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Long-Term Enhancement of Synchronized Oscillations by Adrenergic Receptor Activation in the Olfactory Bulb

David H. Gire and Nathan E. Schoppa
Department of Physiology and Biophysics, and Neuroscience Program, University of Colorado at Denver and Health Sciences Center, Denver, Colorado

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Gire DH, Schoppa NE. Long-term enhancement of synchronized oscillations by adrenergic receptor activation in the olfactory bulb. J Neurophysiol 99: 2021–2025, 2008. First published February 6, 2008; doi:10.1152/jn.01324.2007. The noradrenergic system is widely thought to be important for associative learning in the olfactory system through actions in the first processing structure, the main olfactory bulb (MOB). Here, we used extracellular local field potential (LFP) and patch-clamp recordings in rat MOB slices to examine norepinephrine (NE)-induced long-term changes in circuit properties that might underlie learning. During responses to patterned olfactory nerve stimulation mimicking the breathing cycle, NE induced a long-term increase in gamma frequency (30–70 Hz) synchronized oscillations. The enhancement persisted long after washout of NE (≈ 70 min), dependent on the combined actions of NE and neuronal oscillations. The last effect, increased excitation, was manifested as an increased in evoked long-lasting depolarizations (LLDs) in mitral cells. From a functional perspective, the observed long-term cellular and network changes could promote associative learning by amplifying odor-specific signals.

INTRODUCTION

The noradrenergic system is believed to be involved in various forms of learning in both the main olfactory and vomeronasal systems (reviewed in Brennan and Kendrick 2006; Wilson and Sullivan 1994). Much of the action of norepinephrine (NE) in the main olfactory system seems to be in the main olfactory bulb (MOB). The MOB receives massive projections from the noradrenergic nucleus and the locus coeruleus, and behavioral experiments have shown that adrenergic receptor (AR) agonists and antagonists infused into the MOB can profoundly impact odor preference learning (Doucette et al. 2007; Sullivan et al. 1989). Mechanistic studies have identified several “acute” short-term effects that NE can have in the MOB (Hayar et al. 2001; Jahr and Nicoll 1982; Jiang et al. 1996; Trombley and Shepherd 1992; Wilson and Leon 1988; Yuan et al. 2000), yet the long-term cellular and network changes in the MOB that might underlie NE-mediated learning have not been fully resolved. Here, a combination of extracellular local field potential (LFP) and whole cell patch-clamp recordings were used to examine NE-induced long-term changes in MOB slices from rat neonates. We focused our analysis on two temporally distinct phenomena that dominate MOB responses under both in vivo and in vitro conditions: 1) fast gamma frequency (30–70 Hz) synchronized oscillations (Adrian 1950; Friedman and Strowbridge 2003; Kashiwadani et al. 1999; Lagier et al. 2004), which are widely believed to be critical for odor discrimination (Lledo et al. 2005), and 2) long-lasting depolarizations in output mitral cells (MCs) that are locked to afferent stimuli (Cang and Isaacson 2003; Carlsson et al. 2000; Margrie and Schaefer 2003; Schoppa and Westbrook 2001). These depolarizations, called long-lasting depolarizations (LLDs), bring MCs from their resting potential (approximately −55 mV) to near spike threshold (approximately −45 mV), and thus control the output of the MOB.

METHODS

Horizontal slices (300–400 μm) were prepared from olfactory bulbs of 9- to 14-day-old Sprague-Dawley rats. Experiments were done at 31–34°C. All experiments were approved by the Institutional Animal Care and Use Committee at UCDHSC. 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). LFPs and extracellular spike discharges were recorded using micropipettes filled with extracellular solution (resistance, ~ 5 MΩ). For voltage-clamp recordings of LLD currents, the intracellular pipette solution contained (in mM) 125 Kgluconate, 2 MgCl2, 0.025 CaCl2, 1 EGTA, 2 NaATP, 0.5 NaGTP, and 10 HEPES (pH = 7.3 with KOH). For recordings of IPSCs, Kgluconate was replaced with KCl. For all voltage-clamp recordings, a quite hyperpolarized holding potential (−77 mV) was used to ensure that MCs did not spike during test responses.

For electrical stimulation, a glass patch pipette filled with the extracellular solution (0.5–2 MΩ) was placed in the olfactory nerve (ON) layer. Our standard 4-Hz patterned stimulus consisted of five stimulus bursts (three 100-μs pulses each separated by 10 ms), with 250 ms separating the first pulse in each burst (hence, 4 Hz). This pattern was applied once every 15 s. Recordings were made with a Multi-Clamp 700B patch-clamp (Molecular Devices, Sunnyvale, CA) and were filtered at 0.25–5 kHz using an eight-pole Bessel filter. LFP traces underwent a supplementary filtering off-line (8-pole band-pass Butterworth filter) before further analysis. Spike detection during unit discharges was accomplished by counting events crossing a threshold adapted to each recording (Lagier et al. 2004).

For most recordings, the extracellular solution also included the dopamine (D2) receptor blocker sulpiride (100–200 μM) throughout the experiment to account for possible D2 receptor–mediated inhibitory effects of NE on ON-to-MC transmission. This effect was first reported by Hayar et al. (2001) based on the observation that sulpiride blocked an inhibitory effect of NE on fast glomerular field excitatory postsynaptic potentials (EPSPs) that was not replicated by AR sub-

Address for reprint requests and other correspondence: N. Schoppa, Dept. of Physiology and Biophysics, UCDHSC at Fitzsimons, Mail Stop 8307, PO Box 6511, Aurora, CO 80045 (E-mail: Nathan.Schoppa@UCHSC.edu).

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type-specific agonists. In our hands, sulpiride effects on preventing inhibition of glomerular synaptic transmission seemed to be incomplete, since in eight MC recordings done in the presence of sulpiride, NE still caused a modest (≥13%) acute reduction in evoked LLDs in all cells. Part of this difference might be explained by differences in the particular glomerular synaptic phenomena assayed (LLDs vs. ON-to-MC synaptic transmission). Consistent with the findings of Hayar and co-workers, we did find, in two recordings, that AR subtype-specific agonists (see RESULTS) failed to mimic the effect of NE in acute toxicity reducing LLDs (1 and 5% decreases). This suggests that NE has effects on glomerular synaptic transmission independent of ARs.

Our estimate of NE washout times, 6–7 min, required for interpreting long-term effects of NE, was obtained from separate experiments in which we assessed acute effects of NE on MC inhibitory postsynaptic currents (IPSCs) evoked by stimulation in the granule cell layer. In these studies, NE caused a reduction in the IPSC amplitude (n = 5), from which full recovery took 6–7 min.

Statistical significance was determined via Student’s t-test. Data values are reported as means ± SE.

RESULTS

NE elicits long-term increase in gamma LFP oscillations

For studying gamma oscillations, we stimulated the ON (100–500 µA) with a 4-Hz pattern (see METHODS and cartoon trace in Fig. 1A for details) that was applied once every 15 s. This stimulus, designed to mimic the breathing cycle, has been shown (Schoppa 2006) to be effective in reproducing specific features of odor-evoked responses, including rapid synchronized spiking and stimulus-locked LLDs in MCs (Cang and Isaacson 2003; Kashiwadani et al. 1999; Margrie and Schaefer 2003). Neuronal population activity was monitored from LFP signals (band-pass filtered between 10 and 100 Hz) in the external plexiform layer (EPL) within 50 µm of the mitral cell layer (MCL). The LFP induced by the 4-Hz stimulus showed prominent gamma frequency activity (Fig. 1B; peaks in power spectra between 35 and 45 Hz in 10 recordings), as seen previously in responses to single ON shocks (Friedman and Strowbridge 2003; Lagier et al. 2004).

To examine the effect of NE on gamma oscillations, NE (20 µM) was added to the bath while we continued to apply the 4-Hz stimulus pattern every 15 s. Under these conditions, NE caused a variable but significant increase in gamma oscillations (Fig. 1, A–D; 96 ± 38% increase in integrated power between 30 and 70 Hz, n = 10, P = 0.026; 5- to 20-min test period always recorded ≥25 min following start of NE washout), with an effect that persisted for the duration of the recordings (between 30 and 70 min after washout of NE). The increase in gamma activity was not an acute effect of NE, because the increase in power appeared only after NE had been washed out (Fig. 1C; time estimated to be 6–7 min; see METHODS). Also, extended application of NE for ≥25 min did not produce a long-term enhancement of gamma oscillations as long as NE remained in the bath (4 ± 34% decrease in integrated power, n = 4). The requirement for washout of NE to see LFP enhancement may have been in part caused by modest NE
induced inhibitory effects on ON-to-MC transmission (Hayar et al. 2001; see METHODS). We had some concern that the delayed effects of NE could have been caused by a metabolic breakdown product of NE. However, a similar increase in gamma oscillations (137 ± 53% increase, n = 9, P = 0.030; Fig. 1D) was observed when we substituted NE with a cocktail that included the α-1 AR-agonist phenylephrine (10–20 μM), the α-2 AR-agonist clonidine (3–6 μM), and the β AR-agonist isoproterenol (10–20 μM). Thus the NE-induced enhancement of gamma activity was a specific result of AR activation.

At excitatory synapses within the hippocampus, NE has long been known to facilitate long-term potentiation (LTP) that can occur by electrical stimulation alone (in CA3; Hopkins and Johnston 1984). In the bulb, however, long-term enhancement of gamma oscillations depended completely on a conditioning stimulus that included both NE and electrical stimulation (Fig. 1, C and D). NE applied in the absence of stimulation caused no change in gamma activity (2 ± 24% increase, n = 6; at 25–40 min following washout of NE), nor did long-term electrical stimulation without NE (8 ± 15% increase, n = 7; test period recorded 20–30 min following start of experiment). The reliance of enhanced gamma activity on both electrical stimulation and NE has implications for understanding “acute” actions and functional consequences of NE (see DISCUSSION).

**Long-term cellular events underlying enhanced gamma oscillations**

Mechanistic studies in bulb slices have shown that gamma oscillations in the MOB reflect the rapid back-and-forth synaptic interplay between glutamatergic MCs and GABAergic granule cells (GCs) (Friedman and Strowbridge 2003; Lagier et al. 2004; Schoppa 2006). Importantly, the trigger for this interplay is the prominent LLD in MCs, which controls spike activity in MCs and thus most secondary phenomena in MOB, including gamma oscillations. Thus the enhanced gamma oscillations could represent direct long-term effects of NE on the rapid glutamatergic/GABAergic transmission steps that define each cycle of the oscillation or be the indirect result of enhanced LLDs in MCs. One clue that the enhanced gamma oscillations were at least partly caused by NE effects on LLDs was suggested by the above-mentioned LFP signals, filtered at higher band-pass (300–1,000 Hz; Fig. 2A). Under these conditions, bursts of fast multiunit discharges were evident (see also Lagier et al. 2004), which increased following application and washout of NE (115 ± 30% increase in number of events per unit time, n = 7, P < 0.01). Because the recordings were made near the MC layer, the higher frequency discharge bursts most likely represent LLD-induced MC spiking.

To test more directly whether NE induces long-term enhancement of LLDs, we performed whole cell voltage-clamp recordings of the underlying currents in MCs (Carlson et al. 2000; holding potential = −77 mV). Figure 2B shows examples of MC current responses elicited by a 4-Hz stimulus pattern applied every 15 s. The second control trace shows a characteristic LLD current response, with some phasing with respect to the stimulus, although most control responses were small at the relatively weak stimulus intensity that was used (50 μA). Co-application of NE (20 μM) and an electrical stimulus (≥100 μA) caused significant enhancement of the LLD currents (Fig. 2, B–D; 135 ± 40% increase in charge integral, n = 8, P < 0.01; test period recorded 28–64 min following washout of NE). As in the case of the gamma oscillations, NE was not facilitating enhancement occurring with ON stimulation alone, since when ON stimulation was done in the absence of NE, LLDs did not increase (21 ± 13% decrease, n = 3, P = 0.16; Fig. 2D). The long-term effects of NE on LLDs occurred without effects on MC resting potential recorded in current clamp (n = 8) or spike threshold (n = 8). Thus NE did not alter baseline excitability of MCs, and, instead, seems to have specific effects on one of the complex series of mechanisms that shape evoked LLDs (see DISCUSSION). Voltage-clamp recordings from MCs (n = 6) also showed that NE did not induce a long-term increase in inhibitory postsynaptic currents (IPSCs) in MCs (Fig. 2E; 33 ± 9% decrease in IPSC amplitude, P = 0.031; 48 ± 15% decrease in IPSC frequency, P = 0.053). The absence of an increase in IPSCs provides evidence against the possibility that the enhanced gamma activity was caused by a direct increase in rapid GC-to-MC GABAergic transmission onto single MCs.

**DISCUSSION**

**Long-term and acute effects of NE**

In this study, we identified two major long-term effects of NE: 1) an increase in gamma oscillations and 2) an increase in LLDs in MCs. We propose that the two effects are closely linked, with the primary long-term effect being the enhanced LLDs, which, secondarily, increases gamma oscillations by increasing the drive on the MC/GC network that underlies gamma activity. The evidence for a link at this point is correlative—both effects happened with similar time-courses and depended on co-application of neuronal stimulation and NE—but there are a number of mechanisms by which the enhanced LLD would naturally lead to enhanced gamma oscillations; for example, by increasing the number of MCs and GCs participating in the oscillations. Although our results show quite clear NE-mediated long-term effects on gamma oscillations and LLDs, one unresolved issue is exactly what cellular event underlies the enhanced LLD. LLDs are complex phenomena that are initiated by synaptic transmission from olfactory receptor neurons (ORNs) to MCs and tufted cells (TCs) and are further shaped by the balance between regenerative excitatory mechanisms (e.g., autoexcitation in MCs) and inhibition (Carlson et al. 2000; Christie and Westbrook 2006; Schoppa and Westbrook 2001). We performed preliminary studies that would seem to exclude long-term changes in ON-to-MC/TC transmission as a cause of enhanced LLDs. NE plus electrical stimulation did not induce a long-term increase in the early fast component of the glomerular field EPSP evoked by ON stimulation (20 μA; 26 ± 13% decrease, n = 3, P = 0.18), which is generally taken to reflect ON-to-MC/TC transmission (Aroniadou-Anderjaska et al. 1997).

In addition to NE having specific long-term effects, NE seems to have quite distinct “acute” effects in MOB. Indeed, in our studies, the long-term enhancement of gamma activity did not appear until NE had been washed out (Fig. 1C). Instead, we propose that NE has acute actions that “permit” long-term effects. A good candidate for such an acute permissive effect is NE-induced disinhibition of MCs, which has been widely reported (Jahr and Nicoll 1982; Trombley and Shepherd 1992;
Wilson and Leon 1988). Acute disinhibition, thought to be caused by a reduction in GC-to-MC GABAergic transmission, would also fit well with our data showing that NE long-term effects needed both NE and electrical stimulation. If one assumes a model in which long-term cellular/network changes require a rise in intracellular calcium, sufficient calcium levels might only be achieved if there is both electrical stimulation to drive cellular depolarization, as well as NE-mediated disinhibition to enhance depolarization.

**Functional implications**

How do the long-term synaptic effects that we observed in vitro in MOB relate to functional studies in vivo? In terms of physiological experiments, the most direct link may be between the enhanced LLD and numerous in vivo studies showing learning-associated increases in neural activity within glomeruli (Coopersmith and Leon 1984; Yu et al. 2004; Yuan et al. 2002). LLDs are glomerular in origin and also synchronized in a glomerulus (Carlson et al. 2000; Schoppa and Westbrook 2001); thus their enhancement would naturally lead to enhanced glomerular activity. Ravel et al. (2003) examined training-induced changes in oscillations in MOB, finding reduced activity in the high gamma range (60–90 Hz) but increased “beta” activity (15–30 Hz). Comparisons with our results are made difficult by their different definitions of gamma versus beta frequencies versus ours (gamma = 30–70 Hz) and also because our preparation did not include the olfactory cortex, which has been reported to be the source of beta activity (Martin et al. 2004). It might be noted, however, that, in an intact animal, the enhanced LLDs that we observed in MCs could translate to enhanced centrifugal excitation and hence more beta activity, as seen in vivo.

At a behavioral level, it has long been known that NE is involved in mediating associative learning processes wherein an animal learns to recognize odors to which it has been conditioned (Wilson and Sullivan 1994). Many of these studies...
have been done in rat neonates. The long-term enhancement of synchronized gamma oscillations that we found in MOB slices from rat neonates could promote such learning by increasing the postsynaptic weight of signals coming from a conditioned odor (through summation of EPSPs). It is equally plausible that the key long-term cellular modification for learning is the enhanced LLD, which could underlie increased responsiveness of odor-specific MCs. Regardless of the mechanism, the fact that NE effects in our studies depended on the combined application of NE and electrical stimulation is likely to be critical, since such “use dependence” of NE would mean that learning-associated changes would be specific to a conditioned odor.

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