Coordinated forms of noradrenergic plasticity in the locus coeruleus and primary auditory cortex

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The cerebral cortex is plastic and represents the world according to the significance of sensory stimuli. However, cortical networks are embodied in complex circuits, including neuromodulatory systems such as the noradrenergic locus coeruleus, providing information about internal state and behavioral relevance. Although norepinephrine is important for cortical plasticity, it is unknown how modulatory neurons themselves respond to changes of sensory input. We examined how locus coeruleus neurons are modified by experience and the consequences of locus coeruleus plasticity for cortical representations and sensory perception. We made whole-cell recordings from rat locus coeruleus and primary auditory cortex (A1), pairing sounds with locus coeruleus activation. Although initially unresponsive, locus coeruleus neurons developed and maintained auditory responses afterwards. Locus coeruleus plasticity induced changes in A1 responses lasting at least hours and improved auditory perception for days to weeks. Our results demonstrate that locus coeruleus is highly plastic, leading to substantial changes in regulation of brain state by norepinephrine.

Results

Locus coeruleus plasticity

To determine how locus coeruleus is altered by experience, we first asked how locus coeruleus neurons respond to sensory stimuli. We recorded from these neurons in anesthetized adult rats (Fig. 1 and Supplementary Figs. 1 and 2), and locus coeruleus was identified by response to tail pinch and anatomical identification of electrode position. Intense stimulation (foot shock) produced phasic, high-frequency spiking (Supplementary Fig. 1a), whereas innocuous stimuli (pure tones) did not evoke detectable responses (Supplementary Fig. 1b). However, after tones were repetitively paired with foot shock for 1–5 min, paired tones could evoke locus coeruleus spikes for 1 h (Supplementary Fig. 1b). Spontaneous activity and responses to foot shock were qualitatively similar under both ketamine and pentobarbital anesthesia (Supplementary Fig. 2), although there was a trend for firing rates to be reduced in the presence of ketamine.

We then examined whether pairing auditory stimuli with locus coeruleus activity (locus coeruleus pairing) was sufficient to modify neuronal responses. Pairing was performed either by depolarization through the recording electrode or extracellular stimulation. For single-cell depolarization, we made current-clamp recordings from locus coeruleus neurons and measured responses to pure tones (0.5–32 kHz) 5–20 min before and 5+ min after pairing postsynaptic spike with a pure tone of a specific frequency at 70 dB sound pressure

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Figure 1 Locus coeruleus responses are plastic. (a) In vivo whole-cell or cell-attached recording from locus coeruleus (LC) neurons. (b) Locus coeruleus pairing procedure. Scale bars represent 0.3 mV and 25 ms. (c) Current-clamp recording from locus coeruleus neuron. Dotted line indicates baseline tone-evoked EPSP (0.0 ± 0.1 mV) and the red line represents tone-evoked EPSP after pairing (0.7 ± 0.1 mV, P = 10^{−4}, Student’s unpaired two-tailed t test, z score = 3.0). (d) Recording with AP5 infusion (1 mM). We observed no response to tones before (0.0 ± 0.1 mV) or after pairing (−0.1 ± 0.1 mV, P = 0.3; z score = −0.3). (e) Three locus coeruleus neurons for 7+ h before and after pairing. Left, first recording 10 min before (gray, tone-evoked EPSPs = −0.4 ± 0.1 mV) and 10 min post-pairing (red; tone-evoked EPSPs = 0.3 ± 0.3 mV, P = 0.004, z score = 1.1); third recording 420 min post-pairing (black; tone-evoked EPSPs = 0.6 ± 0.2 mV, z score = 1.8). Right, second cell-attached recording 360 min post-pairing (0.2 ± 0.02 spikes per tone; z score = 3.4). Arrow, paired 16-kHz tone. Arrowhead, maximal response before pairing. (f) Summary of new tonal responses in locus coeruleus neurons after pairing. Left, synaptic responses (33 measurements, n = 14 neurons, N = 9 animals; z score 5–15 min post-pairing = 3.1 ± 0.8, P = 0.0009; z score 3–10 h post-pairing = 2.0 ± 0.5, P = 0.02). Filled diamonds represent experiments pairing with single-cell depolarization instead of extracellular stimulation (ES). Open symbols represent AP5 in locus coeruleus (n = 7, N = 3; z score 5–15 min post-pairing = −0.2 ± 0.1, P = 0.3; z score 3–10 h post-pairing = 0.04 ± 0.05, P = 0.4). Right, spiking (35 measurements, n = 20, N = 10; z score 5–15 min post-pairing = 1.6 ± 0.6, P = 0.02; z score 3–10 h post-pairing = 2.9 ± 0.4, P = 10^{−4}; AP5, n = 13, N = 5; z score 5–15 min post-pairing = −0.7 ± 0.2, P = 0.2; z score 3–10 h post-pairing = −1.0 ± 0.7, P = 0.2). Error bars indicate s.e.m.

Level (SPL). After the baseline period, neurons were phasically depolarized at 20 Hz for 500 ms, repeated at 0.5–1 Hz for 1–5 min (Fig. 1b), similar to firing patterns observed in locus coeruleus neurons during foot shock (Supplementary Fig. 1a). This procedure mimics what can occur when sounds are linked to arousing situations, although locus coeruleus neurons can fire at higher rates and in shorter bursts in some cases. Electrode positions were confirmed by measuring responses to tail pinch (Supplementary Fig. 1a) and post hoc histology (Supplementary Fig. 1c), with some locus coeruleus neurons filled with biocytin through the whole-cell pipette and coabeled with an antibody to tyrosine hydroxylase (TH).

Pairing tones with single-cell depolarization induced responses to auditory stimuli in previously unresponsive locus coeruleus neurons that lasted for the duration of the recordings. An example in vivo whole-cell recording is shown in Figure 1c. Initially, this cell did not respond to sounds. However, after pairing 16-kHz tones with postsynaptic spiking, 16-kHz tones evoked sizable excitatory postsynaptic potentials (EPSPs) at ~30-ms latency, and tone-evoked responses lasted for the recording duration. Locus coeruleus plasticity required NMDA receptors, as local infusion of AP5 (1 mM) prevented emergence of tone-evoked responses after pairing (Fig. 1d). These results are reminiscent of ‘silent synapses’ activated after induction of long-term potentiation, although here it appeared that auditory responses had developed in formerly silent cells.

We tested how long these auditory responses would last after pairing. To simultaneously induce plasticity in multiple locus coeruleus neurons, we paired pure tones with extracellular locus coeruleus stimulation (20 Hz for 500 ms at 0.5–1 Hz, 1–5 min). We confirmed that stimulation was confined to a small area (<500 µm) around the electrode (Supplementary Fig. 1f). We made current-clamp and cell-attached recordings in vivo; after the first recording, 0–4 more recordings were obtained from different neurons in the same animal to assess the degree and duration of changes to other locus coeruleus neurons after a single pairing episode.

Three recordings from the same animal are shown in Figure 1e, a current-clamp recording before and after pairing, a cell-attached recording 5+ h after pairing, and a final current-clamp recording 6+ h afterward. The paired tone was 16 kHz at 70 dB SPL. Locus coeruleus cells continued to respond to paired tones for hours after pairing. In addition, locus coeruleus plasticity could be specific to paired tones, as responses to unpaired tones were sometimes initially enhanced, but not persistently modified after pairing.

The duration of locus coeruleus plasticity as measured by z scores is shown in Figure 1f (see Supplementary Fig. 3 for non-normalized values of synaptic and spiking responses). Substantial increases in synaptic strength and spike generation (Fig. 1f) lasted for at least several hours after pairing, unless locus coeruleus plasticity was prevented with AP5. These findings demonstrate that locus coeruleus neurons can become part of the overall circuit activated by once-innocuous stimuli. Notably, this might lead to noradrenergic modulation of target projection areas in response to tonal presentation alone. Thus, we focused the rest of our experiments on the functional consequences of neuromodulatory plasticity on cortical responses and perceptual learning.

Locus coeruleus pairing modifies A1 responses

A major output of locus coeruleus is the cerebral cortex, where noradrenergic modulation has a key role in sensory processing and control of behavior. To determine how noradrenergic modulation affected cortical sensory representations, we made in vivo recordings from A1 neurons and monitored tone-evoked responses before and after pairing (Figs. 2 and 3, and Supplementary Fig. 4). We made whole-cell recordings from 91 A1 cells (51 current-clamp, 40 voltage-clamp) and 50 cell-attached recordings in 49 adult rats implanted with
Figure 2 A1 plasticity induced by locus coeruleus pairing with electrical stimulation. (a) Setup: stimulation electrode (stim) in locus coeruleus (LC) and recordings (Rec) from A1 neurons. (b) Current-clamp recording of responses to paired 16-kHz and unpaired 4-kHz tones. (c) Synaptic (top) and spiking (bottom) tuning curves from five neurons before and 0–11 h post-pairing from current-clamp (filled) or cell-attached recordings (open). Each recording was from the same A1 location. Upper left, first recording 10 min before (gray) and 15 min after (black) pairing with 16 kHz. After pairing, best frequency shifted to 16 kHz (100% shift) and tuning width increased from 2.4 to 5.3 octaves (221% width). EPSPs increased after pairing. Best frequency shifted to 16 kHz (100% shift) and tuning curve width measured in s.d. (for example, 200% width indicates that best frequency became the original best frequency toward the paired frequency, as well as 100% shift indicates that best frequency became the paired frequency), and by fitting Gaussians and quantifying increase in tuning curve width measured in s.d. (for example, 200% width indicates that s.d. doubled). One set of recordings demonstrating the cortical effects of locus coeruleus pairing with electrical stimulation is shown in Figure 2b.c. We recorded from five neurons from the same region of A1 initially tuned to 4 kHz, for 11 h after pairing. The first cell recorded in current-clamp had a best frequency of 4 kHz (Fig. 2c). The paired frequency was 16 kHz at 70 dB SPL; after pairing, responses to all tones increased (Fig. 2b) across the tuning curve and the best frequency shifted to the paired frequency (Fig. 2c). We measured cell-attached spiking responses 50 min after pairing in this cell; 16 kHz remained the best frequency (Fig. 2c). Over the next 10 h, we obtained four more recordings (two current-clamp, two cell-attached; Fig. 2c). Tuning width recovered, but 16 kHz remained the best frequency. We also examined the effects of optical stimulation in Th-Cre rats expressing the ChETA variant of channelrhodopsin-2 in locus coeruleus neurons via stereotaxic injection of Cre-dependent adeno-associated virus (pAAV5-Ef1a-DIO-ChETA-EYFP). These Th-Cre animals had optical fibers implanted in locus coeruleus for stimulation specifically of the noradrenergic neurons during pairing (Fig. 3a). One set of recordings from the same animal is shown in Figure 3b. Before pairing, the best frequency was 1 kHz and responses were weak. After pairing, responses increased and tuning shifted to the paired 16-kHz frequency up to 7.5 h afterwards for both synaptic and spiking responses. Thus, electrical stimulation and optogenetic stimulation of the locus coeruleus both affect A1 receptive fields, increasing responses to auditory stimuli and at least initially reducing the tuning width of cortical neurons. Pairing with electrical versus optogenetic stimulation share some features, but there may also be some differences in outcomes, reflecting activation of overlapping, but distinct, cell populations with one method compared with the other.

Overall, three general features of cortical plasticity induced by locus coeruleus pairing were apparent: large increases in tone-evoked responses to all stimuli, shifts in best frequency toward paired inputs and return of average tuning width over several hours, with maintained preference at the paired input for the duration of the recordings (Fig. 4 and Supplementary Fig. 4). In individual neurons, responses at paired inputs were substantially larger both 5–10 min and 45–60 min after pairing (Fig. 4a). Responses to unpaired inputs were also enhanced 5–10 min after pairing, but returned toward original levels 45–60 min post-pairing (Fig. 4b). Similar enhancements also occurred for A1 intensity tuning (Supplementary Fig. 5), leading to stronger responses overall, albeit with increased preference around paired levels.

As a consequence, paired inputs tended to become the best input within minutes after pairing, shifting best frequency and broadening tuning. To our surprise, this shift in tuning could last 7–12 h (Fig. 4c and Supplementary Fig. 4a), whereas tuning width recovered and was statistically similar to baseline tuning widths within 3–4 h (Fig. 4d). These changes were also observed for spiking responses in current-clamp and cell-attached recordings (Fig. 4e,f and Supplementary Fig. 4b), as well as multiunit recordings (Supplementary Fig. 6). Similar modifications could be induced under pentobarbital anesthesia (Supplementary Fig. 7).
These effects are qualitatively different from the consequences of nucleus basalis pairing,17,35 which instead induc...cortical excitatory-inhibitory balance in favor of excitation.17 Thus, we next asked whether noradrenergic modulation via locus coeruleus stimulation has a similar effect, using in vivo voltage-clamp recordings from A1 neurons to assess changes to inhibitory postsynaptic currents (IPSCs). We found that locus coeruleus pairing greatly increased tone-evoked EPSCs and IPSCs together (Supplementary Fig. 10). However, pairing decreased spontaneous inhibition for several minutes during and after pairing. This was primarily expressed as a reduction in spontaneous IPSC rate (Fig. 5e), whereas spontaneous IPSC amplitudes (Fig. 5e) and the rates and amplitude of spontaneous EPSCs (Fig. 5f) were unaffected.

Our findings indicate that locus coeruleus activation seems to specifically decrease tonic (spontaneous) inhibition rather than phasic (stimulus-evoked) inhibition. This provides a basic gain control mechanism by which responses to any incoming stimuli would be transiently enhanced after noradrenergic modulation, as a reduction in spontaneous inhibition would affect all subsequent inputs, paired and unpaired. In this manner, locus coeruleus may provide a broadband signal for increasing sensory processing in novel or hazardous environments, where possibly one or more of many environmental cues are important for behavioral performance. Furthermore, these results also suggest that the sources of spontaneous and tone-evoked inhibition are under distinct forms of neuromodulatory control.

We asked whether reducing overall inhibitory tone could lead to similar changes in tone-evoked synaptic responses and frequency tuning. We performed pharmacological experiments to reduce GABAergic inhibition (Supplementary Fig. 11), either by bicuculline ionophoresis or intracelluar perfusion with picrotoxin. We made voltage-clamp recordings to measure synaptic frequency tuning, then presented tones of a given frequency during 5 min of bicuculline ionophoresis. We observed an enduring enhancement of tone-evoked excitatory
and inhibitory responses together 10 min after iontophoresis was over (Supplementary Fig. 11a). In other experiments, GABAergic inhibition was constitutively blocked by including picrotoxin in the whole-cell pipette. Because this disinhibition alone leads to a substantial enhancement of excitatory responses, we waited 10 min for dialysis of picrotoxin through the recording pipette before starting tonal stimulation. We monitored responses to a single tone frequency and observed a progressive strengthening of these synaptic responses in an activity-dependent manner that required NMDA receptors (Supplementary Fig. 11b). Thus, tonic disinhibition can be an effective mechanism for enhancing synaptic responses in a long-lasting manner.

Locus coeruleus plasticity controls cortical plasticity

We next performed a series of pharmacological studies to understand the mechanistic basis of A1 changes induced by locus coeruleus pairing and connect these forms of subcortical and cortical plasticity. To our surprise, we found that locus coeruleus plasticity was both sufficient (Fig. 6) and necessary (Fig. 7) for the induction and maintenance of cortical plasticity.

First, we paired tones with norepinephrine iontophoresis locally in A1 instead of locus coeruleus stimulation (Fig. 6a). Although ‘norepinephrine pairing’ increased responses and shifted best frequency toward the paired input, these changes were temporary and lasted less than an hour. Cortical norepinephrine, paired with sensory input, was not by itself sufficient for the long-lasting changes to A1 responses observed with locus coeruleus pairing.

We then examined whether noradrenergic receptor activation was at all required for the effects of locus coeruleus pairing.

Topical application of the alpha-adrenergic receptor antagonist phentolamine immediately before pairing initially blocked effects of pairing. However, minutes after pairing ended and phentolamine was no longer applied, A1 tuning curves shifted toward the paired frequency, resulting in enduring changes similar to those induced by locus coeruleus pairing (Fig. 6b).

Instead, noradrenergic receptor activation in A1 was required hours after locus coeruleus pairing had ended. To assess the requirement for neuromodulation after pairing, we topically applied phentolamine to A1 continuously over the duration of the experiment starting ~30 min post-pairing. Although locus coeruleus pairing initially produced substantial shifts in tuning curves, tens of minutes later, these changes were diminished and A1 tuning curves shifted back to their original best frequency in the presence of phentolamine (Fig. 6c).

These findings reveal two important features of locus coeruleus pairing and neuromodulatory plasticity. First, alpha-noradrenergic receptor activation is required for long-lasting expression of changes to cortical sensory representations in response to locus coeruleus pairing. Second, this modulatory control over cortical plasticity must occur in A1 itself, as local A1 application of noradrenergic receptor antagonist prevented tuning curve shifts. These results then suggest that plasticity of locus coeruleus directly controls A1 plasticity. In this scenario, each time paired tones are presented after pairing, newly responsive locus coeruleus neurons release norepinephrine into A1, maintaining changes to cortical representations in a selective and powerful manner.

Thus, we asked whether modifications to locus coeruleus neurons were required for cortical plasticity. We recorded from A1 neurons...
Locus coeruleus pairing improves auditory perception

Finally, we asked how these changes to auditory representations induced by locus coeruleus pairing might affect auditory perception. We used a behavioral task involving auditory perceptual learning in adult rats sensitive to plasticity of A1 tuning curves3,5. We examined three predictions suggested by our physiological results. First, behaviorally important stimuli should be easier to detect and recognize after locus coeruleus pairing, as A1 synaptic and spiking responses to tones of all intensities were greatly enhanced. Second, recognition of specific stimuli may initially be impaired, as A1 tuning curves first widened and responses to distinct stimuli became more similar for a few hours after locus coeruleus pairing. Third, improvements to perceptual abilities should persist for hours or perhaps indefinitely even after a single brief pairing episode, given the prolonged maintenance of A1 plasticity by locus coeruleus plasticity.

We operantly conditioned 79 adult rats to nose-poke for a food reward in response to 4-kHz target stimuli of any intensity while withholding responses to six foil tones of other frequencies (Supplementary Fig. 12a). We implanted 22 Sprague-Dawley rats with stimulation electrodes and drug delivery cannulas into locus coeruleus. An additional nine Th-Cre rats expressing ChETA in locus coeruleus with optical fibers implanted for optogenetic stimulation were used. We examined perceptual abilities to detect target stimuli over a range of intensities and recognize target stimuli from non-target foils, assessing performance over several days before and after pairing. In other animals, we examined whether locus coeruleus pairing would affect the learning rate on a reversal learning task.

First we examined detection abilities. Baseline psychophysical performance of a representative animal is shown in Figure 8a. At ≥50 dB SPL, this animal had near-perfect performance detecting target 4-kHz tones and a low number of responses to foils. However, this animal had a low response rate for tones of 20–40 dB SPL. After measuring behavioral responses for 30–60 min, we paired 4-kHz tones at 30 dB SPL with locus coeruleus stimulation for 3 min while the animal was awake. We then re-tested detection abilities 12 h later and found that detection at 20–40 dB SPL was greatly enhanced (Fig. 8a). Similar improvements in detection were observed for 21 animals, including five Th-Cre rats in which optogenetic locus coeruleus pairing was performed (Fig. 8b,c). Notably, enhancements of detection abilities lasted up to 4 d and in some cases 20+ d post-pairing (Fig. 8d).

Behavioral changes were prevented when AP5 (1 mM) was infused into locus coeruleus before pairing (Fig. 8c), indicating that locus coeruleus plasticity is required for behavioral improvement. Thus, locus coeruleus stimulation facilitates detection of previously imperceptible stimuli, and brief episodes of pairing optimize signal processing and formation of sensorimotor associations in a circuit distributed throughout cortex and brainstem.

We then asked how pairing would affect abilities to distinguish targets from foils. Initially, foil stimuli were spectrally dissimilar from the target stimulus of 4 kHz, separated at one octave intervals at 70 dB SPL. Animals easily responded to targets and withheld responses to foils on this ‘wideband’ task before pairing. We then made this task more challenging by compressing the spectral range of foils from A1 to A4, while withholding responses to six foil tones of other frequencies (Supplementary Fig. 12a). We trained 21 animals to respond to 4-kHz target stimuli of any intensity while withholding responses to six foil tones of other frequencies (Supplementary Fig. 12a). We then asked how pairing would affect abilities to distinguish targets from foils. Initially, foil stimuli were spectrally dissimilar from the target stimulus of 4 kHz, separated at one octave intervals at 70 dB SPL. 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**Figure 6** Noradrenergic receptor activation is required for expression of A1 plasticity. (a) Norepinephrine pairing (NE pairing) leads to shorter term but not sustained, A1 changes. Top, experimental design; pure tones were paired with NE iontophoresis (0.1 mM) in A1. Middle, cell-attached recording before and after NE pairing with 2 kHz (arrows). Initially, best frequency (open arrowheads) shifted from 8 kHz to 2 kHz, but returned to 8 kHz 1 h later. Bottom, NE pairing summary (best frequency shift 10 min post-pairing: 95.8 ± 5.1%, n = 4, N = 4, P = 10−5; shift 45+ min post-pairing: 8.3 ± 10.2%, n = 4, N = 4 animals, P = 0.3). Squares, cell-attached recordings. (b) Cortical phentolamine (0.1–1 mM) only during LC pairing prevented shorter term changes, but longer term changes emerged when phentolamine was removed. Top, experimental design. Middle, whole-cell recording before and after pairing, and cell-attached recording 1 h post-pairing. Original best frequency was 4 kHz, tuning was unchanged 10 min post-pairing in presence of phentolamine (0.1 mM), but shifted to paired 16-kHz tone 1 h later when phentolamine was removed. Bottom, phentolamine during pairing summary (shift 10 min post-pairing: 1.5 ± 62%, n = 6, N = 6, P = 0.7; shift 45+ min post-pairing: 67.8 ± 17.7%, n = 8, N = 7, P = 0.0006). Squares, cell-attached recordings. Circles, whole-cell recordings. (c) Phentolamine applied after pairing for hours shortened A1 shift duration. Top, experimental design; phentolamine (0.1 mM) was applied to A1 for subsequent recordings after pairing. Middle, two cell-attached recordings before and after LC pairing. Bottom, phentolamine after pairing summary (shift 10 min post-pairing: 85.0 ± 10.7%, n = 6, N = 6, P = 10−4; shift 3–8 h post-pairing: 6.3 ± 6.3%, n = 8, N = 5, P = 0.3). All comparisons were done with unpaired two-tailed t tests. Error bars indicate s.e.m.

six to one octave, such that foils were similar to the target. Before pairing, behavioral performance on this ‘narrowband’ task was low (Supplementary Fig. 12b), and pairing initially reduced these modest recognition abilities even further for a few hours (Supplementary Fig. 12b,c). However, performance gradually improved, and was improved above baseline levels 12 h post-pairing (Supplementary Fig. 12d). These changes in auditory perception seem similar to A1 modifications observed electrophysiologically: tuning profiles first broadened (leading to similar neural responses for different sensory stimuli) before tuning width recovered and many more A1 neurons responded strongly to paired stimuli (facilitating detection and recognition of target tones). Changes in narrowband task performance were prevented by infusion of AP5 into locus coeruleus (Supplementary Fig. 12c,d).

The duration of these changes induced by a single episode of locus coeruleus pairing was considerably longer than behavioral changes induced by nucleus basalis pairing (Supplementary Fig. 13). Single episodes of nucleus basalis pairing produced improvements in detection (Supplementary Fig. 13a–c) and recognition (Supplementary Fig. 13d–f) that lasted several hours35, but not longer. Instead, lasting improvements with nucleus basalis pairing required multiple episodes of pairing over several days, in contrast with the rapid and enduring behavioral changes produced by a single session of locus coeruleus pairing.

**Figure 7** Locus coeruleus plasticity controls the duration of cortical plasticity. (a) Three A1 recordings for 7+ h pre/post-pairing with AP5 in locus coeruleus. Left, first current-clamp recording 10 min before (gray) pairing; third current-clamp recording 460 min post-pairing (black). 4 kHz was the original best frequency (arrowheads); 1 kHz was the paired frequency (arrows). Right, second cell-attached recording 200 min post-pairing. (b) Summary of A1 best frequency shift with AP5 in locus coeruleus. Left, synaptic A1 best frequency shift. After 2–10 h post-pairing, best frequency returned to baseline (shift = 33.3 ± 21.1%, P = 0.1, n = 6 neurons, N = 3 animals). Shaded area represents mean ± s.e.m. of shifts from Figure 4e. Right, spiking best frequency shift (shift = 20.0 ± 20.0%, P = 0.3, n = 5, N = 4). Shaded area represents mean ± s.e.m. from Figure 4e. Error bars indicate s.e.m.
Figure 8 Locus coeruleus pairing improves sensory perception. (a) Enhanced detection after pairing 30 dB SPL, 4-kHz tones with electrical stimulation (ES) of locus coeruleus. Hits (circles) at 20–40 dB SPL increased after 12 h (pre-pairing, black: 8.3 ± 5.3%, post-pairing, red: 43.7 ± 14.0%, P = 0.04); foil responses (triangles) were unchanged (pre-pairing: 3.6 ± 1.7%, post-pairing: 6.8 ± 1.8%, P = 0.2), increasing d’ (0.5 to 1.4). (b) Enhanced detection after pairing 30 dB SPL, 4-kHz tones with optogenetic locus coeruleus stimulation (opto). Hits at 20–40 dB SPL increased after 12 h (pre-pairing, black: 5.0 ± 5.0%, post-pairing, blue: 38.7 ± 11.8%, P = 0.04); foils were unchanged (pre-pairing: 2.7 ± 1.6%, post-pairing: 3.8 ± 2.5%, P = 0.7), increasing d’ (0.3 to 1.1). (c) d’ values (before: 0.49 ± 0.06, 12 h after: 1.12 ± 0.12, N = 21, P = 10^-5, Student’s paired t-test). AP5 in locus coeruleus prevented improvement (open circles; d’ before: 0.65 ± 0.07, after: 0.57 ± 0.10, N = 6, P = 0.5). (d) Detection was enhanced 4 d (circles; d’ before: 0.49 ± 0.09, after: 0.98 ± 0.12, N = 12, P = 0.004) and 20 d after pairing (stars; before: 0.44 ± 0.09, after: 0.89 ± 0.14, N = 10, P = 0.01). (e) Reversal learning without pairing; rewarded frequency was changed to 16 kHz. Arrowhead, original rewarded frequency (4 kHz). Arrow, paired and rewarded frequency after reversal on day 0. (f) One pairing episode (at 16 kHz) accelerated reversal learning. (g) Accelerated reversal learning after electrical LC pairing. Sliding t tests (width: 2 d) used to determine when performance recovered to baseline (black, control: 22 d, N = 11; red, paired animals: 13 d, N = 6). (h) Accelerated reversal learning after optogenetic LC pairing. Performance recovered faster in paired animals (black, control: 17 d, N = 4; blue, paired animals: 13 d, N = 4). Error bars indicate s.e.m.

In our last experiment, we asked whether locus coeruleus pairing might also accelerate reversal learning. Animals were trained to respond to 4-kHz tones, and the rewarded tone was then switched to 16 kHz (and 4 kHz became a foil). We monitored animals for several weeks after this reversal of reward contingency. The day of reversal, some animals received a single episode of locus coeruleus pairing with 16 kHz. Unpaired animals required 20+ d to recover initial performance levels, but paired animals learned the new association in almost half the time (Fig. 8f,g). Consequently, locus coeruleus pairing improves perceptual abilities for at least hours after pairing, and even longer-lasting gains in performance can emerge over days to weeks after just a single pairing episode. These abilities can be enhanced beyond gains induced by reward-based training alone.

DISCUSSION

The locus coeruleus is the principal source of noradrenergic modulation for the CNS, providing a basic mechanism for adapting cortical circuits to task demands. Previous studies found that locus coeruleus neurons in rodents and primates respond to sensory stimuli after conditioning. We found that enduring contextual associations could be rapidly formed in the locus coeruleus and cells previously unresponsive to sounds could become tonally responsive. The rapid induction and prolonged duration of these changes is reminiscent of changes to brain state and behavior in cases of one-trial learning or post-traumatic stress disorder, suggesting that locus coeruleus plasticity is a fundamental determinant of these memory processes.

Locus coeruleus plasticity depends on NMDA receptors in the locus and can be induced by pairing tones with depolarization of single neurons. This form of long-term synaptic plasticity is likely a result of direct modifications of excitatory transmission of connections into or within locus coeruleus. Given the short latency of these auditory responses from stimulus onset and the speed at which acoustic information can reach A1 (~10 ms), we suspect that auditory inputs from frontal cortex and/or amygdala become sensitized after pairing. Notably, blockade of locus coeruleus plasticity with AP5 was observed in animals anesthetized with ketamine, indicating either that ketamine at these doses does not completely antagonize NMDA receptors or that the anesthetic action of ketamine in the locus coeruleus is independent of NMDA receptors.

Locus coeruleus plasticity exerts a profound effect on downstream modulatory targets, controlling dynamics of cortical plasticity and auditory perceptual learning from induction to long-term maintenance. Noradrenergic modulation seems to provide a specific disinhibitory signal to cortical circuits, transiently reducing spontaneous inhibition in a similar manner as in dorsal cochlear nucleus, a form of modulatory control is distinct from that provided by cholinergic modulation from nucleus basalis. Although both modulators reduce GABAergic inhibition in the cortex, acetylcholine down-regulates stimulus-evoked inhibition, whereas noradrenaline reduces tonic inhibition. There may be an anatomical basis for this distinction depending on the projection patterns of cholinergic versus noradrenergic axons, synaptic versus extrasynaptic localization of receptor subtypes, and other factors governing the spatial and
temporal scales of neuromodulation, but there are limited data on any of these important issues.

Moreover, the physiological and behavioral changes induced by a single episode of nucleus basalis pairing are smaller and briefer than changes triggered by locus coeruleus pairing. The powerful disinhibitory effects of locus coeruleus stimulation on inhibitory tone might naturally lead to stronger and longer-lasting forms of cortical modifications, especially if NMDA receptor activation and postsynaptic spike generation are increased. Indeed, our results demonstrate that even a short amount of locus coeruleus pairing is sufficient to induce tone-evoked responses in previously unresponsive neurons and convert subthreshold responses into spiking responses, similar to a recent study of spike timing–dependent plasticity in rat visual cortex. We hypothesize that the prolonged duration of locus coeruleus stimulation on inhibitory tone might naturally lead to stronger and longer-lasting forms of cortical neuromodulations, especially if NMDA receptor activation and postsynaptic modifications, leading to multiple forms or a composite form of plasticity enabled by cortical neuromodulation. Consequently, for several days following reversal, cortical responses would appear much more ‘confused’ and complex than in highly trained animals. However, the changes triggered by locus coeruleus pairing might provide some latent support for the reorganizations required to learn new associations, ultimately manifesting as an acceleration of the reversal process.

The full neural circuit representing learned associations between external stimuli and internal state is likely to be distributed throughout much of the brain, especially because neuromodulatory systems have extensive recurrent connections. Under natural conditions, many of these systems might be engaged and co-regulated for control of cortical responses and brain state, and modifications to neuromodulator systems provides a powerful mechanism for storing and restoring the most behaviorally important memories.

METHODS

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

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AUTHOR CONTRIBUTIONS

A.R.O.M. performed the experiments. A.R.O.M. and R.C.F. analyzed the experiments and wrote the paper.

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 Institutional Animal Care and Use Committee protocols, in animals kept in a vivarium on a 12/12-h light/dark cycle and housed individually or in pairs. Experiments were carried out in a sound-attenuating chamber. Female 3–5-month-old Sprague-Dawley rats were anesthetized with ketamine (1.2 ml per kg of body weight) and dexmedetomidine (1.0 ml per kg), or pentobarbital (50 mg per kg of body weight). A bipolar stimulation electrode was implanted in the right locus coeruleus using stereotaxic coordinates (from lambda, in mm: 3.6 posterior, 1.2 lateral, 5.6–6 ventral). Location was verified during procedures by measuring responses to noxious stimuli (tail pinch, Supplementary Fig. 1a) and other electrophysiological criteria (spontaneous rates, Supplementary Fig. 1d), and afterwards using histological methods (Supplementary Fig. 1c,e). However, as our study involved making multiple recordings from the same location within the same animals, it was not always possible to resolve which specific recordings came from which cells. A craniotomy was performed over the right temporal lobe and the right auditory cortex was exposed. Pure tones (10–80 dB SPL, 0.3–32 kHz, 50 ms, 3-ms cosine on/offramps) were delivered in pseudo-random sequence at 0.5–1 Hz. Initial studies were performed at 0.5-Hz stimulation rate, but later experiments were performed at 1-Hz rates as there was no significant difference in the amount of plasticity induced at these two frequencies (P = 0.1). A1 location was determined by mapping multiunit responses 500–700 μm below the surface using tungsten electrodes. For infusion of drugs to the locus coeruleus, a hybrid cannula/stimulation electrode was used (Plastics One). For some recordings in locus coeruleus, a hemi-cerebellar-ectomy was performed using gentle suction13, to expose the pons, allowing access of both an extracellular bipolar stimulation electrode (at a 30° angle) and a whole-cell recording pipette (at vertical orientation) to locus coeruleus.

Animals were perfused with 4% paraformaldehyde (vol/vol), brains recovered, and embedded in Optimal Cutting Temperature compound before freezing at −80°C. Afterwards, 40-μm-thick slices were cut from the brainstem and stained using standard immunohistochemistry histological methods. Staining for tyro- sine hydroxylase (primary antibody 1:1,000, Aves Labs catalog number TYH; secondary antibody, DYL488 anti-chicken, 1:500, Life Technologies Labs) was colocalized with either biocytin staining revealed with Alexa Fluor 555–conjugated Streptavidin (1:100, Life Technologies Labs) or YFP (Abcam catalog number ab290).

Electrophysiology. In vivo whole-cell recordings from locus coeruleus or A1 neurons were made with a Multichip 700B amplifier (Molecular Devices). For current-clamp recordings, patch pipettes (5–9 MΩ) contained (in mM): 135 potassium gluconate, 5 NaCl, 5 MgATP, 0.3 GTP, 10 phosphocreatine, 10 HEpes, pH 7.3. For voltage-clamp recordings, pipettes contained: 125 cesium gluconate, 5 TEA Cl, 4 MgATP, 0.3 GTP, 10 phosphocreatine, 10 HEpes, 0.5 EGTA, 3.5 QX-314, 2 CaCl2, pH 7.2. In some cases, 1% biocytin (wt/vol, Sigma) was added to the internal solution for post hoc recovery of recorded neurons. Resting potential of locus coeruleus neurons: −64.2 ± 12.5 mV (n.d.); series resistance (Rs): 31.6 ± 11.0 MΩ; input resistance (Ri): 214.5 ± 131.8 MΩ. Whole-cell recordings from locus coere-uleus neurons were obtained using two different methods, depending on the manner of postsynaptic stimulation during pairing. For single cell stimulation, recordings were obtained 5,500–6,000 μm from the pial surface. During pairing, cells were depolarized through the patch pipette (20 Hz for 500 ms). For extra-cellular stimulation, the cerebellum was aspirated and recordings were obtained ~300 μm below the surface of the pons. Recordings from A1 neurons were obtained from cells located 400–1,200 μm below the pial surface. Resting potential of A1 neurons: −63.0 ± 10.8 mV; Rs: 23.0 ± 12.7 MΩ; Ri: 109.8 ± 55.4 MΩ. Data were excluded if Rs changed >30% or Ri changed >50% from values measured during baseline. Data were filtered at 5 kHz, digitized at 20 kHz, and analyzed with Clampfit 10 (Molecular Devices). For locus coeruleus pairing, after recording baseline responses to the pseudo-random tone sequence for each cell for 5–20 min, a non-preferred tone of a given intensity level and frequency was repetitively presented for 1–5 min (at least 1 min, no more than 5 min, and generally ceased if large changes to responses were observed in between), concurrent with locus coeruleus stimulation (500 ms, 20 Hz, 0.01-ms pulse duration, 20-V stimulation strength) starting at tone onset. Afterwards, locus coeruleus stimulation was ceased and pseudo-random tone sequences were resumed. For nucleus basalis stimulation experiments of Supplementary Figure 8, animals had bipolar stimulation electrodes implanted in nucleus basalis (stereo- taxic coordinates from bregma, in mm: 2.3 posterior, 3.3 lateral, 7 ventral) instead of locus coeruleus. Nucleus basalis stimulation during pairing was at 100 Hz for 250 ms, in the same manner as our previous studies22,23.

For analysis of tonal responses in locus coeruleus neurons in Figure 1f, we computed 2 scores from mean peak EPSPs 20–50 ms after tone onset post-pairing, compared to the mean and s.d. of responses during this same period before pairing. For analysis of tuning curve shifts in Figures 2–4, 6 and 7, best frequency shift was computed as the normalized difference in octaves between the paired frequency and the original best frequency, such that if the best frequency became the paired frequency, this was a 100% shift, whereas if the best frequency stayed the same, this was a 0% shift. To determine tuning curve width, Gaussians were fit to tuning profiles, and the changes in s.d. measured in terms of number of octaves. For determining post-pairing changes in evoked activity in Figure 5, we compared the average response during the baseline period to the average response 6–10 min post-pairing. Spontaneous events were automatically detected using a template, and changes to spontaneous rates and amplitudes were also compared pre-pairing and 6–10 min post-pairing.

For AP5 infusions in Figures 1 and 7, in some experiments we used custom hybrid cannula/stimulation electrodes implanted into locus coeruleus, and AP5 was infused (0.1–1 mM in saline, 1 μl total volume at 0.2 μl min−1). In other experiments in which a hemi-cerebellar-ectomy was performed, AP5 (0.1–1 mM) was topically applied to locus coeruleus. Results of AP5 application at higher (1 mM) and lower (0.1 mM) concentrations were similar (P > 0.7) and so results were combined. For norepinephrine iontophoresis in Figure 6a, double-barreled iontophoresis pipettes (20–30 MΩ) were infused norepinephrine (0.1 mM) in one barrel and saline in the other barrel; for bicuculline iontophoresis experiments of Supplementary Figure 11, one barrel contained bicuculline methiodide (1 mM) and the other barrel contained saline. Iontophoresis electrodes were placed 700 μm below the pial surface, roughly 250–300 μm from the recording pipette; retaining current was ~10 nA and ejection current was +40 nA. For experiments of Figure 6bc, phentolamine (0.1–1 mM in saline) was topically applied to A1. Results of phentolamine application at higher (1 mM) and lower (0.1 mM) concentrations were similar (P = 0.3) and so results were combined. These concentrations were used to ensure that many neurons in the locus coer-uleus or cortex would be affected by pharmacological treatment, but it is important to note that especially near site of application, there may be non-specific effects on excitability or other transmitter systems.

For foot shock experiments of Supplementary Figures 1 and 2, a silver wire was connected to the hindlimb footpad. Foot shock (20–100 Hz, 500 ms duration, 40–150 V) was applied for 2–5 min. To determine the effective activation radius of locus coeruleus stimulation in Supplementary Figure 1f, local field potentials (LFPs) were recorded with a tungsten electrode (0.5–1 MΩ) lowered to 5,500–6,000 μm below the cerebellar surface. Several penetrations were made at different distances from the stimula- tion electrode (100–2,000 μm). LFPs were digitized at 20 kHz and bandpass filtered between 1–100 Hz.

Sterotaxic viral injections were performed in TH-Cre Long-Evans rats. Animals were anesthetized with ketamine/dexmedetomidine, placed into a stereotaxic apparatus, and a craniotomy performed over locus coeruleus. Location was verified by measuring responses to tail pinch with a tungsten electrode, and then injections were performed with a 5-μl Hamilton syringe and a 33 gauge needle. Cre-inducible PAAV5-EF1α-DIO-ChETAYFP virus24 was injected into locus coeruleus at 0.1 nl s−1 for a final injection volume of 1.2–1.5 μl. The virus was given a minimum of 2 weeks to express, and then a second craniotomy performed in the same location, and position was re-verified by recording responses to tail pinch. A calibrated optical fiber ferrule was then implanted in locus coeruleus, and the craniotomy and implant was sealed with dental cement. For optogenetic stimulation in TH-Cre rats expressing the ChETAYFP variant of channelrhodopsin-2, a non-preferred tone of a given intensity level and frequency was repetitively pre-sented for 1–5 min, concurrent with optical stimulation of locus coeruleus (500 ms, 20 Hz, 0.01-ms pulse duration, 1–3 mV) starting at tone onset.

Unless otherwise noted, all statistics and error bars are reported as means ± s.e.m. although normality was not formally tested for all data sets, and P values were determined from Student's paired or unpaired two-tailed t tests. Multiple comparisons were not necessary unless otherwise noted, and studies were not analyzed blind to the conditions of the experiments. No statistical methods
were used to predetermine sample sizes for electrophysiological experiments, as our sample sizes are similar to or larger than what is standard in the field. Electrophysiological experiments were not randomized in terms of which cells were paired under different conditions.

**Behavior.** Rats were lightly food-deprived and pre-trained for 2–6 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 non-target tones. After learning to nosepeck to 4-kHz tones, spectrally wideband foils were also presented (0.5, 1, 2, 8, 16, 32 kHz). Animals that achieved hit rates >80% for targets were then anesthetized with ketamine/dexmedetomidine, had stimulation electrodes or hybrid cannula/stimulation electrodes chronically implanted in right locus coeruleus, and were allowed to recover for a week before being randomly assigned to an experimental group for further study. 16 other animals had electrodes implanted in nucleus basalis instead. Animals were generally tested during the light cycle, but sometimes were tested during the dark cycle as well for measuring the extended time course of behavioral changes. Animals that did not achieve >80% hit rates with 6 weeks or had damaged implants were excluded from further analysis.

Each implanted animal was first tested on the ‘wideband’ recognition task or the detection task for at least 1–2 d. On the wideband and narrowband recognition tasks, tones (wideband target: 4 kHz, foils: 0.5, 1, 2, 8, 16, 32 kHz; narrowband target: 4 kHz, foils: 2.8, 3.2, 3.6, 4.5, 5.1, 5.7 kHz) were presented at 70 dB SPL. For the detection task, tones were presented at 20–90 dB SPL. On the first day at each task, tones were presented for 30–60 min to assess performance at baseline; 4 kHz tones (at 70 dB SPL for the wideband recognition task; 30–45 dB SPL for the detection task; hits binned over 20–40 dB SPL) were then paired with locus coeruleus stimulation in the training box for 2–3 min, and behavior performance assessed and quantified 1, 3, 12 and/or 24 h after one single episode of stimulation. In some cases, animal performance was monitored over the span of weeks, once a day, to examine the duration of behavioral changes induced by locus coeruleus pairing. For reversal learning, 17 Sprague-Dawley rats and eight Long-Evans rats (four wild-type, four Th-Cre) had the rewarded tone switched from 4 kHz to 16 kHz on the wideband task after achieving $d'$ > 2.0. Six of those animals had stimulation electrodes implanted in locus coeruleus and three Th-Cre animals had fiber optics implanted in locus coeruleus, and electrical or optical locus coeruleus stimulation was paired with 16 kHz tones for 5 min just before testing behavioral performance on the first day that 16 kHz was rewarded. Performance was measured daily thereafter. Cannulated animals had AP5 (1 mM in saline, 0.4–1 µl at 0.2 µl min$^{-1}$) or saline infused into the locus coeruleus immediately before stimulation for either task. $d'$ values were computed as the difference in z scores between hits and false positives: $d' = z(\text{hit rate}) - z(\text{false positive rate})$, using the responses between 20–40 dB SPL for detection and responses to 3.6–4.5 kHz for narrowband recognition. Unless otherwise noted, all statistics and error bars are reported as means ± s.e.m. although normality was not formally tested for all data sets, and $P$ values were determined from Student's paired or unpaired two-tailed $t$ tests. Power analysis was performed to determine the number of animals required for statistical significance as in our previous study of the same behaviors in rats. For detection performance after locus coeruleus pairing, effect size was 1.48 and power was 0.99, requiring at least three animals; for detection performance after nucleus basalis pairing over multiple days, effect size was 1.10 and power was 0.82, requiring at least three animals. For recognition performance after locus coeruleus pairing, effect size was 1.00 and power was 0.80, requiring at least 12 animals; for recognition performance after nucleus basalis pairing over multiple days, effect size was 2.03 and power was 0.97, requiring at least two animals. Studies were not performed blind to the conditions of the experiments.

A Supplementary Methods Checklist is available.

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