Amygdalar Gating of Early Sensory Processing through Interactions with Locus Coeruleus

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Fear- and stress-induced activity in the amygdala has been hypothesized to influence sensory brain regions through the influence of the amygdala on neuromodulatory centers. To directly examine this relationship, we used optical imaging to observe odor-evoked activity in populations of olfactory bulb inhibitory interneurons and of synaptic terminals of olfactory sensory neurons (the primary sensory neurons of the olfactory system, which provide the initial olfactory input to the brain) during pharmacological inactivation of amygdala and locus coeruleus (LC) in mice. Although the amygdala does not directly project to the olfactory bulb, joint pharmacological inactivation of the central, basolateral, and lateral nuclei of the amygdala nonetheless strongly suppressed odor-evoked activity in GABAergic inhibitory interneuron populations in the OB. This suppression was prevented by inactivation of LC or pretreatment of the olfactory bulb with a broad-spectrum noradrenergic receptor antagonist. Visualization of synaptic output from olfactory sensory neuron terminals into the olfactory bulb of the brain revealed that amygdalar inactivation preferentially strengthened the odor-evoked synaptic output of weakly activated populations of sensory afferents from the nose, thus demonstrating a change in sensory gating potentially mediated by local inhibition of olfactory sensory neuron terminals. We conclude that amygdalar activity influences olfactory processing as early as the primary sensory input to the brain by modulating norepinephrine release from the locus coeruleus into the olfactory bulb. These findings show that the amygdala and LC state actively determines which sensory signals are selected for processing in sensory brain regions. Similar local circuitry operates in the olfactory, visual, and auditory systems, suggesting a potentially shared mechanism across modalities.

Key words: affective; amygdala; emotional; locus coeruleus; olfactory; sensory gating

Significance Statement

The affective state is increasingly understood to influence early neural processing of sensory stimuli, not just the behavioral response to those stimuli. The present study elucidates one circuit by which the amygdala, a critical structure for emotional learning, valence coding, and stress, can shape sensory input to the brain and early sensory processing through its connections to the locus coeruleus. One function of this interaction appears to be sensory gating, because inactivating the central, basolateral, and lateral nuclei of the amygdala selectively strengthened the weakest olfactory inputs to the brain. This linkage of amygdalar and LC output to primary sensory signaling may have implications for affective disorders that include sensory dysfunctions like hypervigilance, attentional bias, and impaired sensory gating.

Introduction

The sensory areas of the brain receive extensive projections from neuromodulatory centers in the brainstem and basal forebrain. These neuromodulatory regions are thought to play important

roles in sensory information processing by guiding attention, evoking neuroplasticity in sensory structures, and adapting sensory information processing to match the behavioral state of the organism (Aston-Jones et al., 1999; Gu, 2002; Froemke and Martins, 2011; Sara, 2015; Schwarz and Luo, 2015). Dysfunctional neuromodulation has been linked to mental disorders with sensory endophenotypes like hypervigilance, attentional dysregulation, and impaired sensorimotor gating (Maes et al., 1999; O'Donnell et al., 2004; Alsene et al., 2011; Hegerl and Hensch, 2014; Johnston et al., 2014; Hendrickson and Raskind, 2016). However, it remains unclear how the activity of these neuromodulatory structures is directed by other brain regions, and, thus, their role in normal and disordered sensory processing has been difficult to assess.

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One of the major influencers of the neuromodulatory structures of the brain is the amygdala (Price and Amaral, 1981; Aggleton, 1993; Retson and Van Bockstaele, 2013), a subcortical structure that is famously involved in affective processing, including the encoding of stimulus valence (Maren, 2016) and emotional learning about highly valenced stimuli (Johansen et al., 2011; Lee et al., 2013; Paz and Pare, 2013). Amygdala activation by direct stimulation (Chavez et al., 2013) or by naturally aversive stimuli can influence sensory processing (Taylor et al., 2000; Stark et al., 2004). Amygdala-dependent learning about neutral sensory stimuli (such as during fear conditioning) has been found to induce plasticity in virtually all sensory modalities (for review, see McGann, 2015), but it remains unclear how the amygdala influences precortical sensory regions that do not receive any direct amygdalar projections (Maren et al., 2001).

Affective experiences like fear conditioning engage the central, basolateral, and lateral (CBL) amygdalar subnuclei (Goosens and Maren, 2001), which are positioned to broadly influence the rest of the brain through the extensive projections of the central nucleus to neuromodulatory and neuroendocrine structures (Price and Amaral, 1981; Amaral and Price, 1984). Of these, the strong projection from central amygdala (CeA) to the locus coeruleus (LC; Van Bockstaele et al., 1999; Schwarz et al., 2015) is particularly intriguing because the LC has a similarly broad influence across sensory systems (Rogawski and Aghajanian, 1980; Kayama et al., 1982; Sato et al., 1989; George, 1992) and because the LC plays a distinctive role in arousal (Foote et al., 1980), vigilance (Aston-Jones et al., 1994; Rajkowski et al., 1994; Aston-Jones et al., 1998) and sensory gating (Waterhouse et al., 1988; Jiang et al., 1996; Rutter et al., 1998; Devilbiss et al., 2012; Hormigo et al., 2015). The central amygdala → LC circuit includes both excitatory and inhibitory connections (Van Bockstaele et al., 1998, 1999), forming a complex circuit that is activated by fear, stress, and anxiety (Valentino et al., 1992; Van Bockstaele et al., 1998; Berridge and Waterhouse, 2003; Moriceau et al., 2009; Devilbiss et al., 2012; Manella et al., 2013) and shapes learned attachment and fear during development (Landers and Sullivan, 2012).

We explored the interactions of the amygdala, LC, and sensory processing in the olfactory system, where emotional learning produces dramatic changes in sensory processing in both humans and animal models (Jones et al., 2008; Li et al., 2008; Chen et al., 2011; Fletcher, 2012; Kass et al., 2013d; Krusemark and Li, 2013; Dias and Ressler, 2014; Li, 2014) and where noradrenergic signaling has been shown to influence odor perception (Doucette et al., 2007; Escanilla et al., 2010; Linster et al., 2011), olfactory memory (Manella et al., 2013), neonatal olfactory preference (Sullivan and Wilson, 1991; McLean and Harley, 2004), and local circuit function (Jiang et al., 1996; Nai et al., 2009; Eckmeier and Shea, 2014). The olfactory bulb does not receive any direct projections from the amygdala, but we hypothesized that amygdalar influences on early sensory processing could nonetheless be observed in olfactory bulb glomeruli by way of the amygdala → LC → olfactory bulb circuit.

Materials and Methods

Subjects. The present study used a total of 41 experimentally naive adult mice (22 male and 19 female) ranging from 16 to 32 weeks of age. To visualize neural activity in GABAergic periglomerular (PG) interneurons, we crossed mice from the GAD2-IRES-Cre driver line (stock #010802, The Jackson Laboratory) that bicistronically expresses cre recombinase from the promoter for *gad2*, the gene that encodes glutamic acid decarboxylase 65 (GAD65; Taniguchi et al., 2011), with mice from the Ai95D reporter line (stock #024105, The Jackson Laboratory) that

include the calcium indicator GCaMP6f (Akerboom et al., 2012; Chen et al., 2013) sequence under control of the endogenous Gt(ROSA)26Sor promoter/enhancer regions and the CAG hybrid promoter but with a floxed STOP codon (Madisen et al., 2015). In the resulting offspring, cre-mediated recombination excises the STOP codon, resulting in the expression of GCaMP6f in GAD65-expressing cells throughout the brain, including periglomerular interneurons in the olfactory bulb, as previously reported (Kiyokage et al., 2010; Wachowiak et al., 2013). To visualize neurotransmitter release from olfactory sensory neurons (OSNs), we used a line of gene-targeted mice in which the coding region for olfactory marker protein (OMP) has been replaced with the sequence for the fluorescent exocytosis indicator synaptopHluorin (spH) on an albino C57BL/6 background (Miesenböck et al., 1998; Bozza et al., 2004; Czarnecki et al., 2011). These mice were heterozygous for both spH and OMP (Kass et al., 2013b,c). All subjects were maintained on a 12 h light/dark cycle with *ad libitum* access to rodent chow and water. All experiments were conducted in accordance with protocols approved by the Rutgers University Animal Care and Use Committee.

Cannula implantation, infusions, and histology. Mice were implanted with bilateral cannulae (22 g, 4 mm length; Plastics One) targeting the CBL amygdala (1.4 mm posterior to bregma, 3.2 mm lateral from midline, and 4.3 mm ventral from skull). Some mice also received bilateral cannulae (26 g, 1.8 mm center-to-center, 4.2 mm in length) targeting the LC (5.45 mm posterior to bregma, 0.9 mm lateral to midline, and 3.68 mm ventral from skull).

During microinfusion, internal cannulae (CBL, 28 g; LC, 33 g) that extended 0.8 mm beyond their respective guides were affixed to PE-20 polyethylene tubing that was mounted to 10 μ l Hamilton syringes positioned on a dual-channel infusion pump (Harvard Apparatus). Muscimol (Sigma-Aldrich; 2% in 0.1 M PBS solution, filtered at 0.20 μ m) or vehicle (PBS) was infused at 0.25 μ l/min for a 1 min total infusion period for CBL and 0.125 μ l/min for a 1 min infusion period for LC with internals left in place for 1 additional minute to aid in diffusion. Some subjects also received 13.7 mM labetalol (impurity A 2-hydroxy-5-[1-hydroxy-2[(1-methyl-3-phenylpropyl)-amino] ethyl] benzoic acid, LGC) or Ringer's solution vehicle topically applied to the dorsal surface of the olfactory bulb. Following data collection, an equivalent volume and concentration of muscimol with BODIPY TMR-X conjugate (Life Technologies) was infused at each target to enable the estimation of diffusion patterns and localization. Subjects were then perfused intracardially with 4% paraformaldehyde (PFA) followed by 0.1 M PBS, tissue was extracted and stored in PFA until it was sectioned (100 μ m coronal or horizontal) on a vibratome (Ted Pella), mounted on slides for the microscope, treated with ProLong with DAPI (Life Technologies), coverslipped, and viewed on a microscope (Olympus) equipped with the appropriate filter sets. Because of the physical differences between muscimol and BODIPY-tagged muscimol, the fact that the BODIPY-tagged muscimol was administered as a second infusion, and the possibility that the indicator may have spread during tissue processing, these histological methods can be expected to reliably show the centroid of the infusion (see Figs. 2, 4) but only an approximation of the drug spread.

Optical imaging. Mice were prepared for olfactory bulb imaging as previously described (Czarnecki et al., 2012; Moberly et al., 2012; Kass et al., 2013a). Briefly, mice were deeply anesthetized using pentobarbital and dosed subcutaneously with atropine to reduce nasal secretions. An acrylic headcap was implanted to secure the mouse to the custom headholder, and the skull overlying the olfactory bulb was thinned bilaterally and topped with Ringer's solution and a coverslip. For mice receiving direct application of drug to the olfactory bulb, the skull and dura were instead removed and mice received dexamethasone subcutaneously to minimize brain swelling.

Mice were positioned under a custom microscope including an Olympus 4 $\times$ macro objective with a 0.28 numerical aperture with epillumination provided by a blue LED. Green fluorescence images were captured using a low read noise RedShirtImaging SM-256 CCD camera acquiring at 25 Hz at 256 $\times$ 256 resolution. Optical imaging was shot-noise limited. The odor methyl valerate (an ester used commercially as an artificial apple flavor) was presented by vapor dilution olfactometry with nitrogen as the carrier gas at a fixed concentration previously reported as

8 arbitrary units in other studies from our laboratory (Kass et al., 2013d) and standardized daily with a photoionization detector. Odor presentations lasted for 6 s and were repeated a minimum of five times in each condition with a minimum of 60 s between trials. No-odor blank control trials were included to correct for fluorescence bleaching. Respiratory signals were monitored using a piezosensor pressed against the abdominal wall, which conveys the second derivative of intranasal airflow, as previously reported in the study by Kass et al. (2013d, their Supplementary Fig. 1).

Data analysis. Optical imaging data were analyzed as previously described (Kass et al., 2016). Briefly, optical data were spatially low-pass filtered with a 3×3 pixel median filter to minimize shot noise and gently spatially high-pass filtered with a two-dimensional Gaussian filter with an SD of $\sim 240 \mu\text{m}$ to minimize contributions of diffuse metabolic signals or out-of-focus signals originating below the glomerular layer. Low-pass temporal filtering was applied at 9.38 Hz (GCaMP6f) or 6.25 Hz (spH) to prevent aliasing artifacts. No photobleaching was observed in the GCaMP6f data, but spH data were blank subtracted to correct for bleaching. No movement correction was applied. We calculated odor-evoked GCaMP signals as the difference between the fluorescence during odor presentation and baseline fluorescence before odor presentation normalized by preodor baseline ($\Delta F/F$), but for spH we used the raw difference between odor and baseline (ΔF) because baseline fluorescence of spH reflects indicator molecules that are not sequestered to readily releasable vesicles (Bozza et al., 2004). In all GCaMP6f experiments, we tested for a drug-induced change in resting fluorescence, but none was observed, so these analyses are not reported on further. Odor response maps were calculated by subtracting the preodor baseline image from the image during the peak of the odor-evoked fluorescence during the first inhalation of odor (for GCaMP6f, which gives a moment-to-moment readout of neural activity) or at the end of the odor presentation (for spH, which is an indicator of cumulative exocytosis). Pseudocolor scales for response maps were selected to show the full range of glomerular responses except in Figure 7, where the display of the largest glomerular responses was intentionally saturated to better visualize weakly activated glomeruli. The total area under the $\Delta F/F$ curve was also calculated for GCaMP6f signals but gave very similar results (Fig. 1C). Glomerular regions of interest within the image were identified manually for each animal based on pre-drug infusion odor responses by a blind experimenter and applied uniformly to all trials. Responses on individual odor trials were quantified and candidate regions of interest were included if the mean signal over five trials was at least 3 SEs above zero.

Because some of the analyses include changes in small responses, we calculated the mean single trial signal-to-noise ratio (SNR) for each glomerulus for each of the five predrug trials that were averaged to create the scatter plot showing pooled data in Figure 6. SNR was defined as the square of the ratio of the root mean square fluorescence during the first 4 s of odor presentation to the root mean square fluorescence during the 4 s preodor baseline, that is $(\text{RMS}_{\text{odor}}/\text{RMS}_{\text{baseline}})^2$. The average single-trial SNR for these 639 glomeruli was 263 ± 14 , with an average single-trial odor-evoked signal of $4.9 \pm 0.0\% \Delta F/F$ compared with a typical single-trial RMS baseline noise of $\sim 0.2\% \Delta F/F$ before stimulus onset. The smallest individual glomerular response included in the analysis by our statistical methods exhibited a reliable odor response of $0.7\% \Delta F/F$ compared with an RMS baseline noise of $0.2\% \Delta F/F$, while the largest was an odor response of $15.4\% \Delta F/F$. Because these odor-evoked responses were averaged over five trials before and five trials after each experimental manipulation, changes of $<0.1\% \Delta F/F$ could be reliably detected.

Processing and quantification of optical data were performed using custom MATLAB software. Statistical analyses were performed using SPSS and Origin Pro 2016. Comparisons of both central tendency and distributional shape were planned a priori. All reported experiments tested parametric hypotheses, such as the interaction of drug/vehicle infusion with experimental stage or comparisons of group means to the null hypothesis of zero change, so parametric statistics were used throughout. Statistical p values were Bonferroni corrected wherever multiple comparisons were made within the analysis of an individual experiment.

Results

Inactivation of the CBL amygdala inhibits GABAergic periglomerular interneuron populations in the olfactory bulb

The first stage of olfactory processing in the brain happens on the surface of the olfactory bulb, where the axon terminals of OSNs in the nose make synapses onto mitral cells and local interneurons within small spherical structures called glomeruli. Because each glomerulus receives axons only from OSNs expressing one particular odor receptor, odors that bind to different receptors drive activity in different odor-specific subsets of glomeruli, which thus constitute the initial representation of odor identity in the CNS. Previous work has shown that olfactory fear conditioning enhances odor-evoked responding in OSNs (Kass et al., 2013d) and mitral cells (Fletcher, 2012), possibly through alterations in the activity of GABAergic interneurons in the olfactory bulb (Okutani et al., 2002, 2003). We thus initially tested the effect of pharmacological inactivation of the amygdala on the olfactory signaling of PG interneurons in the olfactory bulb.

The activity of glomerular populations of PG cells was visualized in transgenic mice by expressing the fluorescent calcium indicator GCaMP6f in neurons expressing the *gad2* gene, which encodes the GABA-synthesizing enzyme GAD65. Within the olfactory glomerulus, GAD65 is expressed selectively in PG cells (Shao et al., 2009; Kiyokage et al., 2010), resulting in GCaMP6f expression in all glomeruli in these PG-GCaMP mice, as previously reported (Wachowiak et al., 2013). Odor presentation in PG-GCaMP mice evokes respiration-locked increases in fluorescence in odor-specific subsets of olfactory bulb glomeruli. GAD65-expressing granule cell populations in the olfactory bulb likely make little or no contribution to this signal because their depth below the pial surface makes it unlikely that they are illuminated by the LED and because any fluorescence emissions from granule cells would likely produce a diffuse signal unlike the focal fluorescence increases observed here.

Fourteen PG-GCaMP mice were implanted with bilateral guide cannulae targeting the CBL nuclei of the amygdala (CBL amygdala), regions that are involved in affective processing and emotional learning. These mice were then implanted with optical windows over the olfactory bulb, and baseline glomerular PG cell responses to five odorant presentations were observed (Fig. 1A,B). Muscimol (2%), a GABA_A receptor agonist commonly used to silence local activity (Martin and Ghez, 1999; Narayanan et al., 2006), or a vehicle control solution was then slowly infused ($0.25 \mu\text{l}/\text{min}$ for 1 min) into the CBL amygdala and, after allowing 20 min for diffusion, five additional odor presentations were delivered. The effects of muscimol infusion differed from glomerulus to glomerulus (see below) but, on average, produced a significant reduction of odor-evoked responses compared with the preinfusion baseline ($t_{(309)} = -8.177$, adjusted $p < 0.001$; based on 310 glomeruli from 7 mice; Fig. 1C, left) that was replicated in multiple further experiments detailed below. Analysis of subsequent inhalations can be difficult to interpret because they are not necessarily independent of the initial response, but measurement of the total area under the curve for GCaMP signals across the whole odor presentation showed a very similar reduction ($t_{(309)} = -4.952$, adjusted $p < 0.001$; Fig. 1C, right). Vehicle infusion into the CBL amygdala (Fig. 1D–F) induced a slight but statistically significant increase in odor-evoked PG cell activity ($t_{(324)} = 7.172$, adjusted $p < 0.001$; based on 325 glomeruli from seven mice) that was significantly different from the decrease seen with muscimol ($F_{(1,633)} = 74.835$, $p < 0.001$). Figure 1G shows a representative example of the odor-evoked glomerular

Figure 1. Inactivation of the amygdala inhibits GABAergic periglomerular interneuron populations in the olfactory bulb. **A**, Schematic illustration of the first stage of olfactory processing on the surface of the olfactory bulb. Axons terminals from OSNs located in the olfactory epithelium synapse onto mitral cells (which project to the olfactory cortex) and local interneurons, including periglomerular cells (green), within small spherical structures called glomeruli. Odor-evoked activity of olfactory bulb PG interneurons was visualized in transgenic mice that expressed the fluorescent calcium indicator GCaMP6f in GABAergic PG cells. **B**, Mice were implanted with bilateral cannulae targeting the central, basal, and lateral nuclei of the amygdala (CBLA), and odor-evoked activity of PG populations in individual glomeruli was observed through optical windows implanted over the olfactory bulbs. **C**, Infusing the GABA_A agonist muscimol into the CBL amygdala produced a significant reduction ($p < 0.001$) in odor-evoked activity (red bars) both during the first sniff of odorant (left) and integrated over the full 6 s odorant presentation (right). Vehicle infusion into CBL amygdala produced a slight but significant increase in odor-evoked activity (green bars). Error bars represent the SEM. ***Statistically significant change from the preinfusion baseline at the $p < 0.001$ level. **D**, **G**, Pseudocolored activity maps showing the change in fluorescence of the olfactory bulb during the first inhalation of odorant before and after CBL amygdala infusions of vehicle (**D**) or muscimol (**G**). **E**, **H**, Traces indicating the change in fluorescence over time from the glomeruli indicated by arrows in **D** and **G** during odor presentations before (gray trace) and following CBL amygdala infusion of vehicle (**E**, green trace) or muscimol (**H**, red trace). Black bars indicate the delivery of odorant for 6 s. Small black traces indicate respiration frequency, with upticks reflecting inhalation. **F**, **I**, Pseudocolored heat maps depicting the change in fluorescence in all responsive glomeruli (along the ordinate) over time (on the abscissa) before and after vehicle (**F**) or muscimol (**I**) infusion into CBL amygdala for the example mouse shown in **D** and **G**. The arrows denote which line of each heat map corresponds to the glomeruli illustrated in **D** and **G**. Lat, Lateral; Ant, anterior.

PG cell activity during the initial inhalation of odorant before and after CBL amygdala inactivation, along with examples of the time course of calcium dynamics within an individual glomerulus (Fig. 1H,I). To confirm cannula targeting, after completing the imaging experiment we delivered a second infusion that was identical to the first but instead used muscimol tagged with BODIPY, which was not used in the initial infusion to ensure effective pharmacological action at GABA_A receptors, to visualize the approximate spread of infusate. Subsequent histology confirmed effective targeting of the CBL amygdala in both vertical and horizontal planes (Fig. 2A–C) and showed that the spread of muscimol was approximately confined to the relevant nuclei (though this is best viewed as a minimum spread given the larger molecular weight and thus reduced diffusion of the tagged muscimol). The cortical and medial nuclei, which receive projections

from the olfactory bulb (but do not project to the olfactory bulb), were spared.

Inactivation of the locus coeruleus prevents the effect of CBL amygdala inactivation on PG cells

Olfactory bulb glomeruli receive extensive descending noradrenergic projections from the locus coeruleus (Fallon and Moore, 1978; Shipley et al., 1985; McLean et al., 1989), which is richly innervated by the central nucleus of the amygdala (Van Bockstaele et al., 1998, 1999; Reyes et al., 2008; Retson and Van Bockstaele, 2013). We thus hypothesized that the observed effects of CBL amygdala inactivation on PG cells occurred through amygdalar effects on the LC. To test this hypothesis, we examined whether inactivation of the LC via local muscimol infusion would prevent the effect of CBL amygdala inactivation on PG cells in the

Figure 2. Histological confirmation of CBL amygdala cannulation. **A**, Representative cannula placement and the diffusion pattern of BODIPY-tagged muscimol (orange; equivalent volume and rate of experimental solutions) in the left hemisphere of coronally sectioned ($100\ \mu\text{m}$) tissue stained with DAPI (blue) at $4\times$ magnification. Although some backflow can be observed along the cannula tract, the diffusion pattern indicates that all subnuclei of the CeA [only the lateral CeA (CeL) and medial CeA (CeM) are labeled in the image], lateral nucleus (LA), and basolateral (BLA) nucleus of the amygdala were affected, while surrounding areas such as the dorsal endopiriform claustrum (DeN) and ventral endopiriform claustrum (VeN) were spared. **B**, **C**, Schematic illustration (as adapted from the study by Franklin and Paxinos, 2007) of the cannula placement and diffusion patterns observed across all mice that received CBL amygdala infusions. Orange circles indicate the centroid of diffusion observed in BODIPY-tagged muscimol. **B**, Coronal sections. Numbers indicate posterior distance from bregma. **C**, Horizontal sections. Numbers indicate ventral distance from skull. LV, lateral ventricle.

bulb. We implanted mice with four cannulae targeting the left and right LC and the left and right CBL amygdala. Each mouse received five baseline odor presentations, followed by a bilateral infusion of either muscimol or vehicle into the LC and a 20 min

pause to allow diffusion, followed by five more odor trials, which were followed by a bilateral infusion of muscimol into the CBL amygdala and a 20 min pause, followed by five more odor presentations (Fig. 3). As above, at the conclusion of the experiment

all four cannulae were infused with BODIPY-tagged muscimol to confirm infusion targeting, and subjects with any mistargeted cannulae were dropped. Focal inactivation of the LC is challenging, but modest cannula misplacements produced respiratory changes indicating that drug infusion affected the neighboring parabrachial nucleus, thus giving us a functional indicator of mistargeted drug spread. All successful infusions likely inactivated both the LC and Barrington's nucleus, a neighboring structure involved in micturition that has no known projections to the olfactory system or amygdala. In a total of 7 of 19 mice (totaling 298 glomeruli from 4 LC muscimol-infused animals and 3 LC vehicle-infused animals) successfully completed the procedure with correctly placed cannulae and no respiratory changes (Fig. 4).

A 2×3 ANOVA with LC infusate as a between-subjects factor (muscimol vs vehicle) and experiment stage as a within-subjects factor revealed a significant LC infusate $\times$ experiment stage interaction, as hypothesized ($F_{(1,296)} = 106.822$, $p < 0.001$). After the initial infusion into the LC, there were no significant differences in odor-evoked glomerular PG cell activity between muscimol and vehicle infusions ($t_{(296)} = 2.871$; adjusted $p = 0.174$), suggesting that there was little spontaneous norepinephrine (NE) release in the olfactory bulbs in these anesthetized mice. As shown in Figure 3, A, C, E, G, and H, muscimol infusion into the CBL amygdala following vehicle infusion into the LC produced a large decrease in odor-evoked PG cell activity ($t_{(106)} = -12.609$; adjusted $p < 0.001$), replicating the previous result (Fig. 1G–I). However, muscimol infusion into the CBL amygdala after LC inactivation had no significant effect on odor-evoked activity (Fig. 3B,D,F–H; $t_{(190)} = -2.056$; adjusted $p > 1$). These data demonstrate that LC function is necessary for CBL amygdala inactivation to influence PG cells in the olfactory bulb.

Local blockade of noradrenergic signaling in the olfactory bulb prevents the effect of CBL amygdala inactivation on PG cells

Though the LC is the only noradrenergic projection to the olfactory bulb, it also projects to other regions that, in turn, project to the olfactory bulb using other transmitters, including the horizontal limb of the diagonal band of Broca (cholinergic projections to the bulb) and the piriform cortex and anterior olfactory nucleus (glutamatergic projections to the bulb). Merely inactivating the LC therefore does not rule out the possibility that its effects on the bulb could be mediated indirectly. To confirm that CBL amygdala inactivation affects PG cell activity through direct noradrenergic signaling, we tested whether the local application of the broad-spectrum noradrenergic receptor antagonist labetalol to the olfactory bulb could prevent this effect. In these experiments, the initial surgery included implantation of bilateral cannulae targeting CBL amygdala and the removal of the skull and dura mater overlying the olfactory bulbs to allow direct application of drug to the olfactory bulb. In seven mice, we observed odor-evoked PG cell activity during five baseline odorant presentations; then directly applied either vehicle or 13.7 mM labetalol to the olfactory bulb superfusate and waited 5 min to permit diffusion; delivered five more odorant presentations, infused muscimol into the CBL amygdala, and waited 20 min; then delivered five more odorant presentations. As before, cannula position was verified histologically using a postexperiment infusion of fluorescent muscimol (Fig. 2).

A 2×3 ANOVA with olfactory bulb drug as a between-subjects factor (labetalol vs vehicle) and experiment stage as a

within-subjects factor revealed a significant infusate $\times$ experiment stage interaction, as hypothesized ($F_{(2,596)} = 11.799$, $p < 0.001$; based on 300 glomeruli from seven mice). Consistent with the previous experiment showing that LC inactivation alone had no effect in the olfactory bulb (Fig. 3B,D,F–H), labetalol application (Fig. 5B,D,F,G) caused no significant difference compared with preinfusion baseline ($t_{(147)} = -1.744$; adjusted $p = 0.664$) or vehicle control ($t_{(298)} = 2.715$; adjusted $p = 0.056$). Vehicle application (Fig. 5A,C,E,G) also had no significant effect ($t_{(151)} = 2.382$; adjusted $p = 0.144$) compared with baseline. After vehicle application to the olfactory bulb, CBL amygdala inactivation (Fig. 5A,C,E,G,H) evoked a significant reduction in average odor-evoked PG cell activity as in previous experiments ($t_{(151)} = 8.794$, adjusted $p < 0.001$ compared with prebulbar application baseline; $t_{(151)} = 8.479$, adjusted $p < 0.001$ compared with preinfusion baseline). However, after intrabulbar blockade of α - and β -type adrenergic receptors with labetalol (Fig. 5B,D,F–H), the inactivation of CBL amygdala had no effect on odor-evoked PG cell activity, which was not significantly different from its pre-amygdala infusion baseline ($t_{(147)} = 1.974$; adjusted $p = 0.400$) or its prelabetalol baseline ($t_{(147)} = 0.737$; adjusted $p > 1$) but was very different from its vehicle control ($t_{(298)} = 4.005$; adjusted $p < 0.001$). Together with the previous experiments, these results confirm that CBL amygdala inactivation influences PG cell activity by inducing NE release from LC projections to the olfactory bulb.

CBL amygdala inactivation preferentially enhances the weakest odor-evoked OSN outputs

CBL amygdala-driven noradrenergic modulation of PG interneurons demonstrates that these regions can strongly influence early sensory processing, but its implications for odor representations and sensory gating are difficult to interpret. NE release can have complex effects on neural circuits, including directly altering neuronal excitability and presynaptically modulating the release of GABA from interneurons (Nai et al., 2009; Salgado et al., 2012; McGann, 2013; Nakamura et al., 2013; Pignatelli et al., 2013). Moreover, one of the major functions of PG cells is to presynaptically inhibit the synaptic output of OSNs (Aroniadou-Anderjaska et al., 2000; McGann et al., 2005; Murphy et al., 2005; Wachowiak et al., 2005), thus feeding back onto their own inputs. To explore the potentially diverse effects of CBL amygdala inactivation, we pooled data from 16 mice that received muscimol into the CBL amygdala across the three experimental cohorts from Figures 1, 3, and 5. While CBL amygdala inactivation reduced odor-evoked PG responses on average (Figs. 1C, 3H, 5G), Figure 6A shows that the odor-evoked activity of PG cells in some glomeruli was greatly reduced while in a minority of glomeruli it was enhanced. ANOVA revealed that the magnitude of the odor-evoked PG cell response before drug infusion was a significant factor in the size of the effect of amygdalar inactivation (one-way ANOVA: $F_{(2,636)} = 7.24$, $p < 0.001$), with the glomeruli that exhibited the smallest odor-evoked responses at baseline (operationally defined as the bottom third of the baseline response amplitudes) showing a significantly smaller (proportional) reduction on average than glomeruli in the middle ($t_{(424)} = 3.092$, adjusted $p = 0.006$) and top ($t_{(424)} = 3.671$, adjusted $p < 0.001$) thirds of the distribution (Fig. 6B). Please note that while the glomeruli in the bottom third of odor responses by definition exhibited a lower SNR than the others, the average single-trial SNR for the bottom third of glomeruli was nonetheless 64 ± 5 with a mean baseline odor-evoked response of 2.8% and a mean post-drug response of 2.4%, demonstrating that

Figure 3. Inactivation of the locus coeruleus prevents the effect of CBL amygdala inactivation on PG cells. Transgenic mice expressing GCaMP6f in PG cells were each implanted with four cannulae, targeting the left and right LC and left and right CBL amygdala (CBLA). **A**, **B**, Pseudocolored activity maps showing the change in fluorescence of the olfactory bulb during the first inhalation of odorant before any infusion (left), after LC infusion (middle) of vehicle (**A**) or muscimol (**B**), and after subsequent CBL amygdala infusion of muscimol (right). **C**, **D**, Traces indicating the change in fluorescence over time from the glomeruli indicated by arrows in **A** and **B** during odor presentations before any infusion (gray trace), following LC infusion of vehicle (**C**, green trace) or muscimol (**D**, red trace), and after subsequent CBL amygdala infusion of muscimol (rightmost traces). Black bars indicate the delivery of odorant for 6 s. Small black traces indicate respiration frequency, with upticks reflecting inhalation. **E**, **F**, Pseudocolored heat maps depicting the change in fluorescence in all responsive glomeruli (along the ordinate) over time (on the abscissa) before any infusions (left), after LC vehicle (**E**) or muscimol (**F**) infusion, and following CBL amygdala infusion of muscimol for the example shown in **A** and **B**. The arrows denote which line of (Figure legend continues.)

these odor-evoked signals were readily detectable in single trials even within the bottom third of the distribution.

The odor-evoked activity observed in glomerular populations of PG cells reflects the summation of multiple factors, including the direct excitation of some PG cells by OSNs and indirect excitation of PG cells by other OSN-driven neurons in the glomerular circuit (Shao et al., 2009), both of which are influenced by a presynaptic modulation of OSN synaptic output (McGann, 2013). However, previous studies have implicated noradrenergic signaling in the gating of weak sensory input (Waterhouse et al., 1988; Adler et al., 1994; Jiang et al., 1996; Ciombor et al., 1999; Bouret and Sara, 2002; Devilbiss et al., 2012; García-Ramírez et al., 2014), suggesting that the release of NE in the olfactory bulb induced by CBL amygdala inactivation might be preferentially boosting the smallest odor-evoked inputs to the bulb from the OSNs. Should this boosting of weak OSN output occur in combination with a global decrease in PG excitability, it would be reflected in Figure 6 as a smaller reduction of PG cell activity for weakly activated glomeruli, as observed. We thus tested the hypothesis that CBL amygdala inactivation selectively increased odor-evoked OSN output in weakly activated glomeruli.

Odor-evoked OSN synaptic output was visualized *in vivo* in gene-targeted mice expressing the fluorescent exocytosis indicator spH from the promoter for OMP. In these OMP-spH mice, populations of axons from mature OSNs are visible innervating all olfactory bulb glomeruli (Fig. 7A) and linearly indicate cumulative glutamate release from OSNs during each odor presentation (Bozza et al., 2004; Wachowiak et al., 2005). OMP-spH mice were implanted with intra-amygdalar cannulae, and odor-evoked OSN output was measured before and after the intra-CBL amygdala infusion of either muscimol or vehicle control, as above. As shown in Figure 7, C and G–I, muscimol infusion into the amygdala modestly but significantly increased the overall average odor-evoked OSN output ($t_{(218)} = 2.438$, adjusted $p = 0.03$ based on 219 glomeruli in seven mice). More importantly, this effect was significantly different across the population (one-way ANOVA, $F_{2, 216} = 15.77$, $p < 0.001$), with the net change almost entirely attributable to a $36 \pm 10\%$ increase in the response of the most weakly activated third of glomeruli (Fig. 7B; one-sample t test compared with no change: $t_{(73)} = 3.70$; adjusted $p = 0.001$), with no significant change in the middle third of the distribution ($t_{(73)} = -1.32$; adjusted $p = 0.570$) and a slight but significant decrease in the most strongly activated third ($t_{(73)} = -2.75$; adjusted $p = 0.021$). As shown in Figure 7, H and I, within the most weakly activated third of glomeruli, there was a general trend toward the most weakly activated glomeruli showing the largest proportional increases, but even glomeruli with very similar initial responses in the same mouse (Fig. 7H,I, pink and magenta; note that glomerulus 3 is from the olfactory bulb on the other side of the same mouse) could exhibit increases of quite different sizes. Vehicle-infused mice (Fig. 7D–F) showed no significant

←

(Figure legend continued.) each heat map corresponds to the glomeruli illustrated in C and D. G, Summary data across mice showing that inactivating LC prevented the subsequent CBL amygdala inactivation from altering PG activity (red triangles), whereas infusing vehicle into the LC before CBL amygdala inactivation produced a significant decrease in PG activity (green circles) in accordance with the data shown in Figure 1. Notably, no change in odor-evoked activity was observed following LC infusion of either vehicle or muscimol. Error bars represent the SEM. ***A statistically significant difference at $p < 0.001$. H, Cumulative frequency histogram illustrating the distribution of changes in odor-evoked activity after CBL amygdala inactivation across all glomeruli normalized to preinfusion baseline for mice that had received an LC infusion of either vehicle (green points) or muscimol (red points). Lat, Lateral; Ant, anterior.

Figure 4. Histological confirmation of LC cannulation. **A**, Representative cannula placement and diffusion pattern of BODIPY-tagged muscimol (orange; equivalent volume and rate of experimental solutions) in the right hemisphere of coronally sectioned ($100 \mu\text{m}$) tissue stained with DAPI (blue) at $4\times$ magnification. The small volume and rate of infusion minimized backflow along the cannula tract and diffusion into surrounding structures, although Barrington's nucleus (Bar) was likely affected, as shown here. **B**, Coronal illustration (as adapted, with permission, from Franklin and Paxinos, 2007) of infusion locations across animals. Orange circles indicate the centroid of BODIPY-tagged muscimol diffusion. Numbers indicate posterior distance from bregma. MPB, Medial parabrachial nucleus; 4V, fourth ventricle.

Figure 5. Local blockade of noradrenergic signaling in the olfactory bulb prevents the effect of CBL amygdala inactivation on PG cells. Bilateral cannulae targeting the CBL amygdala (CBLA) were implanted, and the skull and dura mater overlying the olfactory bulb were removed in PG-GCaMP6f mice. **A, B**, Pseudocolored activity maps showing the change in fluorescence of the olfactory bulb (OB) during the first inhalation of odorant in preinfusion baseline trials (left) after vehicle (**A**) or the broad-spectrum noradrenergic antagonist labetalol (**B**) was applied to the OB superfusate (middle) and after subsequent CBL amygdala infusion of muscimol (right). **C, D**, Traces indicating the change in fluorescence over time from the glomeruli indicated by arrows in **A** and **B** during odor presentations on baseline trials (gray trace), following OB application of vehicle (**C**, green trace) or labetalol (**D**, red trace), and after subsequent CBL amygdala infusion of muscimol (rightmost trace). Black bars indicate delivery of odorant for 6 s. Small black traces indicate respiration frequency, with upticks reflecting inhalation. **E, F**, Pseudocolored heat maps depicting the change in fluorescence in all responsive glomeruli (along the ordinate) over time (on the abscissa) during baseline trials (left), after OB application of vehicle (**E**) or labetalol (**F**), and following CBL amygdala infusion of muscimol for the example shown in **A** and **B**. The arrows denote which line of each heat map corresponds to the glomeruli illustrated in **C** and **D**. **G**, Summary data showing the mean change in odor-evoked responses across all glomeruli. Replicating the effect of LC inactivation, labetalol applied to the OB prevented the subsequent CBL amygdala (Figure legend continues.)

Figure 6. CBL amygdala inactivation differentially affects PG cell activity depending on baseline odor-evoked responding. We pooled data from 16 mice across three experimental cohorts that received muscimol infused into the CBL amygdala. **A**, Scatter plot of the change in odor-evoked PG cell activity in olfactory bulb glomeruli following CBL amygdala inactivation against preinfusion odor-evoked response amplitude. Each circle represents one glomerulus. Although CBL amygdala inactivation reduced odor-evoked glomerular responses on average, the effect was very heterogeneous and included some glomeruli that showed an increased response. **B**, Summary plot of the mean effect of CBL amygdala inactivation for glomeruli in the bottom, middle, and top thirds of the distribution of preinfusion responses. CBL amygdala inactivation suppressed PG cell activity less in glomeruli that exhibited the weakest odor-evoked response before the CBL amygdala infusion of muscimol.

effect ($99 \pm 1\%$ of control overall; $t_{(166)} = -0.577$; adjusted $p > 1$; based on 166 glomeruli in 6 mice). These data confirm that one consequence of CBL amygdala inactivation is selective enhancement of weak sensory signals from the olfactory nerve to the brain.

Discussion

The present study demonstrated that pharmacological blockade of activity in the central, basolateral, and lateral nuclei of the amygdala changed the response of the early olfactory system to odors, including preferentially enhancing the synaptic output of weakly activated olfactory sensory neuron populations and suppressing the activity of GABAergic periglomerular interneurons in most glomeruli. This influence was mediated through NE released in the olfactory bulb from noradrenergic afferents coming from the LC. The finding that amygdala and LC activity can shape sensory processing as early as the primary sensory input to the brain suggests that the state of these regions influences which sensory signals are selected for neural processing.

Circuit-level mechanisms

How did decreased activity in the CBL amygdala cause excitation of the LC in the present experiments? There are extensive projections from the CeA to the LC (Van Bockstaele et al., 1998, 1999; Retson and Van Bockstaele, 2013; Schwarz et al., 2015). These CeA to LC projections include both symmetric (presumed inhibitory) and asymmetric (presumed excitatory) synapses when viewed using electron microscopy and frequently coexpress neuropeptides like corticotropin-releasing factor (CRF) and dynorphin that can also have inhibitory effects (Valentino et al., 1992; Van Bockstaele et al., 1998; Van Bockstaele et al., 1999; Tjoumakaris et al., 2003; Reyes et al., 2008; Retson and Van Bockstaele, 2013). In our experiments, pharmacological inactivation of the central nucleus (and its inputs from basolateral and lateral nuclei) thus presumably evoked NE release by disinhibiting the LC. As illustrated in Figure 8, the disinhibition of LC led to a suppression of PG cell activity (Fig. 6) and increased OSN output in some glomeruli (Fig. 7). However, it is important to note that these experiments were performed in experimentally naive animals under pentobarbital anesthesia, so the efficacy of inhibitory synapses between CeA and LC was likely enhanced by the anesthesia, and the spontaneous activity of LC was doubtless suppressed compared with the awake state. Nonetheless, this approach proves that CBL amygdala is capable of influencing sensory gating through its connectivity to the LC. Follow-up experiments using attentional tasks, fear conditioning, or stress to explicitly manipulate the state of the amygdala–LC circuit will be necessary to explore the various ways that these regions can influence sensory processing, including in circumstances where amygdala activity excites the LC. Such tasks will also provide the opportunity to explore the broader network interacting with the CBL

(Figure legend continued.) inactivation from altering PG cell activity (red diamonds), whereas OB application of vehicle before CBL amygdala inactivation produced a significant decrease in PG activity (green circles) in accordance with the data shown in Figure 1. **H**, Cumulative frequency histogram illustrating the distribution of changes in odor-evoked activity after CBL amygdala inactivation across all glomeruli normalized to preinfusion baseline for mice that had received intrabulbar application of either vehicle (green points) or labelalol (red points). Together with the previous experiments, these results confirm that CBL amygdala inactivation influences PG cell activity by inducing norepinephrine release from LC projections to the olfactory bulb. Lat, Lateral; Ant, anterior.

Figure 7. CBL amygdala inactivation preferentially enhances the weakest odor-evoked OSN outputs. Odor-evoked output of OSNs was visualized in gene-targeted mice expressing the fluorescent exocytosis indicator spH before and after CBL amygdala (CBLA) infusion of either vehicle or muscimol. **A**, Schematic illustration of the first stage of olfactory processing on the surface of the olfactory bulb with OSN axon terminals highlighted in green to indicate their expression of spH. **B**, Summary across mice showing the change in mean OSN output following CBL amygdala inactivation based on where each glomerulus fell in the distribution of preinfusion odor-evoked response amplitudes (i.e., lowest, middle, and top third of baseline odor-evoked responses). CBL amygdala inactivation selectively enhanced odor-evoked OSN output in glomeruli that exhibited the weakest odor-evoked responses at baseline. Error bars represent the SEM. **C**, Cumulative frequency histogram illustrating the distribution of changes in glomerular inputs normalized to baseline following CBL amygdala infusion of either vehicle (green points) or muscimol (red points). **D, G**, Pseudocolored activity maps showing the change in fluorescence of the olfactory bulb at the end of odorant presentation before and after CBL amygdala infusions of vehicle (**D**) or muscimol (**G**). **E, H**, Traces indicating the change in fluorescence over time from the glomeruli indicated by arrows in **D** and **G** during odor presentations before (gray trace) and following CBL amygdala infusion of vehicle (**E**, green trace) or muscimol (**H**, pink, magenta, and dark red traces). Black bars indicate the delivery of odorant for 6 s. Small black traces indicate respiration frequency, with upticks reflecting inhalation. Glomeruli 1 and 2 refer to the location of the glomeruli in **G**. Note that glomerulus 3 is from the other olfactory bulb of this mouse and so is not visible in **G**; it was included for comparison because of its similar response size to glomerulus 2 at baseline but divergence after muscimol. **F, I**, Scatter plots of odor-evoked glomerular inputs following CBL amygdala infusion of either vehicle (**F**) or muscimol (**I**) as a function of preinfusion magnitude. Glomeruli illustrated in **H** are labeled. As seen with PG cell activity, CBL amygdala inactivation selectively influenced the response magnitude in glomeruli that were weakly driven by the odorant at baseline.

amygdala (Mouly and Di Scala, 2006), including other olfactory regions like piriform cortex (Sadrian and Wilson, 2015), polymodal cortical structures like perirhinal cortex and anterior cingulate cortex (Sripanidkulchai et al., 1984; Kealy and Commings, 2011), and regions involved in modulating amygdalar output like prefrontal cortex (Carmichael and Price, 1995; Murray and Wise, 2010).

Sensory input to the brain uses a local circuit motif that occurs almost identically in the olfactory, visual, and auditory systems, where glutamatergic sensory afferents reach the brain from the olfactory epithelium, retina, or cochlea, respectively, and their neurotransmitter release is regulated by presynaptic GABA_B receptors activated by GABA release from local interneurons (Brenowitz et al., 1998; Chen and Regehr, 2003; McGann, 2013). All

Figure 8. Schematic illustration of amygdala–locus coeruleus–olfactory bulb interactions. Diagram of a subset of interconnections within and between the olfactory system, amygdala, and locus coeruleus responsible for the data reported above. Left, OSNs located in the olfactory epithelium in the nose detect the odorant molecules and project their axons to the glomeruli of the olfactory bulb, where they release glutamate onto mitral and tufted cells (which project to the olfactory cortex) and onto local interneurons called PG cells. PG cells also receive noradrenergic input from the LC, which is in turn inhibited by activity in the CBL nuclei of the amygdala. PG cells release the inhibitory transmitter GABA onto OSN axon terminals (presynaptically inhibiting their transmitter release) and onto mitral and tufted cells. Right, Infusion of muscimol into the CBL amygdala silences local activity (decreased activity shown in red), which disinhibits the LC (increased activity shown in green), which inhibits PG cells (via norepinephrine release), disinhibiting weakly activated OSN axon terminals. The increased OSN output in the glomeruli is presumably reflected in stronger odor-evoked activity in mitral and tufted cells and thus the input to the olfactory cortices.

three regions receive noradrenergic input from the LC (Kromer and Moore, 1980a,b; McLean et al., 1989), suggesting that LC-mediated shaping of the initial sensory input to the brain could be a general feature of sensory information processing. In the present data, we speculate that one consequence of NE suppressing most PG cell activity was decreased tonic presynaptic inhibition of OSN terminals (Pérez and Wachowiak, 2008), which in turn disproportionately boosted weak OSN outputs and evoked stronger PG cell activity in those glomeruli (Fig. 6). Interestingly, it has been proposed that this presynaptic inhibition might serve not only as a gate but as a temporal filter, potentially suppressing initial sensory responses to enable sustained responses (Brenowitz and Trussell, 2001) or temporally structuring neural activity to match phasic sensory dynamics (Wachowiak et al., 2005; Verhagen et al., 2007).

NE release in the olfactory bulb has been previously linked to decreases in GABA release in slice experiments (Nai et al., 2009) and has been shown to preferentially boost the responses of olfactory bulb mitral cells to weak OSN stimulations *in vivo* (Jiang et al., 1996). At the behavioral level, pharmacological activation of the olfactory bulb NE system evokes selectively improved olfactory performance at low odor concentrations (Escanilla et al., 2010). The present data are thus consistent with previous reports that NE release in the bulb opens the gate on weak olfactory sensory input to the brain.

We note that a previous study (Eckmeier and Shea, 2014) reported that LC stimulation-evoked release of NE in the bulb suppressed calcium signaling in OSN terminals, which potentially contradicts our Figure 7. However, their results may not be directly comparable to ours because they used 5 Hz electrical

stimulation of the LC, which is likely to produce an artificially high concentration of NE in the bulb, which might activate different receptor populations (Nai et al., 2009). Furthermore, they used ketamine/xylazine anesthesia, which disrupts noradrenergic tone (Kubota et al., 1999) and directly interacts with NE receptors and HCN-1 channels present in the OSNs and glomerular circuit (Fried et al., 2010; Nakashima et al., 2013). The present study used pentobarbital anesthesia, which enhances the efficacy of GABA at GABA_A receptors (Steinbach and Akk, 2001), which are found throughout the olfactory bulb (though seemingly not on OSN terminals) but do not directly interact with NE receptors. Nonetheless, in our study the use of anesthetic doubtless puts the olfactory bulb circuit into an unnatural state that necessitates follow-up work in awake animals to determine the normal role of the LC → olfactory bulb circuit in olfactory coding.

Relevance to olfactory information processing and amygdala-dependent learning

A key role of sensory brain regions is the selection of sensory stimuli for neural processing by prioritizing stimuli based on the current behavioral or neuromodulatory state of the organism (Hurley et al., 2004; Salgado et al., 2016). This function can have many implementations, including selectively regulating neural responses to weak sensory stimuli, discriminating an ecologically important stimulus from background activity (Gaucher and Edeline, 2015), and adapting out static inputs in favor of dynamic ones. These selection processes use noradrenergic modulation of sensory regions in various ways, including both “opening the gate” for weak sensory inputs in some circumstances (Waterhouse et al., 1988, 1990; Jiang et al., 1996; Ciombor et al., 1999) and suppressing them in others (Manunta and Edeline, 1997; Zitnik et al., 2013). The present data extend our understanding of this system both outwardly, by demonstrating that even the synaptic output of primary sensory neurons can be influenced by LC and CBL amygdala activity (Fig. 7), and inwardly, by showing that the state of the amygdala can indirectly determine the gating of sensory input to the brain through its connections to LC. These findings dovetail with recent evidence that post-traumatic stress disorder (PTSD) patients undergoing aversive visual conditioning exhibit strong functional connectivity among the amygdala, locus coeruleus, and early visual processing areas like the thalamus and primary visual cortex (Morey et al., 2015).

Sensory gating deficits are a key biomarker for attention deficit hyperactivity disorder, schizophrenia, Tourette’s syndrome, and affective disorders like depression, panic disorder, and PTSD (Light and Braff, 1999; Alsene and Bakshi, 2011; Sable et al., 2012; Oranje et al., 2013; Micoulaud-Franchi et al., 2015a,b), conditions that also include explicit sensory attention symptoms like hypervigilance and attentional bias (Felmington et al., 2011; Felmington et al., 2012; Hegerl and Hensch, 2014). These symptoms, some of which can be mimicked in neurotypical subjects by pharmacologically boosting NE release (Adler et al., 1994; Hammer et al., 2007), likely relate to dysfunctional activity in the LC (Bernard et al., 2011; Pietrzak et al., 2013; Morey et al., 2015). In animal models, increased LC activity promotes vigilance behavior (Aston-Jones et al., 1991, 1994; Rajkowski et al., 1994), but high tonic firing rates in LC are related to errors on tasks requiring focused attention on specific stimuli (Aston-Jones and Cohen, 2005). The current finding that LC-driven NE release can regulate sensory input to the brain provides a novel potential target for investigating sensory gating function and dysfunction.

One critical element of the current work is that it shows how differences in amygdala activity could have sensory consequences

mediated by the LC (van Marle et al., 2009; Devilbiss et al., 2012; Chen et al., 2014; Ousdal et al., 2014). Amygdala output is strongly regulated by aversive experiences and by stress, which evoke the release of CRF from central amygdala into the LC (Van Bockstaele et al., 1998, 1999; Reyes et al., 2008). This stress-induced CRF release causes a wide range of sensory dysfunctions, including reduced thalamic and sensory cortical responses to stimuli (Devilbiss et al., 2012) and disrupted sensorimotor gating (Bakshi et al., 2012). The present data suggest that this may underlie a previous report showing that stress impairs olfactory discrimination via noradrenergic action in the olfactory bulb (Manella et al., 2013) and may illuminate recent findings that fear conditioning disproportionately enhances LC and sensory responses to highly emotional stimuli in PTSD patients (Morey et al., 2015).

Models of the biological underpinnings of fear learning prominently feature the CBL amygdala and LC as recipients of sensory information (Fanselow and Poulos, 2005; Johansen et al., 2011, 2014), rather than shapers of it. However, theories of associative learning have always included stimulus-specific parameters intended to capture the “associability” of a sensory cue (Rescorla and Wagner, 1972; Mackintosh, 1975; Pearce and Hall, 1980), which are usually characterized by the salience of the physical stimulus (e.g., loudness of a tone). This stimulus-specific associability has been proposed to be increased by learning itself (Mackintosh, 1975), and evidence is accumulating that the early olfactory system indeed exhibits sometimes profound plasticity during a variety of classically amygdala-dependent experimental tasks, including fear conditioning (Li et al., 2008; Fletcher, 2012; Åhs et al., 2013; Kass et al., 2013d; Krusemark et al., 2013; Dias and Ressler, 2014; Morrison et al., 2015; Parma et al., 2015) and appetitive conditioning (Abraham et al., 2014) in both humans and rodent models. The present study demonstrates how information about amygdala activity can potentially reach even the OSNs during learning or memory retrieval, providing a potential circuit-level substrate for fear learning-induced changes in stimulus salience. Given the well documented role of NE in enabling learning-related plasticity during fear conditioning in the amygdala (Bush et al., 2010; Johansen et al., 2014), it will be important to investigate whether similar mechanisms drive associative neuroplasticity in sensory regions.

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