Adrenergic Receptor-Mediated Disinhibition of Mitral Cells Triggers Long-Term Enhancement of Synchronized Oscillations in the Olfactory Bulb

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INTRODUCTION

Norepinephrine (NE) has been found to be critical for various forms of olfactory learning in rodents, ranging from early learning preference in neonates (McLean and Harley 2004; Sullivan et al. 1989, 2000) to enhanced two-odor discrimination in adults (Doucette et al. 2007). Many of the actions of NE appear to be specific to the first processing 40% of information (Mori et al. 1999; Stopfer et al. 1997), our results that combined brief exposure to NE and local electrical stimulation of a specific odor. There is, however, some question as to whether disinhibition is the dominant action of NE in the rodent bulb (Nai et al. 2009). NE at some concentrations appears to increase, not decrease, GC-to-MC GABAergic transmission during direct recordings of inhibitory currents in dentro-dendritic synapses. Disinhibition due to acute NE exposure has been observed in recordings of inhibitory currents in turtle olfactory bulb slices (Jahr and Nicoll 1982). There is also evidence in rats for AR-mediated disinhibition based on in vivo extracellular measurements of paired-pulse inhibition (Okutani et al. 1998; Wilson and Leon 1988). NE-induced disinhibition is a plausible mechanism for olfactory learning because the enhanced depolarization caused by disinhibition could lead to short-term increases in intracellular calcium of the type that can lead to subsequent long-term synaptic modifications that underlie learning. Also, disinhibition would have the required permissive role in inducing odor-specific cellular changes because the resulting enhanced depolarization would only occur in neurons activated by a specific odor. There is, however, some question as to whether disinhibition is the dominant action of NE in the rodent bulb (Nai et al. 2009). NE at some concentrations appears to increase, not decrease, GC-to-MC GABAergic transmission during direct recordings of inhibitory currents in MCs. In addition, NE can have acute actions on bulb circuit properties other than on inhibition (Hayar et al. 2001; Yuan et al. 2000), which could also underlie its behavioral effects.

Our study tested the hypothesis that NE can lead to specific long-term changes in the cellular properties of the olfactory bulb through acute disinhibitory effects, using electrophysiological recordings in rat olfactory bulb slices. We first tested whether NE can cause disinhibition at MC-GC synapses and the subtype(s) of ARs that mediate this effect. Subsequently, we examined whether NE-induced disinhibition underlies a previously reported long-term increase in synchronized gamma frequency (30–70 Hz) oscillations in extracellular local field potential (LFP) signals in the bulb (Gire and Schoppa 2008). This effect was shown to result from a conditioning stimulus that combined brief exposure to NE and local electrical stimulation of olfactory nerve (ON) afferents. Because synchronized oscillations may be important for integrating olfactory information (Mori et al. 1999; Stopfer et al. 1997), our results
examining the underlying causes of enhanced synchrony in the bulb could have direct implications for understanding olfactory learning mechanisms.

METHODS

The experiments described here were performed in 300–330 μm thick horizontal olfactory bulb slices that were prepared as previously described (Schoppa et al. 1998). Except where noted, slices were taken from rats at postnatal day P9-13. Slices were viewed under differential interference contrast optics at 40x magnification (Axioskop; Carl Zeiss, Thornwood, NY). All experiments were approved by the Institutional Animal Care and Use Committee at the University of Colorado, Anschutz Medical Campus. All experiments were done at 31–34° C.

Electrophysiology

The base extracellular solution for all recordings contained (in mM) 125 NaCl, 25 NaHCO3, 1.25 NaH2PO4, 25 glucose, 3 KCl, 2 CaCl2, and 1 MgCl2 (pH 7.3), and was oxygenated (95% O2:5% CO2). In experiments studying the long-term effects of NE on gamma oscillations, the bath solution included 100 μM sulpiride, a dopamine D2 receptor antagonist, to block possible inhibitory effects of NE on presynaptic ON terminals through D2 receptors (Ennis et al. 2001; Hayar et al. 2001). For whole cell patch-clamp recordings of MC IPSCs in MCs that were evoked by local electrical stimulation (100 μs; single shocks of 50–200 μA) in the granule cell layer, GCL, with a patch pipette (Fig. 1, A and B). Most MC/GC synapses in the bulb are reciprocal synapses in which the site of GABAergic transmission from GC to MC is directly adjacent to the site of glutamatergic transmission from MCs onto GCs. To isolate GABAergic transmission, experiments were done in the presence of glutamate receptor antagonists to block excitatory transmission, either kynurenate (3 mM) or the combination of the AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzof[1]quinoxaline-2,3-dione (NBQX, 20 μM) and the NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (n-AP5, 50 μM). Under these conditions, GCL stimulation resulted in fast IPSC events that occurred with a short onset-time (time to 50% maximum current = 2.3 ± 0.3 ms, n = 6 cells) that were blocked by the GABA_A receptor antagonist gabazine (2–5 μM; 95 ± 1% amplitude decrease, n = 6; Fig. 1A). The peak amplitude of the IPSC evoked by GCL stimulation was not significantly affected (P = 0.11) by whether the MC had an intact or truncated apical dendrite (cut fortuitously during the slice preparation; Supplementary Fig. S1; 60 ± 10 pA for intact, n = 5; 114 ± 21 pA for truncated, n = 3), as expected if the IPSC originated from GCs rather than GABAergic juxtaglomerular cells. As can be seen by the raw current traces and the plot of the response time course (Fig. 1, A and B), bath application of NE (20 μM) caused a reversible reduction in the IPSCs (44 ± 3% decrease, n = 8, P < 0.00005; see Fig. 3E). These results suggest that a dominant effect of NE at 20 μM is a reduction in GABAergic transmission at GC-to-MC synapses.

As a second measure of NE effects on inhibitory transmission from MCs to GCs, we turned to recordings of synaptic activity evoked by patterned stimulation of ON afferents that were separated by 15–20 s. For ON stimulation at theta frequency, stimuli (100 ms; 10–500 Hz) were applied every 30 s. In the pattern consisting of five short bursts (3 pulses at 100 Hz) separated by 250 ms (4 Hz). Each stimulus pattern was applied every 30 s. In the experiments studying the long-term effects of NE on gamma oscillations, the bath solution included 100 μM sulpiride, a dopamine D2 receptor antagonist, to block possible inhibitory effects of NE on presynaptic ON terminals through D2 receptors (Ennis et al. 2001; Hayar et al. 2001). For whole cell patch-clamp recordings of MC IPSCs in MCs that were evoked by local electrical stimulation (100 μs; single shocks of 50–200 μA) in the granule cell layer, GCL, with a patch pipette (Fig. 1, A and B). Most MC/GC synapses in the bulb are reciprocal synapses in which the site of GABAergic transmission from GC to MC is directly adjacent to the site of glutamatergic transmission from MCs onto GCs. To isolate GABAergic transmission, experiments were done in the presence of glutamate receptor antagonists to block excitatory transmission, either kynurenate (3 mM) or the combination of the AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzof[1]quinoxaline-2,3-dione (NBQX, 20 μM) and the NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (n-AP5, 50 μM). Under these conditions, GCL stimulation resulted in fast IPSC events that occurred with a short onset-time (time to 50% maximum current = 2.3 ± 0.3 ms, n = 6 cells) that were blocked by the GABA_A receptor antagonist gabazine (2–5 μM; 95 ± 1% amplitude decrease, n = 6; Fig. 1A). The peak amplitude of the IPSC evoked by GCL stimulation was not significantly affected (P = 0.11) by whether the MC had an intact or truncated apical dendrite (cut fortuitously during the slice preparation; Supplementary Fig. S1; 60 ± 10 pA for intact, n = 5; 114 ± 21 pA for truncated, n = 3), as expected if the IPSC originated from GCs rather than GABAergic juxtaglomerular cells. As can be seen by the raw current traces and the plot of the response time course (Fig. 1, A and B), bath application of NE (20 μM) caused a reversible reduction in the IPSCs (44 ± 3% decrease, n = 8, P < 0.00005; see Fig. 3E). These results suggest that a dominant effect of NE at 20 μM is a reduction in GABAergic transmission at GC-to-MC synapses.

As a second measure of NE effects on inhibitory transmission from MCs to GCs, we turned to recordings of inhibitory synaptic activity evoked by patterned stimulation of ON afferent fibers at the theta frequency (done in the absence of glutamate receptor blockers), which is a stimulus pattern designed to mimic the breathing cycle (see METHODS for details of stimulus protocol). Under these conditions, inhibition appeared
as a barrage of rapid IPSC events that persisted for the duration of the stimulus pattern, reflecting on-going bulb network activity. As was seen for the IPSC evoked by GCL stimulation, NE caused a marked reduction in the barrages of IPSCs evoked by patterned ON stimulation, reflecting on-going bulb network activity. As was seen for the IPSC evoked by GCL stimulation, as measured by the whole cell current variance (Fig. 1, C and D; 50 ± 12% decrease in variance, n = 9, P = 0.002). These results indicate that NE can cause disinhibition of MCs under stimulus conditions that resemble the physiological situation. In addition, they further confirmed that NE can have specific effects on inhibition onto MCs from GCs rather than juxtaglomerular cells because it has been determined that the large majority of IPSCs evoked by patterned ON stimulation reflect GC inputs (Schoppa 2006a).

NE has been reported to have differing effects on GC-to-MC GABAergic transmission, depending on the NE concentration applied (Nai et al. 2009). In our study, we limited our analysis of NE effects at 20 μM because that concentration was previously shown to be effective at inducing long-term physiological changes in the bulb (Gire and Schoppa 2008). The events underlying this long-term change are the main interest of the present study.

Mechanisms of NE-induced acute disinhibition

In addition to recordings of IPSCs evoked by electrical stimulation, we also assayed NE’s effect on spontaneous mIPSCs recorded in the presence of the sodium channel blocker tetrodotoxin (TTX; 1 μM) and glutamate receptor antagonists (NBQX plus d-APV). Recordings of mIPSCs are often done to obtain specific information about the mechanisms of pre- and postsynaptic actions of neuromodulators. In our recordings (Fig. 2, A and B; from our standard animal age group), NE (20 μM) had little effect on mIPSC amplitude (15 ± 5% decrease in amplitude of the average detected event, n = 4, P = 0.07) or frequency (14 ± 15% increase, n = 4, P = 0.40). The absence of an effect of NE on the mIPSC amplitude suggests that the NE-induced reduction in IPSCs due to electrical GCL stimulation (Fig. 1, A and B) was independent of effects on postsynaptic GABA_A receptors on MCs. Meanwhile, the non-effect of NE on mIPSC frequency implies that NE does not have a direct action on presynaptic synaptic vesicle release machinery on GC dendrites. Interestingly, the non-effect of NE on mIPSCs that we observed differs from the recent study by Nai and co-workers (2009), which found that NE increased the mIPSC frequency at a 20 μM concentration. This difference appears to reflect at least in part the ages of the rats used in the studies. Our standard animal age (P9-13) was younger than that used by Nai and co-workers (P14-31). When we conducted experiments in slices from rats at P18-19, NE (20 μM) significantly increased the mIPSC frequency (174 ± 39% increase, n = 4, P = 0.021; Fig. 2, C and D).

We also examined which AR subtype mediated the NE-induced reduction in GC-to-MC transmission, first, by testing...
the effect of different AR sub-type-specific agonists on IPSCs evoked by GCL stimulation (Fig. 3; measured in the presence of NBQX, 20 μM, and d-AP5, 50 μM). The α2 AR-specific agonist clonidine (5 μM) was found, like NE, to be effective in reducing the IPSCs (40 ± 7% decrease, n = 8, P = 0.007; Fig. 3, A, B, and E), although no effect was observed due to either the α1 AR-specific agonist phenylephrine (PE, 20 μM; 2 ± 22% amplitude decrease, n = 5) nor the β AR-specific agonist isoproterenol (Isop, 100 μM; 4 ± 6% amplitude decrease, n = 5; Fig. 3, C–E). The average effect of clonidine on the IPSCs evoked by GCL stimulation (40% decrease) was very similar to that due to NE (average decrease of 44%; see preceding text), suggesting that α2 ARs mediate a large majority of the NE-induced reduction in evoked IPSCs. Consistent with our interpretation, we also found that blockade of α2 ARs with the α2 receptor-specific antagonist yohimbine (20 μM) eliminated the decrease in evoked IPSCs due to NE (20 μM; 1 ± 13% increase in IPSC amplitude due to NE plus yohimbine, n = 5, P = 0.96; Fig. 3F). Finally, as with the NE-induced reduction in inhibition in our standard younger-aged animals (P9-13), α2 AR activation with clonidine in younger animals reduced the evoked IPSC without altering spontaneous mIPSC events measured in TTX (Fig. 3, G and H; 15 ± 10% decrease in amplitude, n = 6, P = 0.20; 6 ± 9% increase in frequency, n = 6, P = 0.55). Unlike NE, clonidine did not increase the mIPSC frequency in older animals (at P23; 3 ± 28% increase in frequency, n = 5, P = 0.91; data not shown). The NE-induced increase in mIPSC frequency observed in older animals (Fig. 2, C and D) was likely due to activation of α1 or β ARs as previously described (Nai et al. 2009).

**NE has no effect on MC-to-GC transmission at dendrodendritic synapses**

As discussed in the preceding text, MCs and GCs make reciprocal connections. Thus NE could affect GABAergic transmission from GCs onto MCs not only due to direct actions at GC-to-MC GABAergic synapses but also indirectly by affecting MC-to-GC excitatory glutamatergic transmission (Trombley and Shepherd 1992). To examine NE actions on MC-to-GC transmission at reciprocal synapses, we performed voltage-clamp recordings from GCs while directly stimulating the external plexiform layer (EPL; 100 μs, 100–500 μA; Fig. 4A).

This stimulation has been reported to elicit a class of excitatory postsynaptic current (EPSC) events with moderate kinetics (τ_decay = 6–8 ms) (Balu et al. 2007; Schoppa 2006b) that reflect glutamatergic transmission from MC lateral dendrites onto the distal apical dendrites of GCs. Recordings of MC-to-GC excitation were done in the presence of bicuculline methiodide (BMI; 10 μM) to block GABA_A receptors. NE (20 μM) had no effect on the EPSCs evoked by stimulation in the EPL (2 ± 6% increase, n = 4; Fig. 4, A and B), indicating that NE actions at reciprocal synapses are confined to GC-to-MC transmission.

We also tested the effect of NE on EPSCs in GCs evoked by electrical stimulation in the GCL. GCL stimulation evokes a class of EPSCs in granule cells with faster decay kinetics (τ_decay = 1–2 ms) than EPSCs evoked by EPL stimulation and that originate at a location relatively close to the granule cell soma (Balu et al. 2007; Schoppa 2006b). We found that NE (20 μM) reversibly inhibited these fast EPSC events (42 ± 6% decrease, n = 5, P < 0.05; Fig. 4, C and D). We did not further explore NE effects at these synapses in the present study. The net effect of an NE-induced reduction in the very fast EPSCs in GCs would be disinhibition of MCs, similar to the NE-induced reduction in GC-to-MC transmission characterized in the preceding text, but this disinhibitory effect may not have been a major contributing factor in the previously-reported increase in synchronized gamma frequency oscillations caused by the combination of NE and ON stimulation (Gire and Schoppa 2008). While some of the fast EPSCs could be derived from axon collaterals of MCs (Schoppa 2006b), a significant fraction of them appear to reflect centrifugal inputs from the piriform cortex (Balu et al. 2007), which, in bulb slices, are likely not activated by ON stimulation.
Conditioning stimulus composed of NE and NMDA leads to long-term changes in gamma oscillations

The main result of our studies thus far is that NE, through activation of $\alpha_2$ ARs, can cause disinhibition of MCs, specifically by reducing GABAergic transmission from GCs onto MCs at reciprocal synapses. The objective of the rest of this study was to test whether this acute disinhibition was the key effect of the conditioning stimulus that combined NE and ON stimulation, which resulted in long-term enhancement of synchronized gamma oscillations. As will be described in the following text, the basic design of the experiments to establish the link between NE-induced disinhibition and long-term effects was to test whether specific drugs mimic NE’s effect in inducing long-term changes, but, as a first step toward this analysis, we wanted to explore altering the ON stimulation component of the conditioning stimulus. The Gire and Schoppa (2008) study found that NE plus ON stimulation led to significant long-term enhancement of the gamma oscillations (96 ± 38% increase), yet the effects were somewhat variable, with large (greater than twofold) increases in gamma oscillations being observed in 3 of 10 LFP recordings.

As an alternative to ON stimulation, we used bath application of a low-to-moderate concentration of NMDA (12.5 $\mu$M) as part of the conditioning stimulus. It has previously been shown that NMDA can elicit slow synchronized depolarizations in MCs that are qualitatively similar to those resulting from electrical ON stimulation (Schoppa and Westbrook 2001). We reasoned that the NMDA-driven depolarizations occur across broader regions of the bulb, and thus should provide a stronger conditioning stimulus. In addition, it has been shown that NMDA infusions into the olfactory bulb in vivo can enhance odor discrimination in a manner similar to prior odor exposure (Mandairon et al. 2006). Figure 5A shows representative LFP records measured in the EPL in response to NMDA (band-pass filtered between 0.5 and 100 Hz), showing the NMDA-evoked slow oscillatory LFP (1–2 Hz) that corresponds to the slow phasic depolarizations seen in published...
Evidence that NE-induced disinhibition drives long-term enhancement of gamma oscillations

Besides causing disinhibition at GC-to-MC synapses, acute exposure of the bulb to NE can cause direct excitation of MCs via $\alpha_1$ AR-mediated closure of potassium channels (Hayar et al. 2001) as well as specific changes in glomeruli that are mediated by $\beta$ ARs (enhanced excitatory transmission from ON terminals and depression of juxtaglomerular cell inhibition) (Yuan 2009; Yuan et al. 2000). To address whether disinhibition at GC-to-MC synapses accounts for the NE-induced long-term enhancement of the synchronized oscillations, we first examined whether the $\alpha_2$ AR-specific agonist clonidine, which causes disinhibition, could mimic NE in causing the long-term effect. We indeed found this to be the case. Clonidine (5 $\mu$M), when combined with NMDA as a conditioning stimulus, had somewhat variable effects on the oscillations, but across 23 experiments, the drug caused a significant long-term increase in the oscillation power (Fig. 6, A–C; $105 \pm 47\%$ increase in integrated power between 30 and 70 Hz, $P = 0.030$; measured 40–60 min after NE washout) with 8 of 23 showing a greater than twofold increase in LFP power. In contrast, neither the $\alpha_1$ AR-specific agonist PE ($20 \mu$M; 4 ± 2% increase in LFP power, $n = 4$, $P = 0.10$) nor the $\beta$ AR-specific agonist Isop ($100 \mu$M; 0 ± 17% change, $n = 5$, $P = 0.98$), when applied in combination with NMDA, altered the oscillations nor did the combination of PE, Isop, and NMDA ($25 \pm 18\%$ decrease, $n = 4$, $P = 0.25$). The average effect of a clonidine-plus-NMDA conditioning stimulus on the oscillatory power in our experiments (105% enhancement) was somewhat less than that of NE-plus-NMDA (196% enhancement); this might suggest that $\alpha_1$ or $\beta$ ARs could facilitate $\alpha_2$ ARs in mediating the NE-induced enhancement in the oscillations. However, the difference in the clonidine and NE-effects was not statistically significant ($P = 0.33$). In addition, we found that NE, when co-applied with the $\alpha_2$ AR-specific antagonist yohimbine ($20 \mu$M) and NMDA, failed to induce any long-term increase in the synchronized oscillations ($27 \pm 8\%$ decrease in integrated power between 30–70 Hz, $n = 7$, $P = 0.018$), further supporting a dominant role of $\alpha_2$ AR-mediated disinhibition for enhancement of synchrony. The small decrease in the oscillatory power following the conditioning stimulus composed of NE, NMDA, and yohimbine suggests that ARs other than the $\alpha_2$ subtype may depress synchrony, though this effect was quite weak.

A second prediction of a mechanism in which NE-induced disinhibition mediates long-term enhancement of the gamma oscillations is that direct pharmacological blockade of inhibitory synapses should mimic the effect of NE. To examine this possibility, we tested the effect of the GABA$_A$ receptor antagonist gabazine, using concentrations (2–5 $\mu$M) that were high enough to block the large majority of the receptors (95% reduction in IPSC amplitude; see Fig. 1A and preceding text), but sufficiently low that the drug could be washed out completely after application of the conditioning stimulus. Washout of gabazine was important because the drug would be expected to cause direct reduction of the gamma oscillations, which depend on GABA$_A$ receptor activation for their inhibitory phase (Lledo et al. 2005). We found that a conditioning stimulus that included gabazine...
(2–5 μM) along with NMDA (12.5 μM) was quite effective in eliciting a long-term enhancement of gamma oscillations (Fig. 7, A and B; 105 ± 36% increase in integrated power between 30 and 70 Hz measured 20–30 min after gabazine wash-out, n = 7, P = 0.028). This result, showing that direct block of inhibition can enhance the oscillations, supports the hypothesis that NE works via disinhibition in inducing long-term changes.

**DISCUSSION**

NE has been shown to be involved in olfactory learning through actions in the main olfactory bulb. In this study, we examined both acute effects of NE on dendrodendritic synapses in the bulb and also long-term circuit-level changes that could underlie olfactory learning.

Acute effects of NE at dendrodendritic synapses

In terms of acute actions, we found that NE caused a reduction in GABA release from GCs onto MCs at dendrodendritic synapses through activation of α2 ARs. This conclusion was based on the fact that both NE and the α2 AR-specific agonist clonidine reduced GC-mediated IPSCs; moreover, the α2 AR-specific antagonist eliminated NE’s acute effect. This effect, α2 AR-mediated reduction in GC-to-MC transmission, fits with long-standing results showing NE-induced disinhibition of MCs (Jahr and Nicoll 1982; Wilson and Leon 1988), although these prior studies did not determine whether the reduced inhibition results from direct effects on GC-to-MC transmission or is a secondary result of reduced MC-to-GC excitatory transmission. We found that NE’s effects at reciprocal dendrodendritic synapses are specific to GC-to-MC trans-
mission (but see Trombley and Shepherd 1992). In our studies, we focused on NE actions at a 20-μM concentration; this was done to fit with our prior results that showed lasting local cellular changes due to NE at that concentration (Gire and Schoppa 2008; see following text). The actual concentration of NE at the site of ARs under physiological conditions is not known. Microdialysis studies have indicated that the bulk concentration of NE in the bulb after stimulation of the locus coeruleus is quite low, just below ~1 nM (El-Etri et al. 1999), but the local concentration of NE at AR sites at dendrodendritic synapses could of course be much higher.

The most likely mechanistic explanation for the α₂ AR-mediated reduction in GC-to-MC transmission is decreased calcium channel activity in GCs. In cultured bulb GCs, it has been shown that NE can reduce calcium channel activity (Trombley 1992), while α₂ ARs mediate reduced calcium currents in many other neuron types (Boehm and Huck 1991; Czesnik et al. 2001; Gollasch et al. 1991; Schofield 1990; Timmons et al. 2004; Trombley 1992). In addition, we found in our studies that neither the amplitude nor frequency of mIPSCs was decreased by NE nor clonidine (Figs. 2 and 3), which would argue against alternate mechanisms of reduced GC-MC transmission, including postsynaptic actions of NE on GABA receptors on MCs or presynaptic effects on vesicle release machinery. Also clonidine did not affect other intrinsic membrane properties of GCs in a manner that could account for decreased GABAergic transmission (2.0 ± 0.5 mV depolarization in resting potential, n = 3, P = 0.042; 8 ± 5% increase in input resistance, n = 3, P = 0.25; 2.4 ± 2.0 mV more depolarized action potential threshold, n = 3, P = 0.29).

There are a few differences in the results we obtained with respect to acute AR-mediated effects on spontaneous mIPSCs as compared with those reported in a recent study by Nai and co-workers (2009). First, they found that the dominant effect of the broad-spectrum agonist NE at a 20-μM concentration was enhanced mIPSC frequency, whereas we often found no effect of NE on mIPSCs. This difference appeared to reflect an animal-age issue, as an NE-induced enhancement of mIPSCs was observed in a subset of our experiments done in slices from older animals similar in age to those used by Nai and co-workers. An additional difference was the action of the α₂ AR-specific antagonist yohimbine (20 μM).

**Link between NE-induced disinhibition and long-term effects on bulb function**

In addition to showing that NE can cause acute disinhibition at dendrodendritic synapses, this study also provided evidence that linked the NE-induced disinhibition to long-term increases in synchronized gamma frequency oscillations (Gire and Schoppa 2008). The link was made based on the fact that specific activation of the α₂-AR subclass, which mediated disinhibition, was able to mimic the effect of NE in causing long-term enhancement of the oscillations. Also blocking α₂-ARs prevented NE from inducing the long-term enhancement.
disinhibition-induced rises in intracellular calcium may have a priming effect on LTP.

Among the issues to be resolved in future studies are the specific steps in the biochemical path that lead to the long-lasting enhancement of the synchronized oscillations, especially the role of calcium. The fact that a strong depolarization in the MC network appears to be required for the long-lasting changes suggests that depolarization-induced calcium rises may be important, happening either in the MCs themselves or in GCs that are excited by MCs. The source of the calcium could be voltage-gated calcium channels or NMDA receptors activated by depolarization-induced removal of magnesium-blockade. Another mechanistic issue is which specific synapse(s) are undergoing the long-term modifications, leading to changes in the gamma oscillations. The relevant synapses could be the MC-GC dendrodendritic synapses that underlie the gamma oscillations (Lledo et al. 2005) or, alternatively, mechanisms that affect neuronal excitability that would secondarily impact activity at MC-GC synapses (Gire and Schoppa 2008).

**Relationship to olfactory learning**

From a functional perspective, an obvious comparison to be made is between our cellular results and AR-dependent forms of olfactory learning in which rodents learn to prefer, or discriminate better, odors with which they associate a reward (Doucette et al. 2007; McLean and Harley 2004; Sullivan et al. 1989, 2000). Our results could in principle provide a cellular basis for learning, since an increase in gamma frequency synchronized oscillations might increase the synaptic weights of specific odor signals in the cortex (Beshel et al. 2007). The key cellular event that underlies olfactory learning may also not be enhanced synchrony per se, but another change in the bulb for which the gamma oscillations provide a read-out. These could include enhancement in network excitation or inhibition (Coopersmith and Leon 1984; Johnson and Leon 1996; Okutani et al. 1998, 1999; Shea et al. 2008; Wilson et al. 1987), either of which could be associated with increased gamma oscillations.

Is there any evidence that could link the cellular changes observed in our study to behavioral changes? One of our findings, suggesting a link, is that NE and clonidine both enhanced gamma oscillations only after the drugs had been washed out (see time plots in Figs. 5E and 6C). Thus α2 ARs appear to be involved in the induction but not maintenance of long-term changes in gamma oscillations. This characteristic is similar to associative olfactory learning, where NE appears to be involved in the acquisition, but not expression, of learned odor preference (Sullivan and Wilson 1991). In addition, there are interesting cellular/behavioral comparisons to be made with respect to animal age. At least certain types of associative learning appear to be most pronounced during a “sensitive period” in newborn rats (Moriceau and Sullivan 2004; Woo and Leon 1987), which fits at least roughly with our data suggesting that NE has disinhibitory acute effects of the type required for long-lasting cellular changes mainly in younger animals. In older animals, acute increases in inhibition due to NE during a conditioning period (see Fig. 2D and (Nai and co-workers 2009)) may prevent learning. There are some mismatches in our cellular effects as compared with reported behavioral ef-

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**FIG. 7.** Direct pharmacological block of GABA<sub>A</sub> receptors leads to long-term enhancement of gamma oscillations. A: consecutive traces of LFP recordings of responses to ON stimulation, made under control conditions and 30 min following washout of a conditioning stimulus comprised of gabazine (GBZ; 5 µM) and NMDA (12.5 µM). Note the large increase in the oscillatory signal following removal of the conditioning stimulus, also shown in the boxed insets at right. Data were filtered at 10–100 Hz. B: time plot of the experiment in A, showing a long-term increase in the integrated power of gamma oscillations between 30 and 70 Hz due to the GBZ-plus-NMDA conditioning stimulus. Note also that, just following the conditioning stimulus, there was a moderate decrease in oscillations that preceded the enhancement (66% decrease during minutes 30–32 vs. control period). This decrease was presumably due to residual GBZ prior to washout. In this experiment, the first 50 ms of data following each ON stimulus burst was ignored in the analysis to avoid a lower-frequency component of the response (see traces in A).

In addition, pharmacological block of GABA<sub>A</sub> receptors to reduce inhibition directly was also able to mimic NE’s effects.

Importantly, we found that NE-induced disinhibition by itself, without concurrent neural stimulation (done here by application of a low concentration of NMDA), was not sufficient for the long-term enhancement of the gamma oscillations (Fig. 5F). This requirement for both disinhibition and neuronal stimulation as a conditioning stimulus leads us to propose a mechanism for long-term changes that depends, most critically, on the generation of a strong depolarization in the MC network. Under natural conditions, the impetus for the strong depolarization would be provided by an odor, which would depolarize a select group of MCs to some extent, but it is only when there is co-occurring disinhibition (resulting from LC activation) that the depolarization would be large enough to induce long-lasting changes. Such a scenario, in which disinhibition acts as a facilitator of long-term cellular changes, has been reported for long-term potentiation (LTP) at hippocampal CA1 synapses (Hsu et al. 1999). In that system,
fects. For example, the age range of our younger rats (P9-13) was slightly older than the newborn rats (<P10) in which associative learning appears to be most pronounced. Also the evidence is strongest for a function for β ARs in associative olfactory learning in newborn rats (Sullivan et al. 1989), whereas α2 ARs were most important in our studies. Resolution to these issues will require additional studies that more carefully examine the role of different AR subtype in rodents of varying age. Cellular studies could reveal a stronger role for β ARs at specific synapses in very young animals (<P10) (Yuan et al. 2000), for example, while additional behavioral studies that focus on α2 ARs could reveal novel roles for these receptors in olfactory learning in young rodents.

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