimulus for one example recording was an 80 dB SPL, 1 kHz pure tone, with strong responses to nearby, 'relative best stimuli' of 1–2 kHz at 60–80 dB SPL (excitatory responses, **Fig. 3a**; inhibitory responses, **Supplementary Fig. 5b**). Tones of 30 dB SPL, 4 kHz were used during pairing for this recording. After pairing, only stimuli of 10–60 dB SPL were played for 10 min (thus excluding absolute best stimuli). We then resumed measuring the frequency-intensity synaptic receptive field with the full stimulus set and found that, although excitatory responses to the paired tone had increased, excitatory responses to the original absolute best stimulus were unchanged (**Fig. 3a**). However, analysis of post-pairing changes throughout the synaptic receptive field revealed reductions to relative best stimuli that were included in the restricted stimulus set. Responses to 60 dB SPL, 2 kHz tones (the relative best stimulus; that is, whichever stimulus of <70 dB SPL evoked the largest pre-pairing responses) were depressed 10–20 min after pairing (**Fig. 3a**).

The reduction of responses at the absolute best or relative best stimuli ('best stimuli depression') thus seemed to require presentation of those tones in a prolonged period after the pairing procedure. For 13 recordings, responses evoked by relative best stimuli at lower intensity levels (usually at 60 dB SPL) were consistently reduced when included in the restricted stimulus set (excitation, **Fig. 3b**, left; inhibition, **Supplementary Fig. 5d**), whereas original absolute best stimuli at higher intensities were unchanged when those stimuli were not

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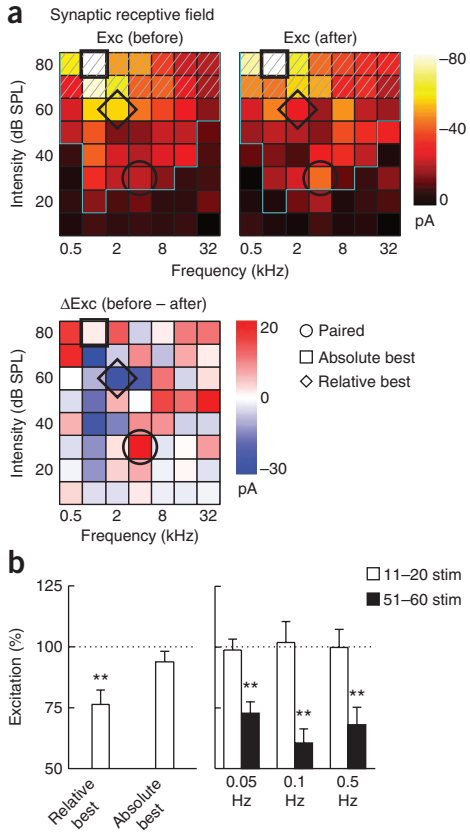

Figure 3 Best stimuli depression depends on recent sensory experience. (**a**) Example recording in which, for 10 min after pairing, no stimuli >60 dB SPL were presented (hatching). Responses to paired tone (30 dB SPL, 4 kHz; circles) increased (before, –21.3 pA; after, –40.7 pA); responses to absolute best stimulus (80 dB SPL, 1 kHz) were unchanged (before, –79.4 pA; after, –81.1 pA; squares); responses to 60 dB SPL, 2-kHz tones (relative best stimuli) decreased (before, –54.3 pA; after, –26.0 pA; diamonds). (**b**) Summary of reduced stimulus set experiments. Left, EPSCs evoked by relative best stimuli were depressed (–23.6 ± 5.9%, $n = 13$ neurons, $P < 0.006$, Student's paired two-tailed t-test), while absolute best stimuli were unchanged (–6.2 ± 4.3%, $P > 0.1$). Right, best stimuli depression was equivalent after 51–60 presentations, regardless of rate (black bars; 0.05 Hz: –27.2 ± 4.7%, $n = 7$ neurons, $P < 0.002$; 0.1 Hz: –39.5 ± 5.8%, $n = 5$ neurons, $P < 0.003$; 0.5 Hz: –31.9 ± 7.2%, $n = 10$ neurons, $P < 0.003$; equivalent magnitudes across rates, $P > 0.4$, Kruskal-Wallis $H = 1.51$). No depression was measurable after 11–20 presentations (open bars). Error bars show s.e.m.

presented. These results indicate that the best stimulus of a neuron is dynamically determined after periods of receptive field reorganization, likely requiring several minutes of stimulus presentation to assess the statistics of sensory input.

This regulatory process of best stimuli depression could require a certain duration (for example, approximately 10 min) to elapse, regardless of the number of stimulus iterations. Alternatively, best stimuli depression could require a certain number of tones to be presented, independent of duration. To resolve this issue, we varied the intervals between pure tone presentation after pairing, playing stimuli either at a slower rate (~1 per 20 s, or 0.05 Hz), a moderate rate (~1 per 10 s, 0.1 Hz) or a faster rate (~1 per 2 s, 0.5 Hz). We then monitored the change in response at best stimuli at two different times: after 11–20 stimulus presentations or after 51–60 stimulus presentations. If best stimuli depression was strictly time dependent, then we expected that, after 60 presentations, responses should be progressively more depressed from faster to slower presentation rates, as more time would have elapsed over the slower rate. Conversely, if best stimuli depression was accretive, then similar amounts of depression should be observed after 60 presentations, irrespective of rate over this range.

We found that the magnitude of best stimuli depression was equivalent after 51–60 stimulus presentations regardless of rate (**Fig. 3b**, right), but not after 11–20 presentations in any of the three cases. These results demonstrate that 20–60 presentations of these tones were required for best stimuli depression, at least when presented within ~1–20 min. Furthermore, these data strengthen the hypothesis that best stimuli depression, and the resulting normalization of net excitation onto A1 neurons after pairing, is input specific and activity dependent. Thus, although mature A1 receptive fields are usually very stable[46], events such as nucleus basalis pairing transiently destabilize cortical tuning by enhancing responses to paired, possibly behaviorally relevant stimuli. To compensate for such changes in excitation, stimulus history appears to be monitored for minutes to hours afterward, allowing cortical networks to preserve excitability and receptive field structure by reducing the empirically determined largest evoked responses among the unpaired stimuli. Furthermore, paired stimuli themselves may be protected from this depression.

Reduced variability improves signal processing

Long-term changes in cortical synaptic receptive field organization might have important consequences for information processing and perception of sensory stimuli. In the remainder of this study, we used three different methods to evaluate changes in cortical function and signal processing after pairing: analytical (computing mutual information and variability of synaptic responses), electrophysiological (examining spiking receptive fields) and psychophysical (performing nucleus basalis pairing in behaving animals).

Two main tasks that sensory systems must accomplish are signal detection and recognition[47,48]. These functions can be challenging even in relatively quiet, controlled environments because, in cortical circuits, sensory-evoked excitatory responses occur in a noisy background of spontaneous activity (**Fig. 4a**, top), with variable single-trial amplitudes close in size to those triggered by other stimuli (**Fig. 4b**, top). We noticed that, particularly after pairing nucleus basalis stimulation with low-intensity tones, the magnitude of initially small responses to paired stimuli increased in parallel with decreases of the largest responses (**Fig. 1b**, top). This observation suggested that while the mean excitatory postsynaptic current (EPSC) size over all stimuli remained unchanged, the total variance of A1 synaptic tuning curves and receptive fields might be reduced.

Detection of sensory input requires the ability to reliably resolve tone-evoked events from spontaneously occurring synaptic events. At the same time, cortical representations of sensory percepts must be statistically distinct for correct recognition of stimuli and discrimination between different inputs. To examine whether changes to signal detection and recognition capacities might be represented at the level of synaptic inputs to A1 and therefore could be maintained for minutes to hours after pairing, we quantified changes to distributions of tone-evoked ('signal') and spontaneous ('noise') EPSCs over frequency-intensity receptive fields. After measuring these distributions before and after pairing, we computed two different metrics: an index of variability q, the variance of synaptic amplitudes normalized by mean amplitude[38], and the mutual information between the signal and noise distributions[48]. In this context, mutual information is related to the probability that, at any time, a given synaptic response was either stimulus evoked or occurred spontaneously.

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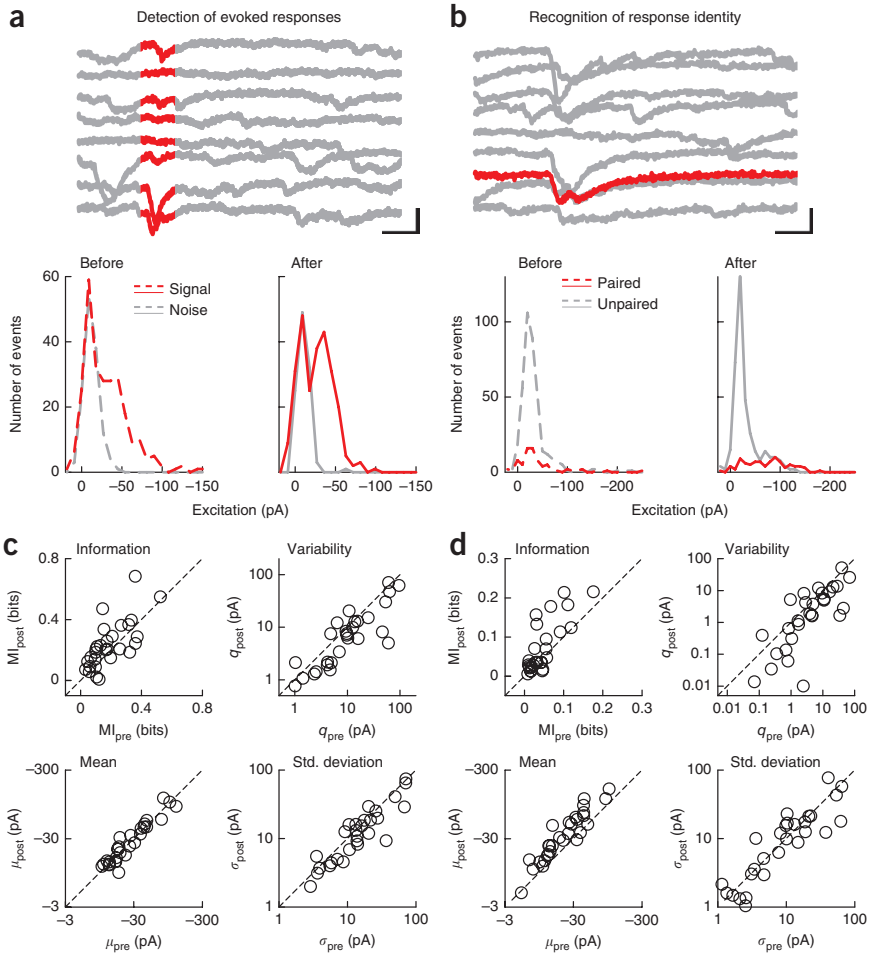

Figure 4 Pairing decreases synaptic variance to enhance detection and recognition of sensory stimuli. (**a**) Example of detection changes. Top, responses to paired frequency (tone presentation, red). Scale bars: 40 pA, 60 ms. Bottom, distributions of tone-evoked and spontaneous EPSCs before (left, dashed) and after (right, solid) pairing. Before pairing, signal and noise distributions overlapped (mutual information MI_{pre}, 0.17 bits) and had higher variability q in signal distribution (q_{pre}, 24.8 pA; mean μ_{pre}, –30.7 pA; σ^2_{pre}, 761.5 pA^2). After pairing, variability decreased and MI increased (MI_{post}, 0.26 bits; q_{post}, 15.1 pA; μ_{post}, –26.2 pA; σ^2_{post}, 396.9 pA^2). (**b**) Example of recognition changes. Top, responses evoked by tones of different frequencies (paired tone, red). Scale bars: 50 pA, 30 ms. Bottom, EPSC distributions for paired (red) and unpaired tones (gray). Initially (left), paired and unpaired responses were similar (MI_{pre}, 0.07 bits; q_{pre}, 69.0 pA; μ_{pre}, –42.2 pA; σ^2_{pre}, 2,911.3 pA^2). After pairing, means increased while variability decreased, enhancing MI between paired and unpaired distributions (MI_{post}, 0.18 bits; q_{post}, 25.9 pA; μ_{post}, –72.2 pA; σ^2_{post}, 1,871.7 pA^2). (**c**) Changes to detection. Top left, MI between signal and noise increased after pairing (before, 0.19 ± 0.02 bits; after, 0.23 ± 0.03 bits; $z = -2.0$, $n = 29$, $P < 0.05$, two-tailed paired Wilcoxon signed-rank test). Top right, q decreased after pairing (before, 19.2 ± 4.6 pA; after, 12.7 ± 3.3 pA; $z = 2.8$, $P < 0.005$). Bottom left, mean amplitudes of signal distributions were unchanged after pairing (before, –33.4 ± 5.3 pA; after, –34.0 ± 5.2 pA; $z = -0.7$, $P > 0.5$). Bottom right, s.d. of signal distributions decreased after pairing (before, 24.0 ± 4.8 pA; after, 19.3 ± 3.8 pA; $z = 2.4$, $P < 0.02$). (**d**) Changes to recognition. Top left, MI between paired and unpaired stimuli increased (before, 0.05 ± 0.01 bits; after, 0.08 ± 0.01 bits; $z = -3.0$, $P < 0.003$). Top right, q decreased after pairing (before, 11.2 ± 3.1 pA; after, 6.2 ± 2.0 pA; $z = 2.5$, $P < 0.02$). Bottom left, mean amplitudes of paired stimuli responses increased after pairing (before, –27.9 ± 4.2 pA; after, –44.5 ± 6.9 pA; $z = -4.4$, $P < 10^{-4}$). Bottom right, s.d. of paired stimuli responses were unchanged (before, 15.6 ± 3.2 pA; after, 14.2 ± 3.1 pA, $z = 0.9$, $P > 0.3$).

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We found that nucleus basalis pairing increased mutual information and decreased q. Distributions of tone-evoked and spontaneous EPSCs for the recording in **Figure 1** are shown in **Figure 4a**, bottom. Before pairing, there was considerable overlap between these distributions (**Fig. 4a**, bottom left). After pairing low-intensity tones with nucleus basalis stimulation, the distribution of spontaneous activity was essentially unchanged, whereas the s.d. of the tone-evoked response distribution subtly but significantly decreased, although mean amplitude was conserved (**Fig. 4a**, bottom right). As a result, the mutual information for signals increased; that is, the uncertainty about the presence of a signal in the noise was reduced, as there was less overlap between signal and noise distributions. Although these effects may be modest in individual recordings, small gains in single cells may have substantial effects at the population level. This suggests that quiet sounds would become easier to detect. Over 29 recordings, mutual information between signal and noise distributions increased (**Fig. 4c**, top left, and **Supplementary Fig. 7a**) and q decreased (**Fig. 4c**, top right), owing to reductions in s.d. (**Fig. 4c**, bottom right) with little change in mean amplitudes (**Fig. 4c**, bottom left). In 22 of 29 recordings, signal distributions became more statistically distinct from noise distributions after pairing (measured with Student's paired two-tailed t-tests). In five cases, signal distributions were initially statistically similar to noise distributions ($P > 0.05$) but became significantly different ($P < 0.05$) after pairing.

Similar analyses indicated that recognition of paired versus unpaired stimuli would also be enhanced by pairing, although by a complementary mechanism: increase in distribution mean (**Fig. 4b**, bottom). Before pairing, responses to tones chosen for pairing were approximately the same as responses to most other tones. After pairing, responses that were initially weak became stronger, increasing mutual information conveyed by paired versus non-paired tones (**Fig. 4d**, top left, and **Supplementary Fig. 7b**). Pairing also decreased q for responses to paired inputs (**Fig. 4d**, top right), by increasing mean amplitude (**Fig. 4d**, bottom left) while s.d. was not significantly affected (**Fig. 4d**, bottom right). As the sizes of paired distributions could be much smaller than the unpaired distributions, we cross-validated this analysis using t-tests. We found that 17 of 29 recordings showed lower P-values between paired and unpaired distributions after pairing (10 of 29 cases changing from statistically similar to statistically distinct at the $P < 0.05$ level), and 16 of 29 recordings showed lower P-values between paired and noise distributions after pairing (7 recordings changing from statistically similar to statistically distinct).

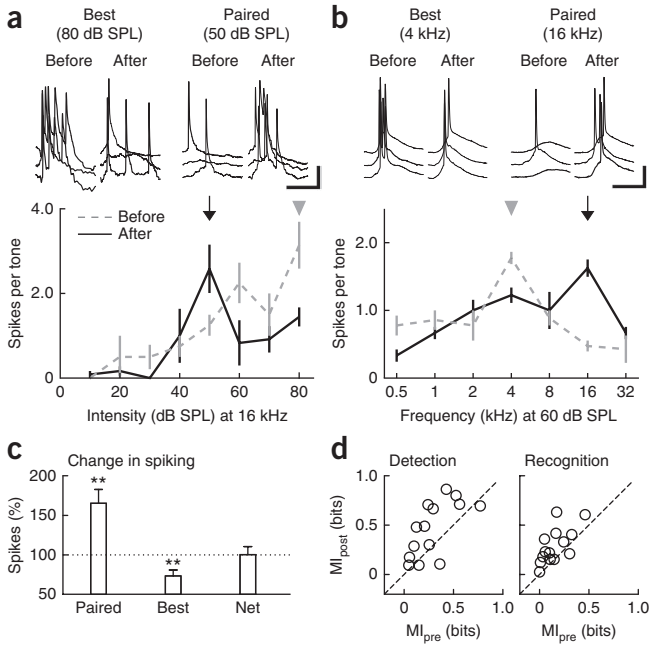

Figure 5 Nucleus basalis pairing modifies spiking receptive fields. (**a**) Suprathreshold intensity sensitivity (at 16 kHz) is modified after pairing. Example recording in which responses at paired intensity (50 dB SPL, arrow) increased (before, 1.3 ± 0.3 spikes per tone; after, 2.6 ± 0.6 spikes per tone; $P < 0.03$, Student's paired two-tailed *t*-test), responses at 80 dB SPL (arrowhead) decreased (before, 3.1 ± 0.6 spikes per tone; after, 1.9 ± 0.4 spikes per tone; $P < 0.05$). Scale bars: 5 mV, 50 ms. (**b**) Example of changes to suprathreshold frequency tuning (at 60 dB SPL) after pairing (for paired frequency 16 kHz: before, 0.5 ± 0.1 spikes per tone; after, 1.6 ± 0.1 spikes per tone; $P < 10^{-4}$; for best frequency 4 kHz: before, 1.8 ± 0.1 spikes per tone; after, 1.2 ± 0.1 spikes per tone; $P < 0.001$). Scale: 20 mV, 25 ms. (**c**) Summary of current-clamp recordings. Spiking responses to paired tones increased (65.2 ± 17.6%, $n = 14$ neurons, $P < 0.003$), responses to original best stimuli decreased (–26.7 ± 7.4%, $P < 0.004$); there was no net change in spiking (0.2 ± 10.3%, $P > 0.9$). (**d**) Summary of changes to MI after pairing. Pairing increased MI for detection (before, 0.29 ± 0.06 bits; after, 0.46 ± 0.08 bits; $z = -2.3$, $P < 0.02$; left) and recognition (before, 0.16 ± 0.04 bits; after, 0.29 ± 0.05 bits; $z = -2.9$, $P < 0.004$; right). Error bars show s.e.m.

Synaptic modifications affect spike output

To be useful for improving perceptual abilities in behaving animals, alterations in cortical receptive fields and signal processing at the level of synaptic inputs should also be represented by changes to spiking receptive fields. To determine whether pairing improved information processing at the level of action potential generation, we made current-clamp recordings from A1 neurons *in vivo* and measured tone-evoked suprathreshold responses.

For intensity sensitivity (**Fig. 5a**) and frequency tuning (**Fig. 5b**), pairing enhanced spike counts evoked by paired tones and reduced spiking evoked by original best stimuli. As with synaptic strength, the net effect of these adjustments was that the total number of

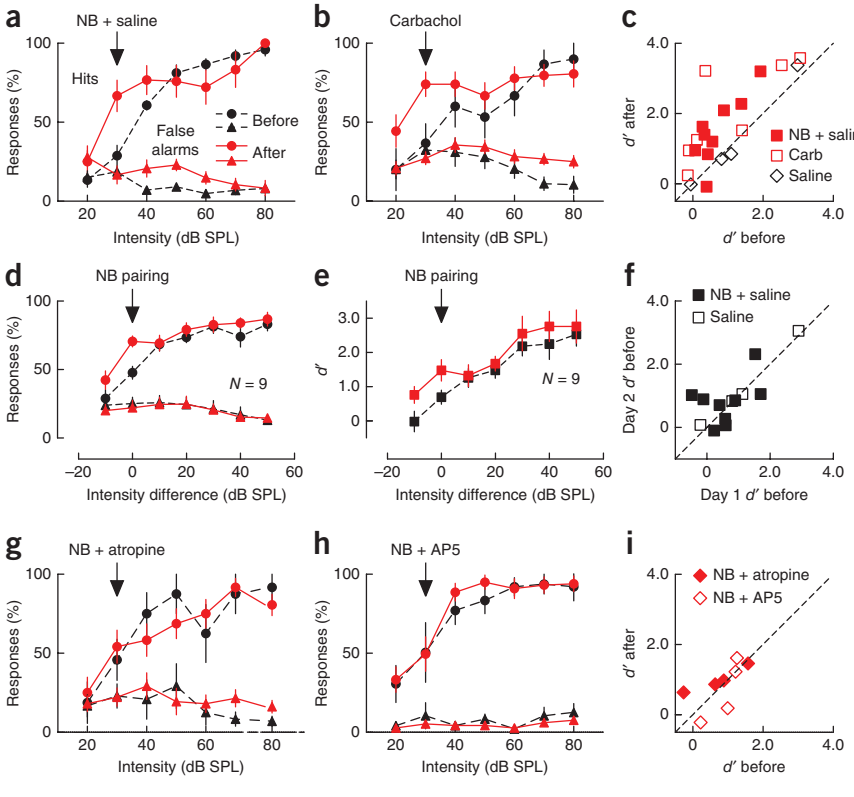

Figure 6 Nucleus basalis pairing improves auditory detection. (**a**) Example of enhanced detection after nucleus basalis (NB) pairing. Hits (circles) at 30 dB SPL) increased after pairing (before pairing, 28.9 ± 6.6%; after, 66.7 ± 10.0%; $P < 0.005$). Responses to foils (triangles) were unchanged (false alarms at 30 dB SPL before, 18.7 ± 3.2%; after, 16.7 ± 5.7%; $P > 0.7$). *d′* increased (0.3 to 1.4). Arrow indicates paired stimulus throughout. (**b**) Carbachol pairing enhanced detection without NB stimulation. Hits increased (before, 36.7 ± 6.6%; after, 74.1 ± 7.8%; $P < 0.001$); false alarms were unchanged (before, 32.4 ± 7.6%; after, 27.2 ± 4.1%; $P > 0.5$). *d′* increased (0.1 to 1.3). (**c**) Summary of *d′* values before and after pairing NB stimulation with saline (*d′* before, 0.7 ± 0.2; after, 1.5 ± 0.3; $N = 9$ animals, $P < 0.003$), or carbachol (carb) pairing without NB stimulation (*d′* before, 1.0 ± 0.5; after, 2.0 ± 0.5; $N = 7$, $P < 0.03$). Saline pairing without NB stimulation had no effect (*d′* before, 1.2 ± 0.6; after, 1.2 ± 0.7; $N = 4$, $P > 0.8$). (**d**) Changes to mean response rate across animals. Response rate increased after pairing at the paired intensity level (hits before pairing, 47.7 ± 4.7%; after, 70.6 ± 4.0%; $N = 9$, $P < 0.002$) and –10 dB SPL from paired level (before, 28.9 ± 6.4%; after, 42.4 ± 6.7%; $P < 0.03$) but not at higher intensities ($P > 0.1$). False alarms were unchanged (before, 25.2 ± 4.6%; after, 22.1 ± 5.5%; $P > 0.3$). (**e**) *d′* for paired stimuli across animals was enhanced after pairing (before, 0.7 ± 0.2; after, 1.5 ± 0.3; $P < 0.003$). (**f**) Comparison of detection between first and second days, before pairing (*d′* day 1, 0.6 ± 0.2; *d′* day 2, 0.8 ± 0.2; $N = 9$, $P > 0.4$) and for animals receiving only saline (*d′* day 1, 1.2 ± 0.6; *d′* day 2, 1.3 ± 0.6; $N = 4$, $P > 0.2$). (**g**) Atropine prevented effects of pairing. Hits, false alarms and *d′* were unchanged ($P > 0.6$). (**h**) AP5 prevented effects of pairing ($P > 0.5$). (**i**) Summary of effects of atropine (*d′* before, 0.7 ± 0.4; after, 1.0 ± 0.2; $N = 4$, $P > 0.2$) and AP5 (*d′* before, 0.9 ± 0.2; after, 0.7 ± 0.4; $N = 4$, $P > 0.4$). Error bars show s.e.m.

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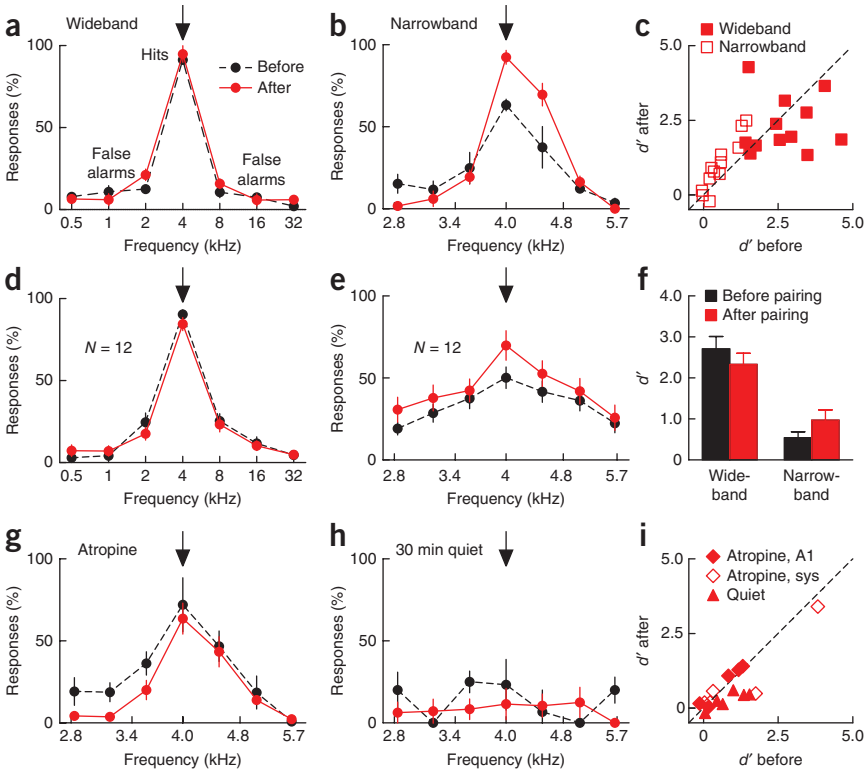

Figure 7 Nucleus basalis pairing improves recognition. (**a**) Responses from one animal. Nucleus basalis pairing did not improve wideband performance (d' before, 3.5; after, 2.8). Arrow indicates paired stimulus throughout. (**b**) Responses from another animal. Pairing improved narrowband performance (d' before, 1.3; after, 2.3). (**c**) Summary of wideband (d' before, 2.7 ± 0.3; after, 2.3 ± 0.3; $N = 12$, $P > 0.3$) and narrowband (d' before, 0.5 ± 0.1; after, 1.0 ± 0.2; $N = 12$, $P < 0.005$). (**d**) Wideband performance was unchanged after pairing (hits before pairing, 90.3 ± 2.8%; after, 84.5 ± 5.0%; $N = 12$, $P > 0.1$). (**e**) Narrowband performance was improved after pairing (hits before pairing, 50.1 ± 6.6%; after, 69.9 ± 9.0%; $N = 12$, $P < 0.005$). (**f**) d' before and after pairing for wideband (before, 2.7 ± 0.3; after, 2.3 ± 0.3; $P > 0.3$) and narrowband (before, 0.5 ± 0.1; after, 1.0 ± 0.2; $P < 0.005$) tasks. (**g**) Atropine infused into A1 prevented pairing from improving narrowband behavior (hits before pairing, 72.0 ± 16.4%; after, 63.6 ± 9.5%; $P > 0.6$; false alarms before, 23.4 ± 6.5%; after, 14.6 ± 6.4%; $P > 0.3$; d' before, 1.3; after, 1.4). (**h**) When only lower intensity (<50 dB SPL) stimuli were presented 30 min after pairing, narrowband behavior task performance was unaffected (hits before, 23.3 ± 15.4%; after, 11.5 ± 9.5%; $P > 0.3$; false alarms before, 11.9 ± 4.5%; after, 7.5 ± 1.8%; $P > 0.3$; d' before, 0.4; after, 0.2). (**i**) Summary of results for A1 atropine (d' before, 0.7 ± 0.3; after, 0.8 ± 0.3; $N = 5$, $P > 0.05$), systemic (sys) atropine (d' before, 1.2 ± 0.7; after, 1.0 ± 0.6; $N = 5$, $P > 0.3$) or when only quiet stimuli were presented after pairing (d' before, 0.7 ± 0.2; after, 0.3 ± 0.1; $N = 6$, $P > 0.05$). Error bars show s.e.m.

evoked spikes across all presented stimuli was kept constant (**Fig. 5c**) and mutual information for both signal detection and recognition increased (**Fig. 5d**).

Synaptic modifications improve perception

Attention and arousal facilitate sensory processing, and the nucleus basalis neuromodulatory system is critically linked to activation of attentive behavioral states[25,40]. Our analyses of synaptic distributions (**Fig. 4**) and measurements of spiking tuning curves (**Fig. 5**) both indicated that the changes to excitatory receptive fields induced by a few minutes of nucleus basalis pairing should improve auditory perception, particularly for subliminal stimuli with initially weak, subthreshold responses. Therefore, in our final experiments, we examined the psychophysical abilities of adult animals to perceive specific tonal stimuli ('targets'), focusing on two separate aspects of sensory perception: detection of target stimuli over a range of intensities and discrimination of target stimuli from non-target tones ('foils') (**Fig. 6**).

Rats were operantly conditioned to nose-poke for a food reward in response to target stimuli (4-kHz tones, any intensity) while withholding responses to foil tones of other frequencies. Animals had stimulation electrodes implanted into right nucleus basalis and cannulas for drug delivery implanted unilaterally into ipsilateral A1. After 2 weeks of training, animals reached performance plateaus for relatively loud, salient target stimuli.

The psychophysical detection abilities of two representative animals are shown in **Figure 6a**,**b**, and the performances of all animals are individually shown in **Supplementary Figure 8**. Animals achieved high hit rates for targets and a low number of false alarm responses to foils (**Fig. 6a**). Consequentially, values of the discriminability index d' were good for louder tones considerably above perceptual threshold, whereas tones below ~50 dB SPL were detected less reliably.

After determining response rates and d', we repetitively paired 4-kHz tones at a single lower intensity (between 30–45 dB SPL) with nucleus basalis stimulation for 3–5 min while animals were awake in the training box. We then retested their perceptual abilities to detect 4-kHz tones. At the paired stimulus intensity, detection was much higher, with average d' more than doubling 30–120 min after pairing (**Fig. 6c**,**e**). This was due to an increase in hit rate at the paired intensity, with no significant change in false alarms (**Fig. 6a**,**d**). Data shown are averaged across 2 d of training; as shown in **Figure 6f**, baseline daily performance of individual animals was approximately the same at the start of the first day and the second day. Thus pairing nucleus basalis activation with presentation of low-intensity sounds makes it easier for animals to perceive and operate on these initially hard-to-hear stimuli, perhaps by a selective reduction in thresholds for spike generation and/or perception.

Changes to A1 networks could lead to substantial perceptual improvements in initially trained animals. We found that, instead of nucleus basalis stimulation, pairing low-intensity tones with direct A1 infusion of the cholinergic agonist carbachol (1 mM) also was effective at increasing detection of the paired tone (**Fig. 6b**,**c**). This demonstrated that changes localized to or initiated directly in A1 could enhance auditory perception, after the basic audiomotor association for the task had been formed. As a control, saline infusion paired with low-intensity tones had no effect (**Fig. 6c**). In contrast, infusion into A1 of either the muscarinic receptor antagonist atropine or the NMDA receptor blocker AP5 (1 mM each) prevented increase in detection abilities after nucleus basalis pairing, indicating that long-term modification of A1 synapses is required for enhanced tone detection (**Fig. 6g–i**).

We further tested whether pairing in awake animals would improve recognition abilities (**Fig. 7**). Initially, foil stimuli were spectrally

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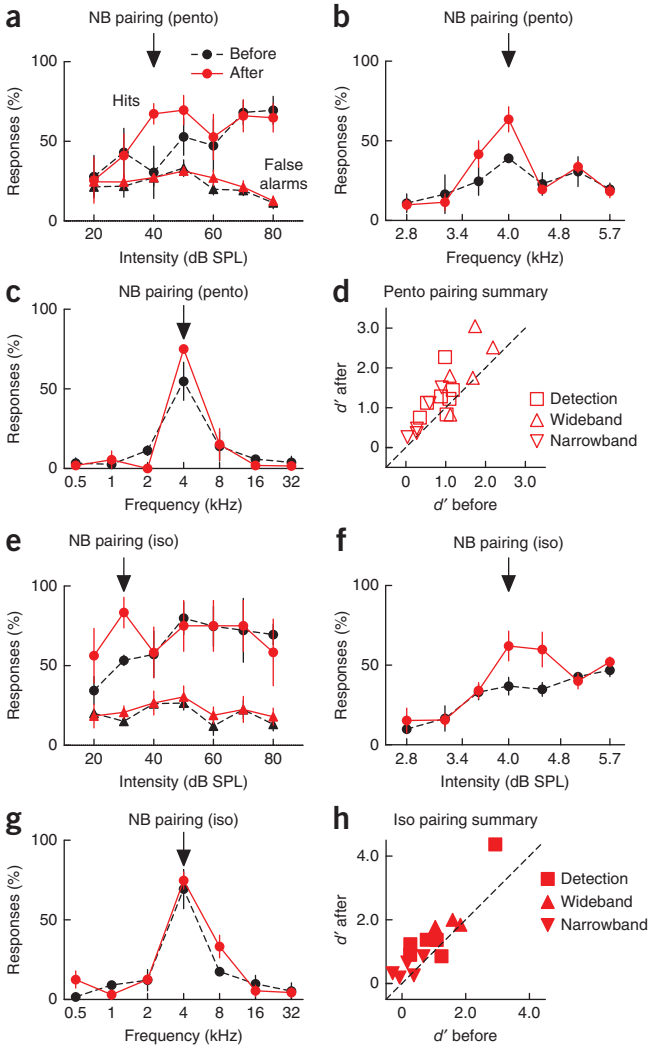

Figure 8 Pairing under anesthesia improves perception. (**a**) Detection; animal anesthetized with pentobarbital (pento) during nucleus basalis (NB) pairing. Hits increased after pairing (before, 30.6 ± 16.3%; after, 67.3 ± 6.5%; $P < 0.04$). False alarms were unchanged after pairing (before, 27.2 ± 5.2%; after, 27.3 ± 1.9%; $P > 0.4$), increasing d' from 0.4 to 0.8. Arrow indicates paired stimulus throughout. (**b**) Narrowband; pentobarbital anesthesia during pairing. Hits increased (before, 39.0 ± 2.7%; after, 63.5 ± 7.9%; $P < 0.03$); false alarms were unchanged (before, 20.9 ± 2.9%; after, 22.4 ± 3.5%; $P > 0.7$), increasing d' from 0.6 to 1.1. (**c**) Wideband; pentobarbital anesthesia during pairing (hits before, 54.8 ± 11.9%; after, 75.1 ± 1.1%; $P > 0.1$; false alarms before, 6.8 ± 1.7%; after, 4.3 ± 2.0%; $P > 0.3$; d' before, 1.7; after: 1.8). (**d**) Summary of experiments with pentobarbital anesthesia during and 1–3 h after pairing. Performance on wideband, narrowband and detection tasks was assessed 30–60 min before pairing and 1–2 h after recovery. Performance improved on detection (d' before, 0.9 ± 0.1; after, 1.3 ± 0.2; $N = 7$, $P < 0.03$) and on narrowband (d' before, 0.4 ± 0.1; after, 0.7 ± 0.2; $N = 5$, $P < 0.02$) but not wideband (d' before, 1.6 ± 0.2; after, 2.0 ± 0.4; $N = 5$, $P > 0.05$) recognition. (**e**) Detection; animal anesthetized with isoflurane (iso) before pairing. Hits increased after pairing (before, 53.3 ± 3.3%; after, 83.3 ± 9.6%; $P < 0.03$) and false alarms were unchanged (before, 15.1 ± 2.6%; after, 20.7 ± 4.0%; $P > 0.1$), increasing d' from 0.8 to 1.4. (**f**) Narrowband recognition; animal anesthetized with isoflurane during pairing. Hits increased (before, 36.8 ± 5.5%; after, 62.1 ± 9.4%; $P < 0.04$) and false alarms were unchanged (before, 30.6 ± 3.6%; after, 36.1 ± 4.2%; $P > 0.1$), increasing d' from 0.2 to 0.7. (**g**) Wideband recognition; animal anesthetized with isoflurane during pairing. Behavior was unchanged (hits before, 69.4 ± 12.3%; after, 74.7 ± 5.4%; $P > 0.7$; false alarms before, 9.2 ± 1.9%; after, 11.9 ± 2.2%; $P > 0.3$; d' before pairing, 1.8; after, 1.8). (**h**) Summary of experiments with isoflurane anesthesia during pairing. Performance improved on detection (d' before, 1.1 ± 0.4; after, 1.7 ± 0.5; $N = 6$, $P < 0.04$) and narrowband (d' before, 0.2 ± 0.2; after, 0.5 ± 0.1; $N = 5$, $P < 0.05$) but not wideband (d' before, 1.5 ± 0.2; after, 1.9 ± 0.1; $N = 3$, $P > 0.1$) recognition. Error bars show s.e.m.

dissimilar from the target stimulus of 4 kHz, separated at 1-octave intervals at 70 dB SPL. Animals could easily respond to targets and withhold responses to foils on this 'wideband' task before pairing, as shown for one example animal (**Fig. 7a**) and for all 12 animals tested on this task (**Fig. 7d**). Unsurprisingly, pairing with 4-kHz tones failed to improve recognition (example animal, **Fig. 7a**; all animals, **Fig. 7c**,**d**,**f**), as animals were already performing close to optimum.

We then made this task more challenging, by compressing the spectral range of the foils from six octaves to one octave, such that the foils were much more similar to the target tone. Before pairing, behavioral performance on this narrowband task was low (example animal, **Fig. 7b**; all animals, **Fig. 7c**,**e**,**f**), but pairing greatly improved frequency recognition (example animal, **Fig. 7b**; all animals, **Fig. 7c**,**e**,**f**). This increase in performance was prevented by administration of atropine, either directly into A1 by means of a cannula (**Fig. 7g**,**i**) or given systemically (**Fig. 7i**).

Overall, 7 of 9 animals showed a significantly higher hit rate ($P < 0.05$, Student's paired two-tailed t-test) in the detection of the target tone at the paired intensity after pairing (**Supplementary Fig. 8**). On the narrowband recognition task, 7 of 12 animals showed a significantly higher hit rate on the target frequency after pairing (**Supplementary Fig. 9**), whereas only 1 of 12 animals improved performance on the wideband recognition task (**Supplementary Fig. 10**).

We then tested whether changes to paired inputs alone could enhance perceptual abilities; alternatively, perhaps best stimuli depression and corresponding changes in full synaptic receptive field variance are required for improved sensory perception. In behaving animals, we found that nucleus basalis pairing enhanced recognition on the narrowband task for tones at 70 dB SPL only when we played the full stimulus set after pairing. If, instead, we presented for 30 min afterward a reduced set of targets and foils that did not contain any tones over 50 dB SPL, pairing failed to improve target recognition at higher intensity levels (example animal, **Fig. 7h**; all animals, **Fig. 7i**). Therefore, although nucleus basalis pairing induced enhancements in responses to paired stimuli, wide-scale receptive field reorganization mediated by large range stimulus exposure—leading to decreased response variability—was required for these changes to be perceptually useful. Given the relatively rapid gains in performance, our results indicate that best stimulus depression is required for cortical receptive field reorganization to have functional significance. Without it, perceptual improvement is limited and the benefits of cortical plasticity may be compromised.

Lastly, although these behavioral changes were observed when nucleus basalis pairing was performed in awake animals, the electrophysiological data were obtained in anesthetized animals. To more closely connect the physiological and behavioral effects of pairing, we conducted further behavioral experiments, performing pairing in animals that were temporarily anesthetized. We monitored baseline behavioral performance of trained, implanted animals for 30–60 min. We then anesthetized them with either pentobarbital (**Fig. 8a**–**d**) or isoflurane (3–5%; **Fig. 8e**–**h**) and performed 5 min of pairing with 4-kHz tones was performed. Animals were allowed to recover (usually after 1–2 h for isoflurane or 3–5 h for pentobarbital) and post-pairing behavioral performance assessed for 1–2 h. We observed significant

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improvements on the detection and narrowband recognition tasks even when pairing was performed in animals that were anesthetized (**Fig. 8**). Thus the changes in neural circuits initiated by nucleus basalis pairing persist and can affect behavioral performance even after major changes in brain state.

DISCUSSION

The central nervous system remains plastic throughout life, adapting to behaviorally relevant changes in the external environment. Previous studies have documented alterations to cortical circuits and elsewhere in the brain that are correlated with periods of behavioral training and conditioning[10,49]. Here we used a different approach to assess the causal value of such modifications to cortical synaptic receptive fields, taking advantage of the powerful neuromodulatory system of the cholinergic nucleus basalis to mimic the neural processes engaged by and important for attention. Nucleus basalis pairing modifies intracortical synapses in absence of changes to thalamocortical transmission[22], allowing us to selectively probe the behavioral and network effects of direct changes to cortical synapses and receptive fields.

Our data demonstrate how excitatory inputs to the cerebral cortex are coordinated *in vivo* to accommodate changes in sensory representations for perceptual learning. Several parameters of A1 receptive fields were rapidly changed by pairing a specific input with nucleus basalis activation. In particular, neurons that initially prefer higher-intensity tones could be retuned to prefer lower-intensity stimuli. Synaptic enhancements at paired inputs were coupled with reductions in responses to previously strong inputs, in a manner that depended on the statistics of the acoustic environment experienced immediately after each episode of nucleus basalis pairing. We predict that these changes serve to transiently increase the similarity between cortical neurons by enhancing responses to the shared, paired input and decreasing responses to originally preferred (possibly distinct) inputs. As a consequence of having more similar receptive fields, we speculate that stimulus and noise correlations between neuronal firing patterns may be higher, possibly improving signal processing and information transmission to downstream stations. Further experiments will be necessary to clarify this issue of the effects of synaptic plasticity and attentional modulation on cortical correlation[23,43–45].

Our experiments indicate that, in addition to the tone presented during nucleus basalis stimulation, the statistics of the post-pairing acoustic environment influence how A1 synapses and tuning curves are adjusted after pairing. In particular, it seems that cells and networks are able to compute their local maximal best stimulus, to reduce those responses and compensate for the increase of excitation at the paired input. The integrative time for this process seemed to be at least 10 min, and future studies are required to determine the duration of this sensitive period for best stimulus depression, the cellular mechanisms and relation to phenomena such as heterosynaptic LTD, and the perceptual impact of changes to input statistics. Notably, a recent study of acoustic perceptual learning in humans demonstrated that passive stimuli presented after training could also influence the degree of learning[50]. However, stimuli presented more than 15 min after a period of practice were less effective, and stimuli presented 4 or more hours after practice were found to be ineffective.

Functionally, changes to A1 synapses alone are presumably insufficient to generate behavioral modification in untrained animals, which require extensive training on the procedural aspects of the tasks. However, once the initial audiomotor associations have been formed, modifications of A1 circuitry can lead to superior behavioral performance for recognizing paired tones from spectrally similar unpaired tones and enhance the perception of liminal, low-intensity stimuli.

METHODS

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

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

R.C.F., I.C., D.B.P., M.M.M. and C.E.S. designed the experiments. R.C.F., I.C. and A.R.O.M. performed the electrophysiological experiments. R.C.F., I.C., A.J.B., K.Y., B.A.S., N.Z. and H.B. performed the behavioral experiments. M.W. and P.A.L. designed and built the wireless device. R.C.F. wrote the manuscript. All authors discussed the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Surgical preparation. All procedures were approved under New York University and University of California, San Francisco Institutional Animal Care and Use Committee protocols. Experiments were carried out in a sound-attenuating chamber. Female Sprague-Dawley rats 3–5 months old were anesthetized with pentobarbital. A bipolar stimulation electrode was implanted in the right nucleus basalis (stereotaxic coordinates from bregma, in mm: 2.3 posterior, 3.3 lateral, 7 ventral) and the right auditory cortex was exposed. Pure tones (10–80 dB SPL, 0.5–32 kHz, 50 ms, 3 ms cosine on/off ramps) were delivered in pseudo-random sequence. A1 location was determined by mapping multiunit responses 500–700 μm below the surface using tungsten electrodes[15,22].

Whole-cell recording. *In vivo* whole-cell recordings were obtained from neurons located 400–1,100 μm below the pial surface. Recordings were made with an AxoClamp 2B (Molecular Devices). For voltage-clamp, patch pipettes (5–9 MΩ) contained (in mM) 125 cesium gluconate, 5 TEA Cl, 4 Mg-ATP, 0.3 GTP, 10 phosphocreatine, 10 HEPES, 0.5 EGTA, 3.5 QX-314, 2 CsCl, pH 7.2. For current-clamp, pipettes contained 135 potassium gluconate, 5 NaCl, 5 Mg-ATP, 0.3 GTP, 10 phosphocreatine, 10 HEPES, 0.5 EGTA, pH 7.3. Resting potential: −65.2 ± 9 mV (s.d.); series resistance (R_s): 25.0 ± 6 MΩ; input resistance (R_i): 108.1 ± 58 MΩ. Data were excluded if R_i or R_s changed >30% from values measured during baseline. Data were filtered at 2 kHz, digitized at 10 kHz and analyzed with Clampfit 10 (Molecular Devices). After recording baseline responses for each cell, a non-preferred tone of a given intensity level and frequency was repetitively presented (1–5 min) during nucleus basalis stimulation (250 ms, 100 Hz). Tone onset occurred 20 or 100 ms after the start of nucleus basalis stimulation. In voltage clamp, we measured peak excitatory current amplitudes at −70 mV and peak inhibitory current amplitudes at either −20 mV or 0 mV. Other aspects of some recordings (15/29 experiments in **Fig. 2**) were previously analyzed in terms of the changes to excitatory and inhibitory frequency tuning for a prior study[22].

Behavior. Rats were lightly food-deprived and pretrained for 1–4 weeks on a frequency recognition go/no-go task. Animals were rewarded with food for nose-poking within 3 s of presentation of a target tone (4 kHz, any intensity) and given a short (~5 s) time-out if they incorrectly responded to nontarget tones. After the rats learned to nosepoke to 4-kHz tones, spectrally wideband foils were also presented (0.5, 1, 2, 8, 16, 32 kHz). Animals that achieved hit rates >66.6% for targets were then anesthetized with ketamine/xylazine, had stimulation electrodes chronically implanted in right nucleus basalis and cannulas implanted into right A1, and were allowed to recover for a week. Each implanted animal was first tested on the wideband recognition task or the detection task for at least 1–2 days. On the wideband task, tones (target: 4 kHz; foils: 0.5, 1, 2, 8, 16, 32 kHz) were presented at 70 dB SPL. On the detection task, tones were presented at 20–90 dB SPL. On each day, tones were presented for 30–60 min to assess baseline performance; 4-kHz tones (at 70 dB SPL for the wideband recognition task; 30–45 dB SPL for the detection task; hits binned over 20–45 dB SPL) were then paired with nucleus basalis stimulation in the training box for 3–5 min and behavior assessed and quantified 30–120 min afterward. Animals were then randomly assigned to be part of an experimental group: detection in which pairing was combined with infusion of saline, atropine or AP5 into the A1 cannula (0.4–1.0 μL, 1 mM), or narrowband recognition (foils: 2.8, 3.2, 3.6, 4.5, 5.1, 5.7 kHz at 70 dB SPL) in which pairing was combined with infusion of saline or atropine, or pairing was followed by 30 min of low-intensity tone presentation ('quiet' stimuli of <50 dB SPL over the same range of frequencies). Choice of experimental group was made irrespective of baseline performance on these tasks, which were statistically similar across groups; for detection: nucleus basalis pairing mean pre d' was 0.7 ± 0.2 and mean pre hit rate was 47.7 ± 4.6%, carbachol pairing mean pre d' was 1.0 ± 0.5 ($P > 0.5$ compared to nucleus basalis paired animals, Student's unpaired two-tailed t-test) and mean pre hit rate was 58.0 ± 7.3% ($P > 0.2$ compared to paired animals), atropine mean pre d' was 0.7 ± 0.4 ($P > 0.9$) and mean pre hit rate was 36.8 ± 5.5% ($P > 0.1$), AP5 mean pre d' was 0.9 ± 0.2 ($P > 0.4$) and mean pre hit rate was 60.1 ± 8.4% ($P > 0.1$); for narrowband discrimination: nucleus basalis pairing mean pre d' was 0.5 ± 0.1 and mean pre hit rate was 50.1 ± 6.6%, atropine mean pre d' was 0.9 ± 0.4 ($P > 0.1$) and mean pre hit rate was 55.7 ± 7.0% ($P > 0.6$), 30 min quiet mean pre d' was 0.8 ± 0.2 ($P > 0.05$) and mean pre hit rate was 66.2 ± 9.8% ($P > 0.1$). For animals exposed to quiet stimuli, responses to 4-kHz target stimuli were still rewarded. Five animals received atropine systemically (1 mM, 2 mg/kg; **Fig. 7i**, open diamonds) and five animals through a cannula implanted in A1 (filled diamonds). For stimulation, three animals wore a miniature custom-built high-amplitude current generator on a backpack, to allow them to move freely around the training box. This device utilizes MICAz wireless sensing devices ('motes') running the TinyOS operating system for online stimulator control and consists of two parts: the 'rat mote', worn by the animal in a backpack, and the 'base mote', placed in the cage. Performances of the three animals who used the backpack-mounted stimulator were similar to the other animals, who were tethered to the current generator for the wideband task ($P > 0.2$).

Statistical analysis. For electrophysiological recordings, Student's paired two-tailed t-test was used for comparison unless otherwise noted. Power analysis was performed to determine the number of cells required for statistical significance. For whole-cell recordings, effect size was 1.40 and power was 0.82, requiring a sample size of at least five neurons (which is satisfied in all electrophysiological experiments summarized in **Figs. 2**–**5** and **Supplementary Figs. 2** and **6**). For multiunit recordings, effect size was 0.94 and power was 0.82, requiring at least nine recordings, satisfied in **Supplementary Figure 1**.

Threshold of synaptic receptive fields (light blue lines in **Figs. 1c**, **3a** and **Supplementary Figs. 3** and **5**) were assessed by measuring the distribution of baseline current noise for each cell (mean trial-by-trial noise level generally <5 pA). We then compared the noise distribution to the distribution of tone-evoked events for each stimulus; threshold was then defined where the tone-evoked peak currents were significantly larger than noise ($P < 0.05$). As paired intensities (30–80 dB SPL) tended to be substantially higher than these thresholds (10–40 dB SPL), nucleus basalis pairing had no significant effect on minimum synaptic response threshold (two neurons increased threshold by 10 dB SPL, two neurons decreased threshold by 10 dB SPL, $P > 0.5$).

To compute the degree of conservation of excitation (**Fig. 2d**), for each cell, we subtracted the baseline response from the post-pairing response for each stimulus. We then normalized these differences by the mean baseline response (over the entire frequency-intensity receptive field, across the paired frequency axis, or across the paired intensity axis) and separately summed the positive values (for increases in response after pairing) and negative values (for decreases). These were then tabulated for each of the 29 whole-cell voltage-clamp recordings, and the absolute values of total normalized increases and total normalized decreases were compared using the Mann-Whitney test.

To compute monotonicity of intensity sensitivity profiles in **Supplementary Figures 4c**,**d**, we first determined the contribution to the decrease in correlation from the changes to three of the eight intensities ('Paired & peak', the paired intensity, the intensity 10 dB SPL lower than paired, and the intensity that evoked the largest EPSC). In this case, we assumed that, after pairing, responses to all other intensities remained at their original values before pairing, and calculated the corresponding linear correlation coefficient. Then, to determine the contribution of changes to all other five inputs ('Other'), we assumed that, after pairing, only the responses to these other stimuli were affected, whereas the responses to the paired, 10 dB SPL lower than paired, and peak intensities remained at their initial levels, and again calculated the change in correlation.

Index of variability q was defined as the variance of all synaptic responses normalized by the mean response amplitude ($q = \sigma^2/|\mu|$) (ref. 38). For signal detection, mutual information was defined as the difference between the synaptic entropy,

$$H(r) = -\sum_r P(r) \times \log_2 P(r)$$

and the synaptic entropy conditioned on whether a response was spontaneous ('noise') or tone-evoked ('signal'),

$$H(r|s) = -P(s = signal) \times \sum_r P(r \mid s = signal) \times \log_2 P(r \mid s = signal)$$

$$+\sum_r P(r \mid s = noise) \times \log_2 P(r \mid s = noise)$$

Mutual information for signal recognition was calculated in a similar manner, except that the noise distribution contained all unpaired tone-evoked responses as well as spontaneous responses, and the signal distribution only contained responses evoked by the paired tone and tones of the same frequency but 10 dB

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SPL lower[48]. Spontaneous events were measured in a 100–500-ms window during silent inter-stimulus intervals, and tone-evoked events measured in a 100-ms window starting at tone onset. To compare population statistics in **Figures 4c**,**d** and **5d**, two-tailed paired Wilcoxon signed-rank tests were used. Values of mutual information, q, means and s.d. were not statistically different between distributions comprising random half-sized subsets of the full distributions, for either signal/noise ($P > 0.1$) or the paired/unpaired data sets ($P > 0.05$).

For behavioral experiments, each animal's performance was averaged over 1–2 d, as detection thresholds and effects of pairing were consistent for at least the first 2 d of stimulation (**Fig. 6f**), and d' values were conventionally computed as the difference in z-scores between hits and false positives: $d' = z(\text{hit rate}) - z(\text{false positive rate})$. For determining the mean performance for each group of animals (**Figs. 6d**,**e** and **7d**,**e**), the individual mean performance curves (shown in **Supplementary Fig. 8** for detection, **Supplementary Fig. 9** for narrowband recognition and **Supplementary Fig. 10** for wideband recognition) were averaged together. Power analysis was performed to determine the number of animals required for statistical significance. For detection performance, effect size was 1.52 and power was 0.87. The required sample size was at least four animals, satisfied in the detection experiments of **Figures 6** and **8**. For recognition experiments, effect size was 1.32 and power was 0.80, requiring at least five animals, satisfied in the recognition experiments of **Figures 7** and **8**.

Error bars represent means ± s.e.m.

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