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Neural activity in the horizontal limb of the diagonal band of Broca can be modulated by electrical stimulation of the olfactory bulb and cortex in rats

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Abstract

Previously published theoretical models of olfactory processing suggest that cholinergic modulatory inputs to the olfactory system should be regulated by neural activity in the olfactory bulb. We tested these predictions using in vivo electrophysiology in rats. We show that the activity of approximately 20% of neurons recorded in the horizontal limb of the diagonal band of Broca (HDB), which is the source of cholinergic projections to the olfactory system, can be modulated by electrical stimulation of either the lateral olfactory tract or the cell body layer of piriform cortex. These data suggest a possible physiological pathway for the proposed regulation of neural activity in the HDB by activity in the olfactory bulb or cortex.

Keywords: Olfactory bulb; Olfactory cortex; Horizontal limb of the diagonal band of Broca; Cholinergic modulation; Electrical stimulation; Units recordings

Understanding the role of neuromodulators for cortical function requires study of their effects at the level of neural activity as well as at the level of behavioral responses. We have recently proposed theoretical models of the modulatory effects of acetylcholine on olfactory processing, which have been shown to play a role in olfactory behavior. In particular, in rats, the muscarinic antagonist scopolamine has been shown to impair short-term memory for odors [18], habituation to familiar odors and odor-based social recognition [8,22], as well as to impair the acquisition of a complex odor discrimination task involving overlapping pairs of odor stimuli [5]. Lesions of the cholinergic and GABAergic basal forebrain neurons that project to the olfactory system have also been shown to influence olfactory habituation and investigation [14,15] as well as odor discrimination [20], and we have recently shown that specifically lesioning only cholinergic neurons projecting to the olfactory system increases generalization between similar odorants in rats [6].

Both the olfactory bulb (OB) and piriform cortex (PC) receive cholinergic and GABAergic input from the horizontal limb of the diagonal band of Broca (HDB), a basal forebrain structure [23]. The HDB contains both cholinergic and GABAergic neurons, and it is known that these two populations are at least partially spatially segregated [2]. Our computational models of the olfactory bulb and cortex have suggested how modulatory inputs from the HDB onto neuronal circuits could influence the processing and associative storage of olfactory stimuli [1,10,11]. Specifically, we proposed a general model of the OB by which cholinergic modulation of inhibitory processes could sharpen the responses of model cells to odor stimuli [10], a prediction supported by recordings from OB output neurons (mitral cells), local interneurons in the glomerular layer, and granule cells [9,13,17]. An important feature of this computational model is that the neural activity of neurons in the OB regulates the activity of the cholinergic neurons that project back to the OB. This feedback regulation ensures that the levels of cholinergic modulation are adaptive in response to a wide variety of odor stimuli at varying concentrations [11]. For example, concentration-independent recognition of odor quality, measured as the population response spectra of olfactory bulb output neurons, can be achieved with such a feedback regulation system. In this report, we investigate whether a physiological pathway exists that could support the regulation of neural activity in the HBD based on activity levels in the olfactory bulb.

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To that purpose, we studied the effects of electrical stimulation of olfactory bulb output fibers in the lateral olfactory tract (LOT) and of the cell body layer of the PC on the spontaneous activity of neurons in the medial (highly cholinergic) part of the HDB. We observed that the activity of approximately 20% of the neurons recorded in the HDB was modulated by electrical stimulation of these olfactory structures.

Adult, male Sprague–Dawley rats (300–400 g) were anesthetized with urethane (1.5 g/kg, i.p.). Levels of anesthesia were monitored and body temperature was maintained at 37°C. Anesthetized animals were placed in a stereotaxic apparatus and holes were drilled in the skull to allow access to the LOT, PC and HDB. Bipolar stimulation electrodes (100 μm stainless steel, Formvar-insulated) were placed in the LOT and in the PC. The PC stimulation electrode was positioned around the point of reversal of the LOT evoked potential, which corresponds to layer II (the cell body layer); to achieve this we recorded from the stimulation electrode during the placement while stimulating the LOT. For the recording of units in the HDB, a recording electrode (25 μm stainless steel, Formvar insulated) was placed slightly dorsal to the coordinates for the HDB (0.0–0.5 mm anterior-posterior, 1.6–2.0 mm medial-lateral and 8.6 mm dorsal-ventral) and then slowly advanced until a unit could be isolated. AC preamplification (Grass Instruments, 10×) was used in all recordings; spike waveforms (4 ms around the trough) were extracted from signals sampled at 30 kHz, bandpass filtered from 600–9 kHz and amplified (2000×) using Neuralynx Lynx-8 amplifiers. Data were recorded using Datawave Experimenters' Workbench. The spike waveforms were analyzed offline to ensure that that they represented the action potentials of single units. Records were checked for a clear refractory period (2 ms) and spike clustering (using Datawave spike sorting software for Workbench) was used to separate stimulation and other artifacts from the recorded action potentials. At the end of the experiment, positive current was passed through the electrodes (5 s via a 9 V battery) to deposit iron in the tissue. Transcardial perfusion was then performed with saline and ferrocyanide in 10% formalin, allowing a Prussian blue reaction. Brains were sectioned at 40 μm and subsequently stained with neutral red for electrode localization. Only animals in which the recording electrodes were clearly located in the medial part of the HDB were included in the analysis.

After isolation of a unit recording in the HDB (Fig. 1A), we studied the modulation of the spontaneous activity of that unit by constructing peristimulus-time-histograms (PSTHs) around the time of electrical stimulation. For a given trial, spontaneous activity was recorded for 500 ms (baseline). After 500 ms, a brief (0.1 ms, 300 μA) pulse was delivered with the stimulation electrode in the LOT or PC. The activity of the HDB units was recorded for 1000 ms after the electrical stimulation. For each cell, 100 such trials were repeated at 5 s intervals and PSTHs were constructed

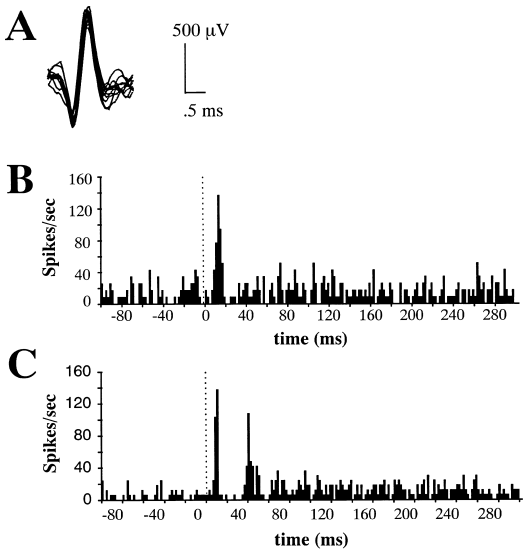

Fig. 1. Effect of electrical stimulation of the LOT on neural activity in the HDB. (A) Superimposed traces of a unit recording in the HDB. B: Peristimulus time histogram of the effect of electrical stimulation (300 μA, 0.1 ms) of the LOT on the spiking activity of an individual neuron recorded in the HDB. The neuron shown in this graph responded to the electrical stimulation with a single increase in spiking. The histogram shows the summed numbers of action potentials (bin size = 2 ms) recorded during 100 successive sweeps. We show only 100 ms before the stimulation and 300 ms after the stimulation. Stimulation occurred at time = 0 and is indicated by the dotted line. (B) The neuron shown in this graph responded to the electrical stimulation with two periods of increased spike rate. The histogram shows the summed numbers of action potentials (bin size 2 ms) recorded during 100 successive sweeps. Stimulation occurred at time = 0 and is indicated by the dotted line.

with a bin size of 2 ms. Means and standard deviations were calculated for the baseline. For analysis purposes, a change from baseline after the electrical stimulation was considered significant when the activity in at least two successive 2 ms bins deviated by more than two standard deviations from this baseline. Both increases (variations above baseline) and decreases (variations below baseline) were considered. Because of the variability in the observed spontaneous activities, the amplitudes of the observed modulation will be reported as the ratio between the peak effect and the averaged baseline activity (both measured as the numbers of spikes in a 2 ms bin) in a given cell. Results are reported as mean ± SE.

We recorded a total of 60 units in 21 animals. The spontaneous activity of the recorded neurons ranged from 0.5 to 16 Hz with an average of 6.7 Hz (±1.19; $n = 60$). In 46 units we examined the effect of electrical stimulation of the lateral olfactory tract. Modulation of the spontaneous activity of HDB units in response to this electrical stimulation was observed in 11 units (24%). Each of these 11 cells responded to a single LOT stimulus with an increase in firing rate; in each case a short-latency increase peaked at 12.36 (±1.34) ms after the stimulus and lasted 7.0 (±0.63)

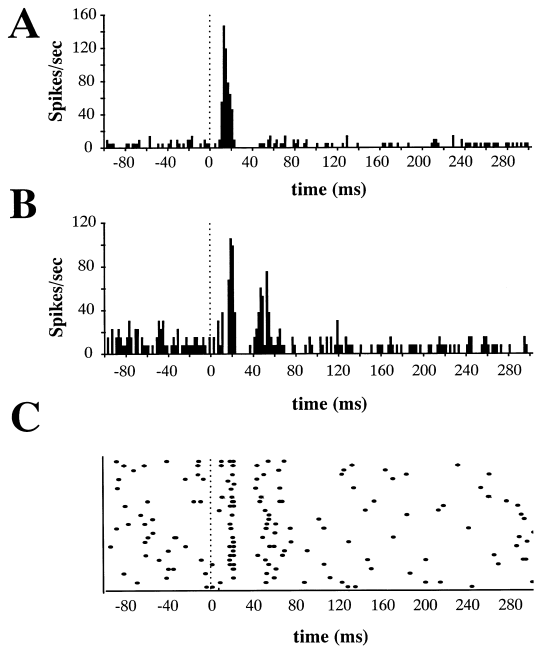

Fig. 2. Effect of electrical stimulation of the PC. (A) Peristimulus time histogram of the effect of electrical stimulation (300 μA, 0.1 ms) of the PC on the spiking activity of an individual neuron recorded in the HDB. The neuron shown in this graph responded to the electrical stimulation with a single increase in spike rate. The histogram shows the summed numbers of action potentials (bin size = 2 ms) recorded during 100 successive sweeps. Stimulation occurred at time = 0 and is indicated by the dotted line. (B) The neuron shown in this graph responded with two periods of increased spiking to the electrical stimulation. (C) Dot raster showing the occurrence of action potentials during the 30 individual trials which were part of the summed histogram shown in B. Stimulation occurred at time = 0 and is indicated by the dotted line.

ms (Fig. 1B). In one unit, we additionally observed a second, longer-latency increase in activity peaking at 40 ms and lasting 10 ms (Fig. 1C). The amplitudes of the peaks of increased activity ranged from an 8-fold to a 40-fold increase from baseline with an average of 19.0 (±4.2) times baseline.

We examined the effect of electrical stimulation of the cell body layer of PC in 42 units. The spontaneous activity of eight units (19%) was modulated by electrical stimulation of layer II of the piriform cortex. Each of these eight units showed a short-latency activation similar to that observed after stimulation of the lateral olfactory tract, with an average latency to peak of 9.14 (±1.46) ms and an average duration of 7.4 (±1.05) ms (Fig. 2A). The amplitudes of the peaks varied between a 5.2-fold increase from baseline to a 60-fold increase with an average spike rate increase of 24.2 (±7.9) times baseline. In three of these eight units (37%), a second period of increased activity, similar to that described above, was also observed, with a latency to peak of 52.6 (±4.8) ms and duration of 17.3 (±6.3) ms (Fig. 2B). Visual inspection of the PSTHs of these recordings suggests that all these cells are inhibited between the two periods of increased activation (Fig. 2B), but the change below baseline was never significant (this is probably due to the low spontaneous activity of these cells). Inspection of the individual trials of all recordings in which an average effect had been observed showed that the electrical stimulation evoked a single, or sometimes two spikes during the short latency responses in each trial, as is illustrated in Fig. 2C for a cell which responded to electrical stimulation of the PC. Fig. 3 compares the average latencies and durations of the observed periods of higher spiking (Fig. 3A,B) and the amplitudes of the peak effects (Fig. 3C,D) for the two stimulation sites (LOT and PC).

We have shown that the activity of neurons in the horizontal limb of the diagonal band of Broca can be modulated by electrical stimulation of the olfactory system. Our results suggest the existence of a physiological pathway which could support the regulation of neural activity in the HDB

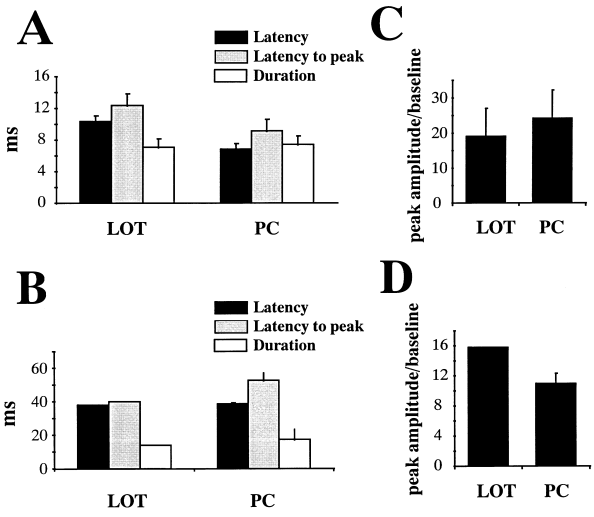

Fig. 3. Average results from all experiments. (A) Average latencies and durations (mean ± SE) of the shorter-latency modulation effect in all neurons responding to LOT stimulation (LOT, 11/46) and to PC stimulation (PC, 8/42). We here include all responding cells, both those which responded with a single change in firing and those which responded with two periods of increased firing. Latency: latency from the stimulus to the onset of the effect; latency to peak: latency from the stimulus to the peak of the effect; duration: duration of the modulation. (B) Average latencies and durations (mean ± SE) of the second, longer-latency increase in firing in all neurons in which this effect was observed (LOT stimulation (LOT, 1/46) and PC stimulation (PC, 3/42)). Latency: latency from the stimulus to the onset of the activation; latency to peak: latency from the stimulus to the peak of the activation; duration: duration of the activation. (C) Average size of the amplitude increase during the first, short amplitude response in to electrical stimulation of the LOT and PC. The graph shows the average normalized peak spike rate amplitudes (i.e. the peak of the effect was divided by the average baseline activity for each neuron). (D) Distribution of the peak amplitudes of the second, longer latency response in all four neurons in which this late activation was observed in response to LOT or PC stimulation. The graph shows the average normalized peak amplitudes for each neuron (the peak of the effect was divided by the average baseline activity).

by olfactory pathways, as predicted by our theoretical studies [11]. We only included neurons in this study in which the recording electrode was located in the medial part of the HDB, shown to contain a high density of cholinergic neurons projecting to the olfactory system [2,12,23]; while this ensures a high probability of recording from cholinergic neurons, we cannot exclude that recordings from GABAergic neurons were included in this study. Both direct and indirect pathways between the olfactory system and the HDB have been described in the literature [3,16,19,21], which could underlie the rather long latency of the effect of electrical stimulation of the LOT and PC on the neural activities recorded in the HDB. While antidromic stimulation of HDB neurons cannot be ruled out as the cause of the effects observed here, the relatively long latencies to onset (approximately 10 and 7 ms for LOT and PC stimulation, respectively; Fig. 3A) and the long duration of the increase of the firing rate (approximately 7 ms; Fig. 3A) argue against direct antidromic activation. In addition, stimulating the LOT is very unlikely to recruit incoming modulatory fibers, as the LOT contains mainly output fibers from OB mitral cells. However, in this study we cannot rule out antidromic stimulation of HDB axons which could then synaptically activate the recorded neurons within the HDB. This concern could be addressed by using electrical stimulation of the olfactory nerve as well as olfactory stimulation of the epithelium paired with unit recordings in the HDB. Similar mechanisms of feedback modulation of neural activity in modulatory neurons by their target areas have been reported in the medial septum - hippocampal complex [4]; in this system, computational models have also proposed the existence of a feedback regulation of levels of cholinergic modulation by neural activity in its target areas [7].

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