Behavioral/Systems/Cognitive

Noradrenergic Induction of Odor-Specific Neural Habituation and Olfactory Memories

Stephen D. Shea,[1] Lawrence C. Katz,[1,2†] and Richard Mooney[1]

[1]Department of Neurobiology and [2]Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina 27710

For many mammals, individual recognition of conspecifics relies on olfactory cues. Certain individual recognition memories are thought to be stored when conspecific odor cues coincide with surges of noradrenaline (NA) triggered by intensely arousing social events. Such familiar stimuli elicit reduced behavioral responses, a change likely related to NA-dependent plasticity in the olfactory bulb (OB). In addition to its role in these ethological memories, NA signaling in the OB appears to be relevant for the discrimination of more arbitrary odorants as well. Nonetheless, no NA-gated mechanism of long-term plasticity in the OB has ever been directly observed *in vivo*. Here, we report that NA release from locus ceruleus (LC), when coupled to odor presentation, acts locally in the main OB to cause a specific long-lasting suppression of responses to paired odors. These effects were observed for both food odors and urine, an important social recognition cue. Moreover, in subsequent behavioral tests, mice exhibited habituation to paired urine stimuli, suggesting that this LC-mediated olfactory neural plasticity, induced under anesthesia, can store an individual recognition memory that is observable after recovery.

Key words: **noradrenaline; olfactory bulb; neuromodulation; ceruleus; memory; behavior; electrical stimulation**

Introduction

Accurate recognition of mates, kin, and other familiar conspecifics is critical for survival; in many animals, behavioral responses otherwise evoked by strangers are specifically suppressed for familiar individuals. Neural habituation of sensory responses evoked by familiar individuals may be one mechanism underlying this behavioral habituation (Horn, 1985). Many mammals achieve recognition of conspecifics through the detection of species-specific chemical cues using their olfactory systems. It is widely held that certain olfactory memories for cues related to mating, birth, or maternal care involve structural and functional changes in the olfactory bulb (OB) triggered by noradrenaline (NA) (Brennan and Keverne, 1997), which may result in subsequent suppression of aversive behaviors toward the remembered individual and related stimuli. NA release in the OB also apparently plays a role in conditioning or discrimination tasks involving arbitrary odorants (Brennan et al., 1998; Doucette et al., 2007). Nonetheless, long-term physiological plasticity of olfactory bulb activity gated by NA has never been directly demonstrated *in vivo*.

Several lines of evidence indicate that social olfactory memories are stored as persistent changes to the circuitry of the OB, which is the first CNS station for odor processing. Inactivation of the OB, but not its downstream targets, impairs memory storage during encounters (Kaba et al., 1989). Postencounter electrophysiology (Wilson et al., 1987; Sullivan et al., 1989; Binns and Brennan, 2005), imaging (Yuan et al., 2002), and 2-deoxyglucose labeling (Coopersmith and Leon, 1984; Sullivan et al., 1989) imply changes in OB responses to memorized odors. Nonetheless, single time-point comparisons of population activity across animals, as were made in these studies, could not reveal the initial response of a given cell, or how it changes dynamically during memory formation. Olfactory memory formation is accompanied by persistent increases in bulk GABA content in the OB (Kendrick et al., 1992; Brennan et al., 1995; Rangel and Leon, 1995), as well as increased numbers of inhibitory synaptic profiles evident in EM material (Matsuoka et al., 2004), consistent with the idea that there is a lasting increase in inhibition onto cortically projecting OB neurons activated by the familiar conspecific (Brennan and Keverne, 1997). Despite these important observations, dynamic changes in odor responses that could underlie olfactory memories have not been directly observed.

The circuit mechanisms of olfactory memories are not well understood; however, it is likely that NA is a key participant in the underlying synaptic modifications. Memory formation is marked by a dramatic surge of NA in the OB (Kendrick et al., 1992; Brennan et al., 1995; Rangel and Leon, 1995), which is likely caused by a release from axons that arise from neurons in the brainstem nucleus locus ceruleus (LC) (Shipley et al., 1985; McLean et al., 1989), and which is apparently triggered by intensely arousing social events such as mating, birth, or maternal care (Kendrick et al., 1992; Brennan et al., 1995; Rangel and Leon, 1995). Application of NA antagonists (Sullivan et al., 1989, 1992)

Received Aug. 13, 2008; revised Sept. 1, 2008; accepted Sept. 1, 2008.

This work was supported by National Institutes of Health Grants DC005671 to L.C.K. and R.M. and DC007804 to S.D.S. L.C.K. was an Investigator in the Howard Hughes Medical Institute; he passed away on November 26, 2005 during the completion of this study. We thank R. Irving for histology assistance and D. Fitzpatrick, D. Anderson, Y. Ben-Shaul, I. Davison, B. Arenkiel, G. Laurent, and R. Axel for thoughtful comments on an earlier version of this manuscript.

†Deceased, Nov. 26, 2005.

Correspondence should be addressed to Stephen D. Shea, Department of Neurobiology, Box 3209, Duke University Medical Center, Durham, NC 27710. E-mail: shea@neuro.duke.edu.

DOI:10.1523/JNEUROSCI.3853-08.2008

 0270-6474/08/2810711-09$15.00/0

or ablation of noradrenergic terminals (Keverne and de la Riva, 1982; Pissonnier et al., 1985; Sullivan et al., 1994) block memories, and evoking NA release is sufficient to alter behavioral responses to accompanying odors (Sullivan et al., 2000). Thus, NA is likely to modulate synaptic function in the OB. Indeed, previous studies showed that NA transiently enhances excitability and responses to sensory input in OB mitral/tufted (M/T) cells through direct and disinhibitory mechanisms (Jahr and Nicoll, 1982; Trombley and Shepherd, 1992; Jiang et al., 1996; Ciombor et al., 1999; Hayar et al., 2001). However, these studies did not assess whether NA can gate long-term synaptic plasticity.

To assess whether NA can have long-term effects on the encoding of social and nonsocial odorants, and whether these effects can cause changes in subsequent behavior, we developed a preparation using stimulation of LC to precisely coordinate NA surges with odor exposure in anesthetized mice and directly observed the neural and behavioral consequences. We report that pairing LC stimulation with odor presentation causes long-lasting suppression of odor responses in the main OB (MOB). The suppression specifically attenuates responses to paired stimuli, and is attributable to local noradrenergic mechanisms that may modulate inhibition from nearby granule cells. LC pairing suppressed neural responses to food stimuli and, importantly, urine from other mice. Finally, in subsequent behavioral tests (24 h after stimulation), mice exhibit habituation specifically to paired urine stimuli relative to unpaired urine, suggesting that our experimentally induced olfactory plasticity stores a highly selective long-term individual recognition memory. The induction of a memory under anesthesia opens the cellular substrates of this memory for detailed study.

Materials and Methods

All experiments were performed in strict accordance with National Institutes of Health and Duke University Institutional Animal Care and Usage Committee guidelines.

Electrophysiology. Adult male sexually naive C57/Black6 mice (Charles River Laboratories) were initially anesthetized with 100 mg/kg ketamine and 20 mg/kg xylazine. Subsequently, anesthesia was maintained with sevoflurane (1–3% in pure O_2). LC stimulation was performed >2.5 h after initial injection. Odor responses under this anesthetic are sparse and selective, as they also are in awake animals (Rinberg et al., 2006; Davison and Katz, 2007). Fifty-seven well isolated neurons were recorded throughout the MOB with tungsten microelectrodes (1–1.5 MΩ; Micro Probe); only presumptive mitral/tufted cells (based on depth, background activity, spike amplitude, conspicuous respiratory coupling, and in 32 of 52 cases, electrolytic marking lesions) were considered for analysis (supplemental Fig. 1*A*,*B*, available at www.jneurosci.org as supplemental material). Moreover, our population of extracellularly recorded neurons was physiologically indistinguishable from a set of intracellularly recorded and morphologically identified M/T cells (supplemental Fig. 1*C–E*, available at www.jneurosci.org as supplemental material). Extracellular neural data were recorded with an AC differential amplifier (Model 1800; A–M Systems), and intracellular data were recorded with an Axoclamp 2B (Molecular Devices) in bridge mode. Signals were band-pass filtered between 300 and 5000 Hz and digitally acquired to disk at 10 kHz.

Odor stimuli were delivered to the nose (2 s delivery, 30 s interstimulus interval, 60 s for urine) via flow dilution into the oxygen stream (10% into 1.5 L/min O_2) using a custom 64-channel olfactometer. Stimuli were drawn from a panel of natural food odorants acquired from local grocery stores (peanut butter, chocolate, apple, banana, sesame oil, cheddar cheese, lemon, onion, vanilla, orange, coffee, cumin, clove, cardamom, nutmeg, tea leaves, and liquid smoke) and were flow diluted from their full strength form. For urine, 200 μl was placed in a vial with a small piece of tissue and was used for only 10 presentations. Only cells exhibiting excitatory responses to at least one stimulus were selected for analysis. For pseudorandom presentation of multiple odors, stimuli were presented in blocks in which each odor was presented once per block in random order. Depending on its random position in a given block, "antipaired" LC stimulation was separated from the preferred odor by 30, 60, or 90 s.

Electrical stimuli were applied through monopolar tungsten microelectrodes (0.5–1 MΩ; Micro Probe) and consisted of 5 s, 5 Hz trains of 200 μs, 35–60 μA biphasic pulses delivered to LC ipsilateral to the recording electrode and beginning 2 s before stimulus onset. Activation of LC substantially increases NA release in the olfactory bulb (El-Etri et al., 1999). Although that study did not assess release as a function of LC firing, tonic activation at this frequency is at or near the point of maximal NA release as measured in prefrontal cortex (Florin-Lechner et al., 1996; Berridge and Abercrombie, 1999). Chemical stimulation of LC was achieved with 20–50 ms, 10 psi puffs of 100 μM carbachol applied with a Picospritzer III (Intracel) through a glass pipette (inner diameter tip, 10–20 μm), and preceding the odor by 1 s. Local NA receptor blockade in the MOB was similarly applied with 1–2 larger bolus injections (200–400 ms, 3–5 psi) within 200 μm of the recorded cell. In addition to online physiological criteria (supplemental Fig. 2, available at www.jneurosci.org as supplemental material), all stimulation and many recording sites were confirmed by observation of electrolytic marking lesions in the target structure (20 s, −10 μA DC) (see Fig. 1*B*; supplemental Fig. 1*A*, available at www.jneurosci.org as supplemental material).

Data analysis. All data were analyzed with Matlab (Mathworks). Odor-evoked response strength for each trial was defined as the mean firing rate during the stimulus and 1 s after (3 s total), subtracting the mean firing rate during the previous 6 s. Changes in response strength and spontaneous firing rate were assessed for each pairing with an unpaired *t* test comparison of 20 prestimulation responses with 20 poststimulation responses. Suppressed sites were assessed for persistent suppression by comparing the same prestimulation responses with the last 20 responses before the end of the record for that cell, or where applicable, the next stimulation. In cases where <20 responses were available, at least 10 were used, and for multiple odor experiments (see Fig. 2), 10 responses from each period were compared. For population data, prestimulation responses for each site were normalized to 1. As a result, the prestimulus response population had no variance, and the population of mean poststimulation responses was tested for significant difference from 1 using a Wilcoxon signed rank test. Some cells (14 of 57) were stimulated twice and contributed two data points to the population. Exclusion of these extra data points did not change any of the results.

The phase and strength of respiratory coupling to neuronal firing was calculated as follows. Cells with at least 30 continuous seconds of spontaneous activity and sufficient quality of the breathing measurement signal (44 of 57 neurons) were selected for analysis. For each inspiratory cycle, the time from the preceding to the subsequent inspiratory peak was normalized to -2π to $+2\pi$, and spikes were binned between $-\pi$ and $+\pi$ at a bin size of $\pi/10$. Bins were considered vectors with a phase angle and magnitude, with the angle and magnitude of their vector sum denoting the phase and strength of coupling for that cell. The angle was expressed as degrees, and the magnitude was normalized to the summed magnitude of all bins regardless of phase.

Urine collection. Adult female BalbC, 129, and FVB (Friend virus B) mice confirmed by vaginal cytology to be in estrus were placed overnight in custom metabolic cages. A fine, stainless steel mesh caught feces and other solid material, whereas urine fell into a receptacle and was frozen immediately on dry ice. Urine was pooled from 2 to 4 individuals of the same strain. Urine used for physiology was collected and pooled regardless of estrus cycle.

Behavioral testing. Male sexually experienced C57/Black6 mice (Charles River Laboratories) were anesthetized (100 mg/kg ketamine and 5 mg/kg xylazine) and delivered odors as above. Two strains of urine were presented 20 times each in interleaved blocks of 10. For stimulation mice, one urine was designated to be paired with 5 s, 5 Hz trains of 200 μs, 40 μA biphasic pulses to the left LC beginning 2 s before odor onset. The selection of stimulus strains and the assignation of the paired stimulus were random and balanced across mice. Control mice were treated identically, including placement of the electrode in LC; however, no stimula-

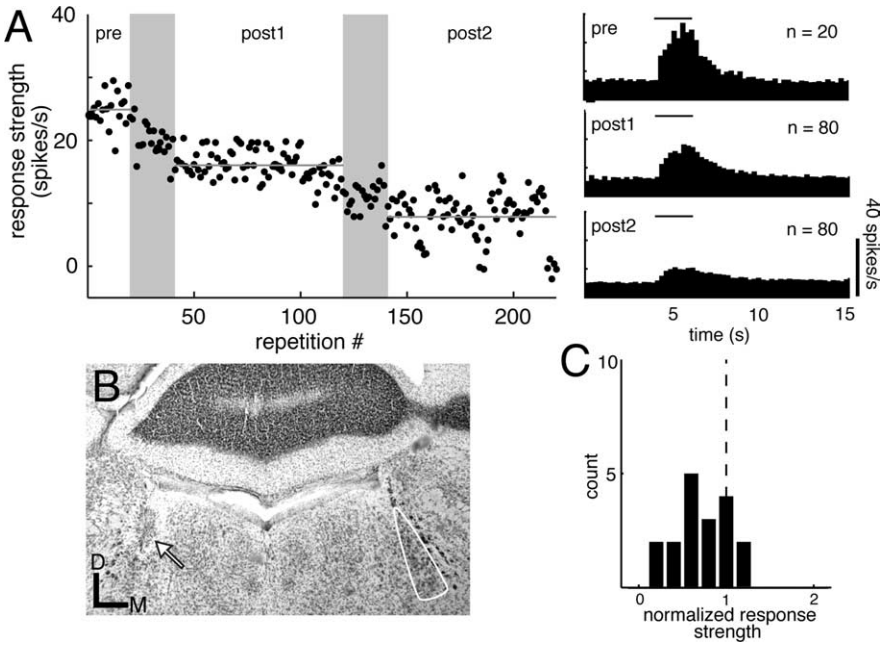

Figure 1. Pairing odors with LC stimulation suppresses odor responses in the MOB. ***A***, Odor response suppression. Left, Odor-evoked firing rate above spontaneous discharge is plotted for a series of 220 presentations of cumin. During the shaded periods, each of 20 presentations was paired with a 5 s, 5 Hz, 40 μA train of biphasic electrical pulses applied to LC. Each pairing period resulted in a significant lasting suppression of the response to the odor. Right, Peristimulus time histograms show the mean response in each denoted epoch. ***B***, Micrograph of an LC-stimulation site. One intact LC (right) is visible, outlined in white in this Nissl-stained coronal section. A marking lesion denoting the stimulation site in ***A*** is evident in the opposite LC (left, arrow). D, Dorsal; M, medial. ***C***, Histogram of effects of LC stimulation. Here and in similar graphs, the poststimulation response (normalized to baseline) is plotted for each of 18 electrical LC-stimulation cases with a single odor.

tion current was applied for either stimulus. Nonetheless, for comparative analysis, one stimulus was randomly and arbitrarily designated the paired stimulus.

After 24 h recovery, interest in the stimuli presented under anesthesia was assessed in a custom Y-maze. Tests were performed as described (Lin et al., 2005), in darkness, and recorded to videotape under infrared illumination. Mice were allowed access to urine volatiles without direct contact for 5 min. Dwell time (time spent in the stimulus arm) and sniff time (time spent with the nose pointing at the port and within 1 cm) was measured for each stimulus during the full 5 min trial. Mean absolute investigation times were compared for the two stimuli with a paired *t* test. Differential interest between the two stimuli was also quantified with a normalized response bias measure calculated from sniff and dwell times as follows: (paired investigation time − unpaired investigation time)/(paired investigation time + unpaired investigation time). These measures were assessed for significant deviation from 0 with a Wilcoxon signed rank test.

Results

The MOB and accessory OB (AOB) are both functionally implicated in social recognition. We focused our study on the MOB, which processes olfactory social cues (Schaefer et al., 2001; Lin et al., 2005, 2007; Liberles and Buck, 2006) and is likely to participate in individual recognition through the detection of social signals in urine (Pankevich et al., 2004; Mandiyan et al., 2005; Keller et al., 2006; Spehr et al., 2006; Wang et al., 2006). We developed a preparation to allow controlled manipulation of odors and LC activity and assessment of subsequent neural and behavioral responses. Using acutely anesthetized mice, we recorded odor-evoked activity of individual M/T cells in the MOB (supplemental Fig. 1, available at www.jneurosci.org as supplemental material) (see Materials and Methods), which receive direct input from olfactory receptor neurons in the nasal epithelium and thus constitute the first CNS station for odor processing (Shepherd and Greer, 1998). In initial experiments, to increase the likelihood of locating responsive M/T cells, odor stimuli were drawn from a panel of food odorants (see Materials and Methods). We achieved temporal control of endogenous NA release with electrical stimulation of LC and measured the strength of the odor-evoked response in M/T cells before and after pairing odorants with LC stimulation.

LC pairing reduces odor responses through NA release

Pairing odorant delivery with LC stimulation often (12 of 18 stimulations) resulted in a lasting suppression of the odorant-evoked response in the recorded M/T cell (Fig. 1*A*). Across all cells and stimulations, LC pairing during repeated presentation of single odors led to significant suppression of responses to the paired odorant (Fig. 1*C*) ($n = 18$; $26 \pm 7.5\%$ suppression relative to normalized baseline responses; Wilcoxon signed rank test, $p < 0.001$). The strength of the suppressive effect was not related to anatomical location in the LC, absolute magnitude of the baseline odor response, or spontaneous firing rate ($p > 0.05$) (supplemental Fig. 3, available at www.jneurosci.org as supplemental material). LC-mediated suppression of the odor-evoked response was long-lasting; of 44 cases exhibiting suppression reported here and in other experiments below, 39 remained suppressed for the duration of the recording (mean: 63 min; range: 14–260 min). Thus, pairing odor delivery with electrical activation of LC triggers a long-lasting form of olfactory plasticity in M/T cells.

Spontaneous discharge rates in M/T neurons were not systematically affected by LC stimulation, when measured either acutely during stimulation ($n = 59$; $1.1 \pm 3.2\%$ suppression; Wilcoxon signed rank test, $p = 0.32$, excluding one low-firing rate outlier which exhibited a low absolute, but high relative, increase) or after the end of stimulation ($n = 59$; $7.2 \pm 4.2\%$ suppression; Wilcoxon signed rank test, $p = 0.12$). These observations were unaffected by considering only sites that showed a suppression of odor responses ($n = 43$; acute effects, $2.6 \pm 3.3\%$, $p = 0.39$; lasting effects, $12 \pm 5.0\%$, $p = 0.06$). Jiang et al. (1996) observed a transient but substantial decrease in spontaneous activity of M/T cells shortly after the initiation of activation of LC. It is not clear why we did not observe this effect, but it is possibly related to the strong and sustained nature of their manipulation (lasting minutes, as opposed to seconds).

Habituation of odor responses was not observed in the absence of LC stimulation. Control recordings made for a time span equal to that over which the above cells were tested (60 repetitions) showed no significant suppression of response strength ($n = 13$ cells; $3.9 \pm 4.5\%$; Wilcoxon signed rank test, $p = 0.60$). Additionally, in cells receiving LC stimulation reported here and below, responses were stable before LC pairing, exhibiting a mean decrease of $<3\%$ over their baseline period ($n = 57$; Wilcoxon signed rank test comparing the first and second halves of a mean baseline period of 28 repetitions, $p = 0.08$). At some sites, rapid

habituation was observed over the first 2–3 repetitions, but this period was always excluded from analysis. Thus, with these stimuli, repeated presentation of an odor alone was insufficient to cause habituation.

We next used complementary pharmacological activation of LC to exclude a contribution of axon fibers from other sources that passed near to our stimulating electrode. Injections of cholinergic agonists selectively activate LC neurons (Adams and Foote, 1988; el-Etri et al., 1993; Jiang et al., 1996) and not passing fibers; therefore, we substituted electrical stimulation in LC with brief puffs of the muscarinic cholinergic agonist carbachol (100 μM). Carbachol injections caused brief neuronal excitation comparable in magnitude to our electrical stimulation (Fig. 2*A*). Pairing carbachol with odor stimulation consistently (10 of 12 stimulations) decreased responses to the paired odors (Fig. 2*B*). Across all sites ($n = 12$), chemical stimulation of LC caused a significant suppression of responses to the paired odorant ($38 \pm 8.1\%$ suppression; Wilcoxon signed rank test, $p < 0.01$) that was indistinguishable from the suppression for odorants paired with electrical stimulation of LC (Fig. 2*C*) (Kolmogorov–Smirnov test, $p = 0.69$). Thus, LC-mediated olfactory plasticity results from specific activation of LC neurons and is likely to be largely insensitive to the precise temporal pattern of LC activity. This is consistent with the observation that olfactory memories are linked to sustained surges of NA release that presumably lack precise temporal structure (Kendrick et al., 1992; Brennan et al., 1995; Rangel and Leon, 1995).

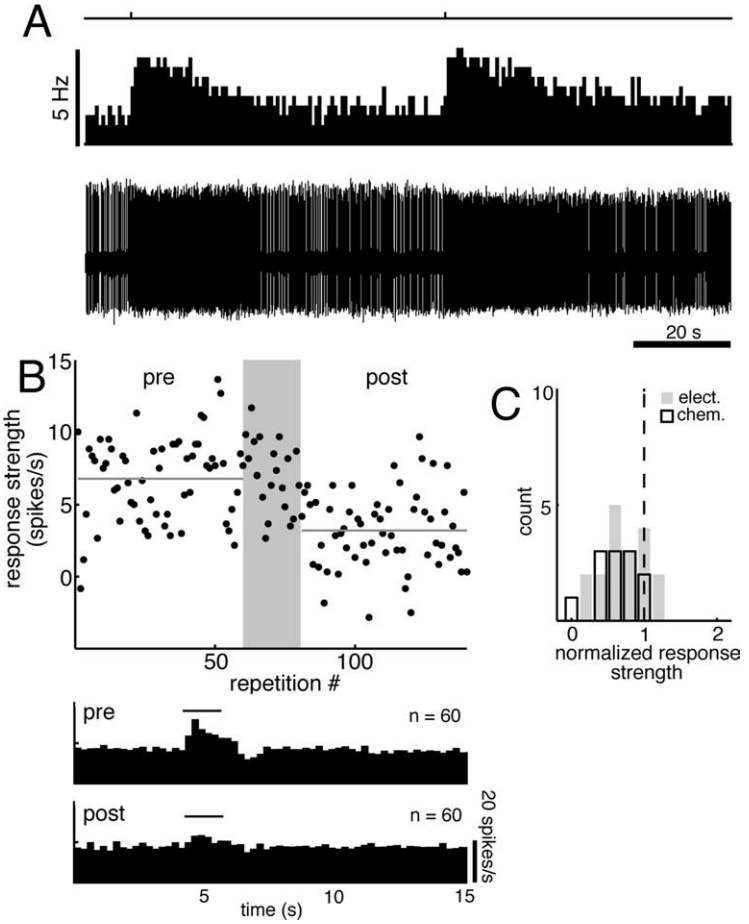

Figure 2. Pairing an odor with chemical LC stimulation suppresses odor responses in the MOB. ***A***, LC response to carbachol injection. Top, The trace shows the timing of two 20 ms, 10 psi puffs of 100 μM carbachol applied to LC. Bottom, The spike rate histogram and raw trace reflect the response of a nearby LC neuron. ***B***, Odor response suppression. Odor-evoked firing rate above spontaneous discharge is plotted for a series of 140 presentations of clove. During the shaded period, each of 20 presentations was paired with a 20 ms, 10 psi puff of 100 μM carbachol applied to LC. Pairing resulted in a significant lasting suppression of the response to the odor. Peristimulus time histograms below show the mean prepairing and postpairing response. ***C***, Histogram of effects of LC stimulation. Effects of all chemical LC stimulations with a single odor are shown, compared with similar experiments using electrical stimulation.

Along with direct NA release in the MOB, LC stimulation is likely to trigger release of NA as well as other neurotransmitters in many additional brain regions. We assessed the necessity of noradrenergic receptor activation local to the MOB for LC-mediated olfactory plasticity by performing LC-odor pairing after a focal injection of α- and β-type noradrenergic receptor antagonists (1 mM phentolamine and 1 mM propranolol, respectively) near the recorded M/T cell in the MOB (Fig. 3*A*); acute modulation of MOB circuitry is observed with activation of both receptor types (Hayar et al., 2001). Notably, NA antagonist injections consistently abolished the suppression of odor responses by LC pairing (1 of 6 sites showing suppression) (Fig. 3*C*); population data showed no difference between prepairing and postpairing responses (Fig. 3*B*) ($2.1 \pm 5.5\%$ suppression; Wilcoxon signed rank test, $p = 0.84$). In contrast, sites that received identical control injections of saline still showed significant suppression in response to LC-odor pairing (Fig. 3*B*) ($n = 7$; $18 \pm 0.5\%$ suppression; Wilcoxon signed rank test, $p < 0.05$). Sites with antagonist injections significantly differed from electrical stimulation without injections (Fig. 3*C*) (Kolmogorov–Smirnov test, $p < 0.05$), whereas sites with saline injections did not (Kolmogorov–Smirnov test, $p = 0.44$). Thus, although our stimulation undoubtedly evoked widespread NA release and may have effects on olfactory processing in deeper brain structures (Bouret and Sara, 2002; Best and Wilson, 2004), LC-mediated odor response plasticity requires NA receptor activity in the MOB close to the recorded cell. This finding indicates that LC stimulation modifies odor responses through a noradrenergic mechanism acting on the local MOB circuit, but does not resolve whether the proximal target of NA input is the M/T cells and/or the inhibitory granule cells. Both cell types show acute responses to NA *in vitro* (Jahr and Nicoll, 1982; Hayar et al., 2001).

LC-mediated suppression is specific to paired odors

Olfactory memories involve selective suppression of behavior toward specific individuals, so if LC-mediated plasticity underlies specific memories, LC stimulation should selectively affect responses to the paired odor. The preceding experiments do not resolve whether LC-mediated plasticity of olfactory responses was global or specific to cells responding to paired odorants. They also do not assess its activity dependence, that is, whether the LC stimulation can modulate olfactory responses for a given cell when separated in time from stimulus-driven firing. To answer these questions directly, we presented 3–4 odorants in pseudo-

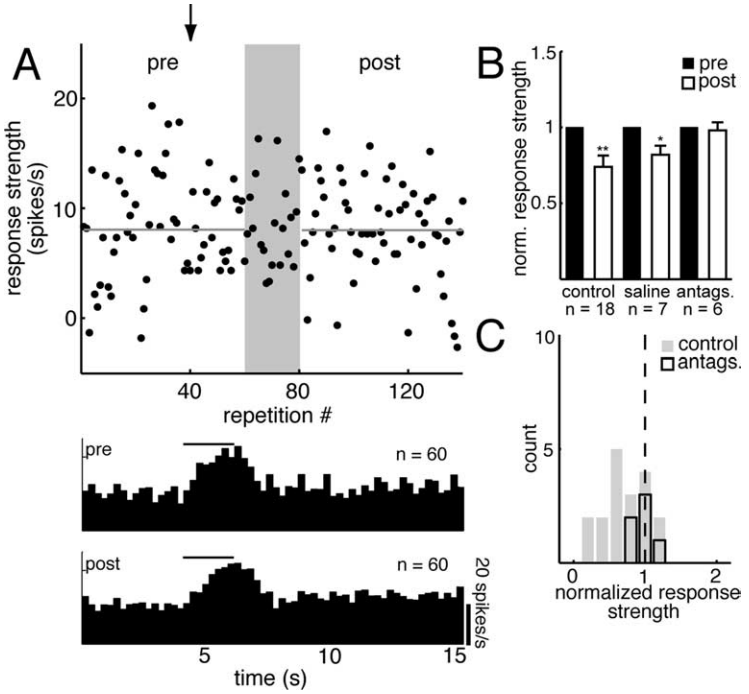

Figure 3. Pairing an odor with LC stimulation after local injection of NA antagonists fails to suppress odor responses in the MOB. ***A***, Odor response is not suppressed. Top, Odor-evoked firing rate above spontaneous discharge is plotted for a series of 140 presentations of cardamom. At the arrow, two 400 ms, 3 psi puffs of 1 mM phentolamine and 1 mM propranolol were made local to the recorded cell. During the shaded period, each of 20 presentations was paired with a 5 s, 5 Hz, 40 μA train of biphasic electrical pulses applied to LC. The odor response was unaffected by pairing. Bottom, Peristimulus time histograms show the mean prepairing and postpairing response. ***B***, Mean population effects of LC stimulation with NA antagonists. Normalized baseline odor responses (black; 1 for all sites by definition) are compared with mean post-LC stimulation responses (white) for control electrical stimulation, stimulation with saline injections, and stimulation with NA antagonist injections. Bars show mean and SEM (Wilcoxon signed rank test, $^{**}p < 0.01$, $^{*}p < 0.05$). ***D***, Histogram of effects of LC stimulation. Effects of all LC stimulations with antagonist treatment are shown and compared with control electrical stimulation.

random order; one odorant elicited a response in the recorded M/T cell (preferred odor), and the others elicited no response (nonpreferred odors). Importantly, other recordings demonstrated that all odorants in our set were effective at driving responses in the MOB (data not shown). We varied the timing of LC stimulation so that it coincided with either the preferred odorant ("pair" condition) or one of the nonpreferred odorants ("antipair" condition, separated by 30–90 s; see Materials and Methods) (Fig. 4*A*). As expected, LC pairing with the preferred odorant consistently suppressed (11 of 16 stimulations) the response to the preferred odor (Fig. 4*B*) (52 ± 8.7% suppression; Wilcoxon signed rank test, $p < 0.001$). Notably, identical LC stimulation, when applied in conjunction with a nonpreferred odorant that elicited no response in the cell, never (0 of 11 stimulations) resulted in suppression of the response to the preferred odor (Fig. 4*B*; supplemental Fig. 4, available at www.jneurosci.org as supplemental material) (−13 ± 14% suppression; Wilcoxon signed rank test, $p = 0.97$). These data significantly differed from those obtained in the pair condition (Fig. 4*C*) (Kolmogorov–Smirnov test, $p < 0.001$). All odors evoked robust responses in the MOB, and for 4 of 11 antipaired cases, we even observed plastic responses to the same odor in separate experiments, and thus, we can infer that suppression occurred at other locations. Furthermore, in a few cases, pairing to a nonpreferred stimulus significantly increased responses to the preferred stimulus (Fig. 4*A*,*C*), raising the possibility that some lateral inhibition to the recorded cell was removed. Nonetheless, we conclude that the suppression of odor responses is limited to stimuli that are coupled to NA release in the MOB. The joint requirement for sensory drive in the mitral cell and exposure to NA thus confers stimulus specificity to the effects and has the consequence that LC stimulation and an odor must coincide for responses to that odor to be altered.

LC pairing reduces neural responses to urine volatiles

We tested whether LC-mediated olfactory plasticity extends to social signals such as urine, which contains cues important for individual recognition (Brennan, 2004; Restrepo et al., 2004). We targeted electrodes to a lateral region of the MOB that includes a cluster of urine-responsive M/T cells and glomeruli (Schaefer et al., 2001; Lin et al., 2005). As seen with food stimuli, when urine exposure was paired to LC stimulation, M/T cell responses to urine were consistently (6 of 7 sites) suppressed (Fig. 5*A*). Considering the population of all urine-selective cells, LC pairing during repeated presentation of urine led to a significant suppression of responses ($n = 7$; 47 ± 18% suppression relative to normalized baseline responses; Wilcoxon signed rank test, $p < 0.05$) that was indistinguishable from the suppression seen for food stimuli (Fig. 5*B*) (Kolmogorov–Smirnov test, $p = 0.27$). We conclude that LC activity, when coupled to odor presentation, leads to selective changes in the representation of a diverse array of stimuli, including potential social signals present in urine.

LC pairing leads to specific behavioral habituation for urine volatiles

One intriguing idea is that LC-mediated olfactory plasticity leads to long-lasting olfactory memories. Specifically, we predict that the suppression of M/T cell responses to an LC-paired odor should also result in a corresponding habituation of behavioral responses to the same odor. To directly test whether LC-mediated plasticity might contribute to olfactory memories, we assessed how LC/odor pairing affects subsequent behavioral responses of male mice to female urine (Fig. 6*A*). Anesthetized male mice were exposed in interleaved blocks of trials to urine from two different strains of female mice in estrus, but presentation of only one urine type was accompanied by an LC stimulus train. Twenty-four hours after LC-stimulus pairing, and after recovery from anesthesia, mice were allowed to explore a Y-maze; one test arm contained a sample of the paired female urine and the other test arm contained the unpaired female urine. Our design was fully counterbalanced for the designation of the paired urine strain and the maze arm in which it was presented.

We found that LC-stimulated mice exhibited behavior consistent with acquisition of a long-term (≥24 h) habituative memory specific for the paired urine. Stimulated mice ($n = 11$) spent significantly less time sniffing the paired stimulus (paired t test, $p < 0.01$) and dwelling in the arm containing the paired stimulus (paired t test, $p < 0.05$) compared with the time spent investigating the unpaired stimulus (Fig. 6*B*,*C*). Sniffing and dwelling were

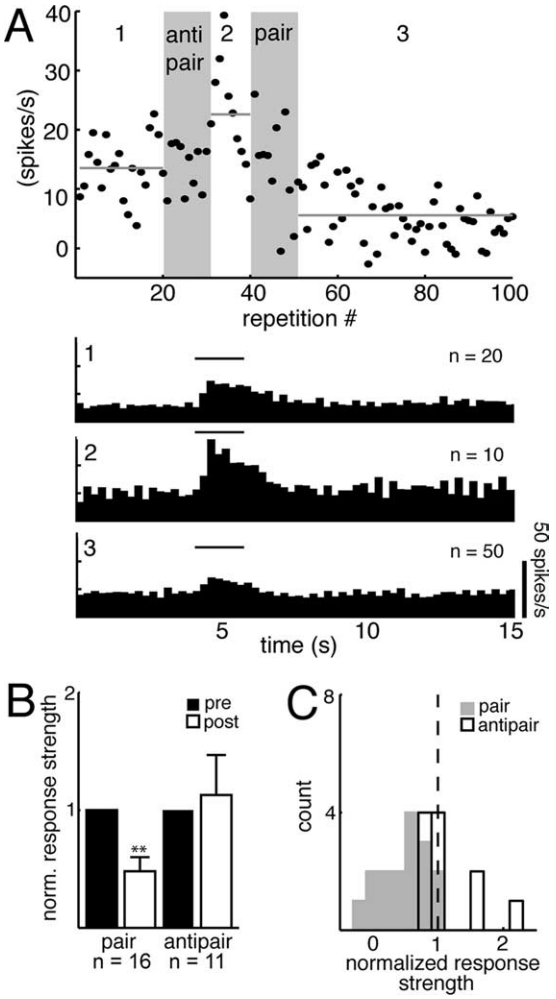

Figure 4. LC-mediated odor response suppression is stimulus-specific. ***A***, Stimulus-specific odor response suppression. Top, Odor-evoked firing rate above spontaneous discharge in response to the preferred odor (lemon) of this cell is plotted for a series of 100 presentations. Lemon was interleaved with presentation of three nonpreferred odors. During the shaded periods, each of 10 presentations of either a nonpreferred odor (antipair) or the preferred odor (pair) was paired with a 5 s, 5 Hz, 50 μA train of biphasic electrical pulses applied to LC. Only the pair condition significantly suppressed the response. Bottom, Peristimulus time histograms show the mean response in each denoted epoch. ***B***, Mean population effects of LC stimulation. Normalized baseline odor responses (black; 1 by definition) are compared with mean post-LC stimulation responses (white) for pair and antipair stimulation. Bars show mean and SEM (post-stimulation responses significantly lower than 1, Wilcoxon signed rank test, $^{**}p < 0.001$). ***C***, Histogram of effects of LC stimulation. Effects of all LC stimulations are shown for electrical stimulation in the pair (black) and antipair (gray) conditions.

57 and 67% greater, respectively, for the unpaired stimulus compared with the paired stimulus. In contrast, unstimulated mice ($n = 12$) that were passively exposed to both stimuli under anesthesia showed no differences in investigation of the two stimuli in the Y-maze (paired t test; sniffing, $p = 0.67$; dwell time, $p = 0.21$) (Fig. 6*B*,*C*) (for these mice the "paired" stimulus and "unpaired" stimulus are arbitrary and random designations). We also measured differences in investigation of the stimuli with a normalized measure of bias (see Materials and Methods). Although data from LC-stimulated animals showed significant bias away from the paired urine stimulus as assessed by both sniffing and dwell time (Wilcoxon signed rank test, $p < 0.05$) (Fig. 6*D*,*E*), control animals did not significantly differ from zero on either measure (Wilcoxon signed rank test; sniffing, $p = 0.62$; dwell, $p = 0.23$) (Fig. 6*D*,*E*).

The reduced investigation of the paired stimulus suggests that mice were habituated to the stimulus by coupling its presentation with LC activation. Like the neural habituation seen with food odorants, this behavioral habituation also was highly selective, because it was not evident for the unpaired urine. This experiment allows us to bridge our anesthetized physiology with wakeful behavior, despite potential differences in coding between the two states (Rinberg et al., 2006). In certain forms of olfactory memory, stimulus-specific habituation is proposed to underlie the suppression of behavioral responses to familiar stimuli (Brennan and Keverne, 1997). Thus, when an odor is paired with LC activation, the animal appears to acquire a long-lasting memory of that stimulus, subsequently treating it as though it were a familiar odor.

Discussion

We have shown that odor-evoked activity and coincident NA release in the main olfactory bulb immediately trigger stimulus-specific neuronal habituation, and over a longer time course, result in behavioral habituation to the paired stimulus. Other stages of odor processing may also reflect NA-dependent modulation (Bouret and Sara, 2002; Best and Wilson, 2004), but notably the odor-specific behavioral habituation after LC/odor pairing directly parallels the odor-specific suppression of neuronal responses in the MOB. This compelling correlation suggests that LC-dependent neuronal habituation in the MOB is a substrate for specific olfactory memories.

Forebrain and brainstem neuromodulatory systems are widely associated with short-term and long-term modifications of sensory representations (Rasmusson, 2000; Hurley et al., 2004). These systems thus serve as a link between attention and arousal and state-dependent modification of sensory activity. Typically, the behavioral significance of such phenomena is unclear; however, our study demonstrates similar effects for natural and social stimuli, and furthermore, links these effects to the storage of behaviorally observable implicit memories. Interestingly, the noradrenergic system has been linked to the memory of emotional events in general, including in humans (Cahill et al., 1994; de Quervain et al., 2007), suggesting the mechanism here may also have counterparts in other modalities and species.

Behavioral significance of LC-mediated MOB plasticity

The LC-mediated olfactory plasticity we observed exhibits properties that could make it suitable for supporting specific and long-lasting olfactory memories. First, the effects of LC/odor pairing are highly specific, exerting differential effects on behavioral responses to two highly similar urine samples; these samples were taken from female mice in the same reproductive state and with very small genetic differences between them. Second, the behavioral effects of LC/odor pairing are long lasting, persisting for at least 24 h poststimulation. Although it remains to be seen whether this memory exhibits the indelibility of a natural olfactory memory, it persists long enough to suggest that the short-term physiological changes seen under anesthesia are consolidated for long-term storage. Finally, the electrical stimulation we used to trigger NA release mimics surges of NA in the olfactory bulb that are evoked by mating, birth, and maternal odor presentation (Kendrick et al., 1992; Brennan et al., 1995; Rangel and Leon, 1995). Thus, noradrenergic modification, like we report here, will be engaged under naturalistic learning conditions.

The joint requirement of activity and NA release constitutes a particularly elegant mechanism for enforcing stimulus specificity that is "bottom up" rather than instructive. This means that the

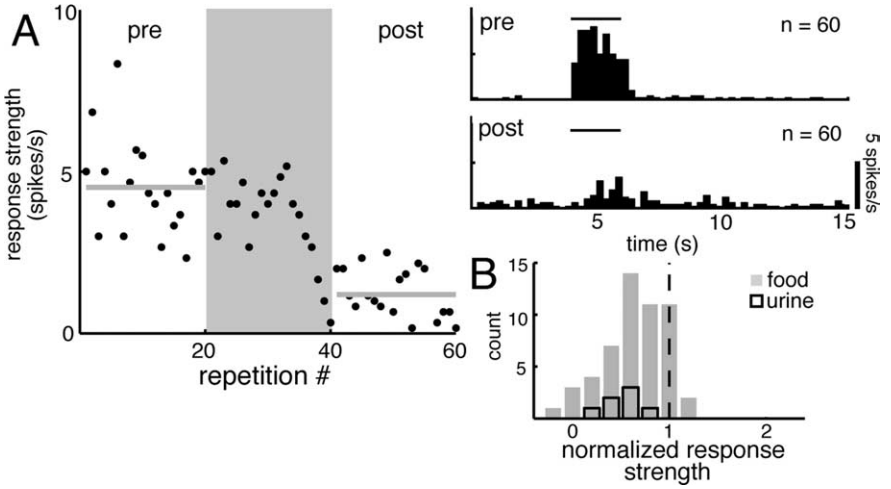

Figure 5. Pairing urine with LC stimulation suppresses responses to urine. ***A***, Urine response suppression. Left, Odor-evoked firing rate above spontaneous discharge is plotted for a series of 60 presentations of urine. During the shaded period, each of 20 presentations was paired with a 5 s, 5 Hz, 40 μA train of biphasic electrical pulses applied to LC. Pairing resulted in a significant lasting suppression of the response to urine. Right, Peristimulus time histograms show the mean response in each denoted epoch. ***C***, Histogram of effects of LC stimulation. Effects of all electrical LC stimulations with urine are shown, compared with all electrical stimulations with food stimuli.

olfactory representations of any odorants that are correlated with a social encounter-induced NA surge will be affected. Further experiments could reveal the requirements for tightness of that correlation, but our data set a lower bound of 30 s. It is also noteworthy that we observed no relationship between intrinsic spontaneous discharge and the magnitude of LC suppression (supplemental Fig. 3, available at www.jneurosci.org as supplemental material), suggesting that the requirement for sensory drive cannot be replaced by ongoing activity.

Three forms of natural olfactory learning in various species at different developmental stages require NA (Brennan and Keverne, 1997). The Bruce effect, seen in newly mated female mice, is a suppression of pregnancy block specific for pheromonal cues from the stud male; all other male pheromones evoke a failure of implantation (Bruce, 1959). After birth, ewes exhibit a suppression of their innate aversion to lamb odors that is specific for their own young, thus restricting access to nursing (for review, see Lévy et al., 2004). Finally, during a developmental critical period, rat pups can be conditioned to a novel odor, with the result that they later spend more time dwelling in the presence of the remembered odor (for review, see Moriceau and Sullivan, 2005). The first two phenomena clearly involve suppression of innately aversive responses. The latter has been interpreted as a conditioned attraction; however, aspects of the conditioned behavior may be consistent with removal of novelty avoidance, possibly relating to observed inhibitory responses to the conditioned odor in M/T cells (Wilson et al., 1987). Thus, these memories could in principle be subserved by NA-dependent habituation of olfactory cues that evoke aversive behaviors. Alternatively, it is known that circuitry related to this neonatal conditioning, including that in LC itself, undergoes rapid and dramatic developmental modification, defining a critical period for certain responses to conditioned stimuli (Moriceau and Sullivan, 2004). These differences may make comparisons to adult animals more complicated.

One question is how LC plays a specific role in highly selective individual recognition memories while also participating in more general memory, attention, and arousal functions (Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005). Indeed, even with respect to olfaction, NA is released in the MOB during behavioral conditioning (Brennan et al., 1998) and has been implicated in nonsocial discrimination of arbitrary odorants (Doucette et al., 2007). Moreover, the balance among the various roles of LC is likely to be dynamic across the life of an animal, defining specific critical periods for phenomena such as imprinting. Thresholds for aversive and attractive olfactory learning are regulated by development and social context, partly via changes in LC circuitry (Moriceau and Sullivan, 2004).

To address these issues, it is necessary to determine with fine resolution what conditions lead to dramatic surges of NA during important social encounters and to compare their magnitude with NA fluctuations during other behaviors. Biochemical assays suggest that NA release during an arbitrary olfactory conditioning task is approximately fivefold lower than that seen during social memory formation (Brennan et al., 1995, 1998). Chronic electrophysiological recordings from LC neurons during social encounters, mating, or birth may more directly and precisely reveal activity that is distinct in pattern or amplitude from that occurring during nonsocial tasks, thus reflecting coding solutions for compartmentalizing the diverse functions of LC. Such experiments may also reveal whether and how LC firing may interact with odors that receive positive reinforcement, rather than habituation.

It is interesting that similar habituation was evoked by LC stimulation for both food odorants and urine stimuli. This demonstrates that LC input is capable of sculpting responses to a diverse array of odors, not only uniquely social signals. It is also consistent with the observation that LC input to the bulb is relatively uniformly distributed (McLean et al., 1989) and not apparently targeted to specialized glomeruli. Furthermore, mouse urine contains many compounds that are found in a wide range of contexts, including food (Schwende et al., 1986). Recent data analyzing the response to naturally occurring mixtures suggests that food and urine are similarly encoded in the MOB by selective glomeruli that independently signal the presence of one or a few specific compounds, regardless of context (Lin et al., 2005, 2006). For this reason, all such components may be equally affected by LC input. Additionally, although we were able to affect responses to food with artificially generated LC activity, it may be the case that this mechanism is only naturally engaged in the presence of conspecific stimuli. If so, then control of the sensory signals that are reshaped by LC is exerted by context-dependent LC activation, not anatomically restricted access of LC input to MOB targets. Indeed, NA input to the OB is capable of conferring apparent social significance to an arbitrary novel odor such as peppermint (Sullivan et al., 1989). It would be interesting to determine whether LC odor pairing can change the behavioral response to food.

Synaptic mechanisms of LC-mediated MOB plasticity

Although systemic application of epinephrine can facilitate classical conditioning under anesthesia (Weinberger et al., 1984), our study specifically implicates neural plasticity at a defined anatom-

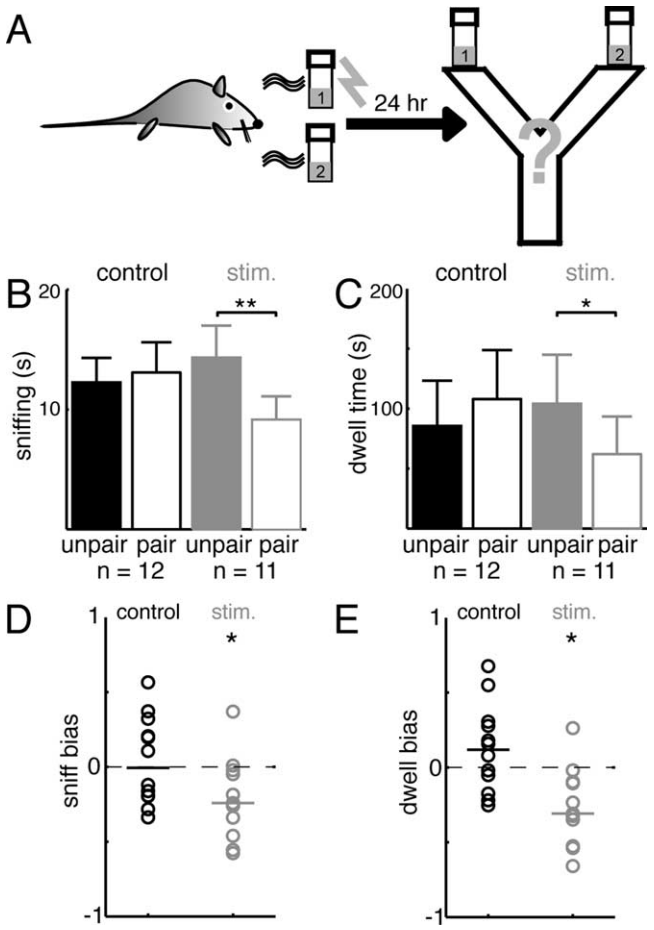

Figure 6. Mice show behavioral habituation to LC-stimulation-paired urine. ***A***, Behavioral habituation testing paradigm. Mice were exposed to two urine samples under anesthesia, and one was paired to LC electrical stimulation. The mice were placed 24 h later in a Y-maze containing both urine samples, and investigation time was measured for both stimuli. ***B–E***, Behavioral data. ***B***, ***C***, Mean absolute investigation time is compared between stimuli for control and stimulated (stim.) mice, quantified as direct sniffing (***B***) or dwelling (***C***). Bars show mean and SEM (paired *t* test, $*p < 0.05$, $**p < 0.001$). ***D***, ***E***, A normalized measure of bias is shown for control and stimulated mice, calculated from direct sniffing (***D***) or dwelling (***E***). Lines show median values (bias significantly different from 1, Wilcoxon signed rank test, $*p < 0.05$). The design was balanced for the urine stimuli used, and subjects showed no systematic preference for any of the three strains contributing urine (supplemental Fig. 5, available at www.jneurosci.org as supplemental material).

ical locus, namely the local circuit encompassing M/T cells, in the formation of memories of ethologically relevant stimuli. Thus, the induction of olfactory memories under anesthesia opens the possibility to study the cellular mechanisms of olfactory memories in detail. One attractive idea is that olfactory memories result from long-term potentiation of inhibitory feedback from MOB granule cells onto M/T cells (Brennan and Keverne, 1997), which could be initiated by transient NA-mediated disinhibition of M/T cells (Jahr and Nicoll, 1982; Jiang et al., 1996; Ciombor et al., 1999). Indirect evidence for such a mechanism includes the observation that memory formation is accompanied by augmented inhibition in the olfactory bulb, as assessed by microdialysis (Kendrick et al., 1992; Brennan et al., 1995), electron microscopy (Matsuoka et al., 2004), and postencounter electrophysiology (Wilson et al., 1987; Sullivan et al., 1989; Binns and Brennan, 2005). NA application can also lead to changes in local field potential oscillations in MOB slices (Gire and Schoppa, 2008), possibly consistent with enhanced inhibition. It may seem surprising that we did not observe enhanced responses to odorants during LC pairing. However, NA-enhanced responses to olfactory nerve input were selectively observed for early response components and only near threshold (Jiang et al., 1996; Ciombor et al., 1999). Strong odor-driven responses with a temporal profile dictated by respiration are likely to evoke excitation and inhibition with different balance and dynamics. In any case, intracellular recordings and direct recordings from granule cells could directly resolve the role of inhibitory processes in neuronal habituation and define their relationship to the formation of olfactory memories. It also remains to be seen whether similar *in vivo* plasticity mechanisms operate in the AOB, which is also important for social recognition (Stowers et al., 2002); NA mechanisms in that structure differ in some respects (Araneda and Firestein, 2006).

Finally, we see relatively rapid changes in the physiological response to paired odors in anesthetized mice, yet our behavioral changes persisted at least 24 h after recovery. The consolidation of rapid synaptic plasticity into physiological changes lasting more than several hours typically involves gene expression and morphological restructuring (Reymann and Frey, 2007). As such, LC-mediated plasticity may allow us to visualize the synaptic changes underlying long-term modifications of neural circuitry and behavior.

References

Adams LM, Foote SL (1988) Effects of locally infused pharmacological agents on spontaneous and sensory-evoked activity of locus coeruleus neurons. Brain Res Bull 21:395–400.

Araneda RC, Firestein S (2006) Adrenergic enhancement of inhibitory transmission in the accessory olfactory bulb. J Neurosci 26:3292–3298.

Aston-Jones G, Cohen JD (2005) An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. Annu Rev Neurosci 28:403–450.

Berridge CW, Abercrombie ED (1999) Relationship between locus coeruleus discharge rates and rates of norepinephrine release within neocortex as assessed by in vivo microdialysis. Neuroscience 93:1263–1270.

Berridge CW, Waterhouse BD (2003) The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Brain Res Rev 42:33–84.

Best AR, Wilson DA (2004) Coordinate synaptic mechanisms contributing to olfactory cortical adaptation. J Neurosci 24:652–660.

Binns KE, Brennan PA (2005) Changes in electrophysiological activity in the accessory olfactory bulb and medial amygdala associated with mate recognition in mice. Eur J Neurosci 21:2529–2537.

Bouret S, Sara SJ (2002) Locus coeruleus activation modulates firing rate and temporal organization of odour-induced single-cell responses in rat piriform cortex. Eur J Neurosci 16:2371–2382.

Brennan PA (2004) The nose knows who's who: chemosensory individuality and mate recognition in mice. Horm Behav 46:231–240.

Brennan PA, Keverne EB (1997) Neural mechanisms of mammalian olfactory learning. Prog Neurobiol 51:457–481.

Brennan PA, Kendrick KM, Keverne EB (1995) Neurotransmitter release in the accessory olfactory bulb during and after the formation of an olfactory memory in mice. Neuroscience 69:1075–1086.

Brennan PA, Schellinck HM, de la Riva C, Kendrick KM, Keverne EB (1998) Changes in neurotransmitter release in the main olfactory bulb following an olfactory conditioning procedure in mice. Neuroscience 87:583–590.

Bruce HM (1959) An exteroceptive block to pregnancy in the mouse. Nature 184:105.

Cahill L, Prins B, Weber M, McGaugh JL (1994) Beta-adrenergic activation and memory for emotional events. Nature 371:702–704.

Ciombor KJ, Ennis M, Shipley MT (1999) Norepinephrine increases rat mitral cell excitatory responses to weak olfactory nerve input via alpha-1 receptors in vitro. Neuroscience 90:595–606.

Coopersmith R, Leon M (1984) Enhanced neural response to familiar olfactory cues. Science 225:849–851.

Davison IG, Katz LC (2007) Sparse and selective odor coding by mitral/tufted neurons in the main olfactory bulb. J Neurosci 27:2091–2101.

de Quervain DJ, Kolassa IT, Ertl V, Onyut PL, Neuner F, Elbert T, Papassoti-

ropoulos A (2007) A deletion variant of the alpha2b-adrenoceptor is related to emotional memory in Europeans and Africans. Nat Neurosci 10:1137–1139.
Doucette W, Milder J, Restrepo D (2007) Adrenergic modulation of olfactory bulb circuitry affects odor discrimination. Learn Mem 14:539–547.
el-Etri MM, Ennis M, Jiang M, Shipley MT (1993) Pilocarpine-induced convulsions in rats: evidence for muscarinic receptor-mediated activation of locus coeruleus and norepinephrine release in cholinolytic seizure development. Exp Neurol 121:24–39.
El-Etri MM, Ennis M, Griff ER, Shipley MT (1999) Evidence for cholinergic regulation of basal norepinephrine release in the rat olfactory bulb. Neuroscience 93:611–617.
Florin-Lechner SM, Druhan JP, Aston-Jones G, Valentino RJ (1996) Enhanced norepinephrine release in prefrontal cortex with burst stimulation of the locus coeruleus. Brain Res 742:89–97.
Gire DH, Schoppa NE (2008) Long-term enhancement of synchronized oscillations by adrenergic receptor activation in the olfactory bulb. J Neurophysiol 99:2021–2025.
Hayar A, Heyward PM, Heinbockel T, Shipley MT, Ennis M (2001) Direct excitation of mitral cells via activation of alpha1-noradrenergic receptors in rat olfactory bulb slices. J Neurophysiol 86:2173–2182.
Horn G (1985) Memory, imprinting, and the brain: an inquiry into mechanisms. Oxford: Clarendon.
Hurley LM, Devilbiss DM, Waterhouse BD (2004) A matter of focus: monoaminergic modulation of stimulus coding in mammalian sensory networks. Curr Opin Neurobiol 14:488–495.
Jahr CE, Nicoll RA (1982) Noradrenergic modulation of dendrodendritic inhibition in the olfactory bulb. Nature 297:227–229.
Jiang M, Griff ER, Ennis M, Zimmer LA, Shipley MT (1996) Activation of locus coeruleus enhances the responses of olfactory bulb mitral cells to weak olfactory nerve input. J Neurosci 16:6319–6329.
Kaba H, Rosser A, Keverne B (1989) Neural basis of olfactory memory in the context of pregnancy block. Neuroscience 32:657–662.
Keller M, Douhard Q, Baum MJ, Bakker J (2006) Destruction of the main olfactory epithelium reduces female sexual behavior and olfactory investigation in female mice. Chem Senses 31:315–323.
Kendrick KM, Lévy F, Keverne EB (1992) Changes in the sensory processing of olfactory signals induced by birth in sleep. Science 256:833–836.
Keverne EB, de la Riva C (1982) Pheromones in mice: reciprocal interaction between the nose and brain. Nature 296:148–150.
Lévy F, Keller M, Poindron P (2004) Olfactory regulation of maternal behavior in mammals. Horm Behav 46:284–302.
Liberles SD, Buck LB (2006) A second class of chemosensory receptors in the olfactory epithelium. Nature 442:645–650.
Lin DY, Zhang SZ, Block E, Katz LC (2005) Encoding social signals in the mouse main olfactory bulb. Nature 434:470–477.
Lin DY, Shea SD, Katz LC (2006) Representation of natural stimuli in the rodent main olfactory bulb. Neuron 50:937–949.
Lin W, Margolskee R, Donnert G, Hell SW, Restrepo D (2007) Olfactory neurons expressing transient receptor potential channel M5 (TRPM5) are involved in sensing semiochemicals. Proc Natl Acad Sci U S A 104:2471–2476.
Mandiyan VS, Coats JK, Shah NM (2005) Deficits in sexual and aggressive behaviors in Cnga2 mutant mice. Nat Neurosci 8:1660–1662.
Matsuoka M, Kaba H, Moriya K, Yoshida-Matsuoka J, Costanzo RM, Norita M, Ichikawa M (2004) Remodeling of reciprocal synapses associated with persistence of long-term memory. Eur J Neurosci 19:1668–1672.
McLean JH, Shipley MT, Nickell WT, Aston-Jones G, Reyher CK (1989) Chemoanatomical organization of the noradrenergic input from locus coeruleus to the olfactory bulb of the adult rat. J Comp Neurol 285:339–349.
Moriceau S, Sullivan RM (2004) Unique neural circuitry for neonatal olfactory learning. J Neurosci 24:1182–1189.
Moriceau S, Sullivan RM (2005) Neurobiology of infant attachment. Dev Psychobiol 47:230–242.
Pankevich DE, Baum MJ, Cherry JA (2004) Olfactory sex discrimination persists, whereas the preference for urinary odorants from estrous females disappears in male mice after vomeronasal organ removal. J Neurosci 24:9451–9457.
Pissonnier D, Thiery JC, Fabre-Nys C, Poindron P, Keverne EB (1985) The importance of olfactory bulb noradrenalin for maternal recognition in sheep. Physiol Behav 35:361–363.
Rangel S, Leon M (1995) Early odor preference training increases olfactory bulb norepinephrine. Brain Res Dev Brain Res 85:187–191.
Rasmusson DD (2000) The role of acetylcholine in cortical synaptic plasticity. Behav Brain Res 115:205–218.
Restrepo D, Arellano J, Oliva AM, Schaefer ML, Lin W (2004) Emerging views on the distinct but related roles of the main and accessory olfactory systems in responsiveness to chemosensory signals in mice. Horm Behav 46:247–256.
Reymann KG, Frey JU (2007) The late maintenance of hippocampal LTP: requirements, phases, 'synaptic tagging', 'late-associativity' and implications. Neuropharmacology 52:24–40.
Rinberg D, Koulakov A, Gelperin A (2006) Sparse odor coding in awake behaving mice. J Neurosci 26:8857–8865.
Schaefer ML, Young DA, Restrepo D (2001) Olfactory fingerprints for major histocompatibility complex-determined body odors. J Neurosci 21:2481–2487.
Schwende FJ, Wiesler D, Jorgenson JW, Carmack M, Novotny M (1986) Urinary volatile constituents of the house mouse, *Mus musculus*, and their endocrine dependency. J Chem Ecol 12:277–296.
Shepherd GM, Greer CA (1998) Olfactory bulb. In: The synaptic organization of the brain (Shepherd GM, ed), pp 159–204. New York: Oxford UP.
Shipley MT, Halloran FJ, de la Torre J (1985) Surprisingly rich projection from locus coeruleus to the olfactory bulb in the rat. Brain Res 329:294–299.
Spehr M, Kelliher KR, Li XH, Boehm T, Leinders-Zufall T, Zufall F (2006) Essential role of the main olfactory system in social recognition of major histocompatibility complex peptide ligands. J Neurosci 26:1961–1970.
Stowers L, Holy TE, Meister M, Dulac C, Koentges G (2002) Loss of sex discrimination and male-male aggression in mice deficient for TRP2. Science 295:1493–1500.
Sullivan RM, Wilson DA, Leon M (1989) Norepinephrine and learning-induced plasticity in infant rat olfactory system. J Neurosci 9:3998–4006.
Sullivan RM, Zyzak DR, Skierkowski P, Wilson DA (1992) The role of olfactory bulb norepinephrine in early olfactory learning. Brain Res Dev Brain Res 70:279–282.
Sullivan RM, Wilson DA, Lemon C, Gerhardt GA (1994) Bilateral 6-OHDA lesions of the locus coeruleus impair associative olfactory learning in newborn rats. Brain Res 643:306–309.
Sullivan RM, Stackenwalt G, Nasr F, Lemon C, Wilson DA (2000) Association of an odor with activation of olfactory bulb noradrenergic beta-receptors or locus coeruleus stimulation is sufficient to produce learned approach responses to that odor in neonatal rats. Behav Neurosci 114:957–962.
Trombley PQ, Shepherd GM (1992) Noradrenergic inhibition of synaptic transmission between mitral and granule cells in mammalian olfactory bulb cultures. J Neurosci 12:3985–3991.
Wang Z, Balet Sindreu C, Li V, Nudelman A, Chan GC, Storm DR (2006) Pheromone detection in male mice depends on signaling through the type 3 adenylyl cyclase in the main olfactory epithelium. J Neurosci 26:7375–7379.
Weinberger NM, Gold PE, Sternberg DB (1984) Epinephrine enables Pavlovian fear conditioning under anesthesia. Science 223:605–607.
Wilson DA, Sullivan RM, Leon M (1987) Single-unit analysis of postnatal olfactory learning: modified olfactory bulb output response patterns to learned attractive odors. J Neurosci 7:3154–3162.
Yuan Q, Harley CW, McLean JH, Knöpfel T (2002) Optical imaging of odor preference memory in the rat olfactory bulb. J Neurophysiol 87:3156–3159.