Air Movement Evokes Electro-Olfactogram Oscillations in the Olfactory Epithelium and Modulates Olfactory Processing in a Slug

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Ito, Iori, Satoshi Watanabe, and Yutaka Kirino. Air movement evokes electro-olfactogram oscillations in the olfactory epithelium and modulates olfactory processing in a slug. J Neurophysiol 96: 1939–1948, 2006. First published July 12, 2006; doi:10.1152/jn.00323.2006. In many animals, neurons in the olfactory system have been shown to respond not only to odorants but also to air movements. However, the manner in which the mechanical dynamics of odor stimulation affect olfactory processing remains poorly understood. Using a series of flow rates and odor concentrations from clean air to high-concentration vapors, we systematically analyzed the effects of air movement and odor concentration on olfactory processing. We extracellularly recorded local field potentials and spike units from the olfactory epithelium (OE) and tentacular nerve (TN), which connects the first and second relay centers of olfactory information, in the terrestrial slug Limax marginatus. We found that clean air puffs at a flow rate of 0.18 ml/s (gentle wind), but not high-concentration odor puffs at lower flow rates, induced electro-olfactogram (EOG) oscillations in the OE with a constant frequency (2.5 Hz), regardless of the odor. Surgically isolated OE preparations also showed these EOG oscillations, indicating that the oscillations arose from the OE independently of the downstream circuits. The EOG oscillations entrained the slower spontaneous TN oscillations (1–2 Hz) to the fixed rhythm (2.5 Hz). Spontaneous and odor-evoked units were phase-locked to the TN oscillation peaks. This TN oscillation entrainment by the EOG oscillations caused stronger phase-locking, specifically TN oscillation entrainment by the EOG rhythm (2.5 Hz). Spontaneous and odor-evoked units were phase-locked to the fixed rhythm (2.5 Hz) of odor evoked in the OE, which shows robust oscillatory activity in the primary olfactory system (Ito et al. 2003a) and olfactory center (Gelperin and Tank 1990; Ito et al. 1999), as a simple animal model for studying the functional roles of oscillation in olfaction. We stimulated the OE using a series of air flows and odor concentrations and analyzed their effects on the induction of EOG oscillations systematically. We found that only fast air movements were able to induce EOG oscillations. To determine the conditions for inducing EOG oscillations, we used the terrestrial slug, Limax marginatus, which shows robust oscillatory activity in the olfactory system (Ito et al. 2003a) and olfactory center (Gelperin and Tank 1990; Ito et al. 1999). As a simple animal model for studying the functional roles of oscillation in olfaction, we stimulated the OE using a series of air flows and odor concentrations and analyzed their effects on the induction of EOG oscillations. We found that only fast air movements were able to induce EOG oscillations. To determine the conditions for inducing EOG oscillations, we used the terrestrial slug, Limax marginatus, which shows robust oscillatory activity in the primary olfactory system (Ito et al. 2003a) and olfactory center (Gelperin and Tank 1990; Ito et al. 1999), as a simple animal model for studying the functional roles of oscillation in olfaction. We stimulated the OE using a series of air flows and odor concentrations and analyzed their effects on the induction of EOG oscillations. We found that only fast air movements were able to induce EOG oscillations.
tentacular ganglion as a local field potential (LFP) (Ito et al. 2003a) and output spikes to the procerebral lobe (Ito et al. 2003b), the cut end of the TN was created ~400 \mu m away from the tentacular ganglion and sucked into a blunt glass electrode (~150 \mu m OD). The EOG was recorded from the surface of the OE using one or two glass suction electrodes (25 or 150 \mu m OD) filled with saline or 3\% gelatin saline. In experiments involving surgical isolation of the OE from the tentacular ganglion, succinylcholine (0.1–10 \mu M) was added to the bath solution to avoid artifacts from muscle movements. The neural activity was passed on to a high-impedance probe (JB-101J, Nihon Kohden, Tokyo, Japan) and an AC amplifier (Bioelectric Amplifier MEG-1200, Nihon Kohden), sampled at 2,000 Hz and stored for off-line analysis as described previously (Ito et al. 2001).

**Odor application**

Before each odor application, the OE was exposed to air for a few minutes by lowering the saline level (Fig. 1A). A Pasteur pipette (1.2 mm OD) was positioned about 2 mm apart from the OE. The proximal end of the Pasteur pipette contained a small wad of Kimwipe soaked with 50 \mu l of an odor source and was connected to an electrically activated valve and a peristaltic pump delivering active carbon-filtered saline or 3\% gelatin saline. The odorant was dissolved in distilled water and freshly prepared before use. Similar volumes of distilled water were used as controls. Clean air stimuli and up to four odor stimuli at concentrations of 3\% (1 odor) or 0.10, 0.14, and 0.18 ml/s, except for experiments involving surgical isolation of the OE from the tentacular ganglion, in which only 0.18 ml/min was used.

The sequential process exhausted 56\% of the headspace volume of the Pasteur pipette (3 ml). The OE was perfused with saline between trials (over 3 min). Two trials were performed for each odor.

**Data analysis**

Signal analyses were carried out off-line using custom-made programs developed with MATLAB (The MathWorks, Natick, MA). The signals were digitally filtered with band-pass filters of 0.1–30 Hz for the LFP and 150–1,000 Hz for spike units in the TN. The noise level of the unit recording for each trial was estimated by averaging the absolute amplitude of the band-pass filtered signal (150–1,000 Hz) over time (whole recording period of 90 s including before, during, and after the odor stimulations). A spike was detected when the amplitude of the signal exceeded four times the noise level. Cross-correlation and power spectrum analyses were performed on the LFP signals (0.1–30 Hz). For phase analysis, the signal was filtered with a narrow band-pass filter of 0.5–3 Hz for better estimation of the peaks and troughs. Peaks, troughs, and zero crossings were assigned phases (0, \pi/2, 3\pi/2, 0 (0 crossing in the rising phase), and \pi (0 crossing in the falling phase). To examine the phase relationships of units and LFP oscillation cycles, the phase of each unit was estimated by interpolating between the previous peak (or trough) and the next trough (or peak) as follows

\[ P_{\text{peak}} = \frac{t_{\text{peak}} - T_{\text{pre}}}{T_{\text{post}} - T_{\text{pre}}} \times \pi + P_{\text{pre}} \]

where \( P_{\text{peak}} \) is the phase of the unit (rad), \( t_{\text{peak}} \) is the time of the unit (s), \( T_{\text{pre}} \) is the time of the previous trough (or peak), \( T_{\text{post}} \) is the time of the next peak (or trough), and \( P_{\text{pre}} \) is the phase of the previous peak (\( \pi/2 \) or trough (3\pi/2)).

The ratio of the spike numbers between a preferred phase and its antiphase was obtained to estimate the strengths of the phase-locking for spontaneous, 0.10 and 0.18 ml/s stimulation. Spikes were separately pooled for each condition. The highest bin and one of its neighboring bins were chosen to obtain the highest combination for

**FIG. 1.** Spontaneous oscillatory activity and odor-evoked responses in the olfactory epithelium (OE) and tentacular nerve (TN). A: recording method. Olfactory receptor neurons (ORNs) are located at the tip of the digitate extensions of the tentacular ganglion (beneath the OE) and terminate in synapse projection neurons in the tentacular ganglion, which in turn send olfactory information to the higher olfactory center through the TN (Ito et al. 2000). TN, tentacular ganglion. B: spontaneous oscillations in the OE (top trace) and TN (bottom trace) in the absence of an odor. Cross-correlograms between the OE and TN calculated from 26 superior tentacles are shown below. Overlapping curves indicate a coherent activity at ~2 Hz. Peaks of TN oscillations correspond to the 0-crossings in the falling phases of OE oscillations (about \( \pi/2 \) phase difference). C: odor responses in the OE (EOG) to clean air (left) and 10\% rat chow (right) at flow rates of 0.10 (top), 0.14 (middle), and 0.18 ml/s (bottom). Horizontal bars indicate the time-course of odor application. D: summary of EOG response amplitudes. Gray bar represents control (no stimulation). Inset: definition of peak voltage of EOG response. Low-frequency component (black line; 0.1–1 Hz) of the EOG response (gray lines; 0.1–30 Hz) was used to calculate mean voltage at 1 s before onset (1 s) and at each negative peak value during stimulation. EOG responses to 10\% rat chow (\( n = 9 \)), 10\% 2-ethyl-3-methoxy-pyrazine (EMOP; \( n = 11 \)), 10\% amyl acetate (AA; \( n = 9 \)), and 10\% AA (\( n = 10 \)) were examined. *\( P < 0.05 \).
the preferred phase. Antiphase bins were chosen by shifting $\pi$ from the preferred phase. The phase range of the two combined bins was $\pi/3$.

The statistical significance of differences between the EOG responses of the control (basal spontaneous activity without stimulation) and odor conditions was determined by ANOVA followed by Fisher’s LSD test ($P < 0.05$).

RESULTS

Spontaneous and stimulus-induced oscillations in the OE

First, we characterized the spontaneous activity in the OE because the slug primary olfactory systems display spontaneous oscillations in the absence of odor stimuli (Ito et al. 2001, 2003a,b, 2004). We extracellularly recorded the spontaneous activities from the surface of the OE and the cut end of TN simultaneously using blunt glass electrodes. We detected weak spontaneous LFP oscillations at 1–2 Hz in the OE that were always highly correlated with the simultaneously recorded TN oscillations (Fig. 1B).

Next, we applied clean air and odor puffs to the OE with a series of flow rates (0.10, 0.14, and 0.18 ml/s) for 4 s. At the lowest flow rate (0.10 ml/s), a clean air puff did not evoke an EOG response (slow negative potential), whereas all the odor stimuli at the same flow rate and clean air puffs at higher flow rates evoked significant EOG responses (Fig. 1, C and D). This effect has also been observed in Limax maximus, in which a clean air puff at a flow rate of 0.10 ml/s does not evoke an EOG response (Gervais et al. 1996). At the maximum flow rate (0.18 ml/s), clean air and odor puffs elicited EOG oscillations riding on a slow negative potential, whose frequency and amplitude were faster and larger than the spontaneous oscillations in the OE (Figs. 1C and 2). The peak frequency of the EOG oscillations was constant (2.5 Hz) across odors and concentrations in most cases (Fig. 3). In contrast to 0.18 ml/s, 2.5-Hz EOG oscillations in the OE were very rare at 0.10 and 0.14 ml/s (Fig. 3B). Strong odor puffs ($10^{-2}$% AA) at flow rates <0.18 ml/s failed to evoke the 2.5-Hz EOG oscillations in the OE (20% at 0.10 ml/s and 0% at 0.14 ml/s), although weaker odor puffs ($10^{-3}$% AA) and even clean air puffs at the highest flow rate frequently evoked them (89% for $10^{-3}$% AA and 69% for clean air; Fig. 3B). Moreover, undiluted AA also failed to induce the 2.5-Hz EOG oscillations at 0.10 ml/s (data not shown). These results indicate that the mechanical aspect of the odor puff stimulation, rather than the odor concentration as usually assumed, is the dominant factor that evokes EOG oscillations in the slug.

Among the 49 trials pooled over all the odors, including clean air, the 2.5-Hz activity was less frequently observed in the TN (2/49 at 0.10 ml/s, 2/49 at 0.14 ml/s, and 16/49 at 0.18 ml/s) than in the OE (4/49 at 0.10 ml/s, 5/49 at 0.14 ml/s, and 35/49 at 0.18 ml/s; Fig. 3B). Whenever the TN showed the 2.5-Hz oscillations (16/49 at 0.18 ml/s), the OE always had the peak frequency at 2.5 Hz with two exceptions (14/16; Fig. 3C). In these two exceptions, the second highest peak in the power spectrum of the EOG oscillations was at 2.5 Hz (data not shown). These results suggest that the 2.5-Hz oscillations in the TN originate in the OE, similar to the case for vertebrates (Dorries and Kauer 2000).

Central origin of spontaneous oscillations and local origin of the EOG oscillations

We hypothesized that the spontaneous oscillatory activity in the OE originates from the tentacular ganglion because of the high synchrony between the OE and TN oscillations (Fig. 1B, where all the odor stimuli at the same flow rate and clean air puffs at higher flow rates evoked significant EOG responses (Fig. 1, C and D). This effect has also been observed in Limax maximus, in which a clean air puff at a flow rate of 0.10 ml/s does not evoke an EOG response (Gervais et al. 1996). At the maximum flow rate (0.18 ml/s), clean air and odor puffs elicited EOG oscillations riding on a slow negative potential, whose frequency and amplitude were faster and larger than the spontaneous oscillations in the OE (Figs. 1C and 2). The peak frequency of the EOG oscillations was constant (2.5 Hz) across odors and concentrations in most cases (Fig. 3). In contrast to 0.18 ml/s, 2.5-Hz EOG oscillations in the OE were very rare at 0.10 and 0.14 ml/s (Fig. 3B). Strong odor puffs ($10^{-2}$% AA) at flow rates <0.18 ml/s failed to evoke the 2.5-Hz EOG oscillations in the OE (20% at 0.10 ml/s and 0% at 0.14 ml/s), although weaker odor puffs ($10^{-3}$% AA) and even clean air puffs at the highest flow rate frequently evoked them (89% for $10^{-3}$% AA and 69% for clean air; Fig. 3B). Moreover, undiluted AA also failed to induce the 2.5-Hz EOG oscillations at 0.10 ml/s (data not shown). These results indicate that the mechanical aspect of the odor puff stimulation, rather than the odor concentration as usually assumed, is the dominant factor that evokes EOG oscillations in the slug.

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Central origin of spontaneous oscillations and local origin of the EOG oscillations

We hypothesized that the spontaneous oscillatory activity in the OE originates from the tentacular ganglion because of the high synchrony between the OE and TN oscillations (Fig. 1B), whereas the stimulus-evoked 2.5-Hz activity arises from the OE itself because of the higher occurrence rate of EOG oscillations in the OE (Fig. 3, B and C). We tested this hypothesis by gradual surgical separation of the tentacular ganglion from the OE (Fig. 4). The digitate extensions of the tentacular ganglion (digits) can be classified into three groups (left, central, and right) (Ito et al. 1999). We simultaneously recorded from two recording sites (300 μm apart) on the area of OE innervated by the central group of digits. The OE preparations that retained the intact tentacular ganglion (Fig. 4B) and those that lacked the right and left groups of digits (Fig. 4C) showed spatially coherent spontaneous oscillations.

Air  Rat chow  EMOP  AA  10x AA

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FIG. 2. Stimulus-evoked oscillatory activities in the OE and TN at flow rates of 0.10 (top) and 0.18 ml/s (bottom). To eliminate the slow deflection (<0.5 Hz), signals simultaneously recorded from OE and TN were filtered with a narrow band-pass filter (0.5–10 Hz).
The specific peaks in the power spectra indicated a periodic activity (Fig. 4, B and C). Next, we cut the most proximal region of the central group of digits at the first branching point to ablate the possible neural circuits connecting the two recording sites through interneurons in the digits (Ito et al. 2003b). The OE preparations without the basal region did not show any periodic oscillations, but did show some fluctuation of the LFP signals (Fig. 4 D). The mean values of coherence significantly decreased after the basal region of the digits was cut (ANOVA followed by the Bonferroni post hoc test, \(P < 0.05\); Fig. 4 F). Filled symbols indicate 2.5-Hz oscillations. A cross-correlation analysis revealed no synchronized activities between the two recording sites (Fig. 4 D). These results show that the spontaneous oscillatory activity in the OE originates from the digits and that the ORNs alone do not oscillate spontaneously.

Next, we cut the isolated OE preparations containing only the lobules with odors. All seven superior tentacles examined showed EOG responses to \(10^{-4}\)% EMOP at 0.18 ml/s (Fig. 4 G). Furthermore, four of the seven tentacles also showed the 2.5-Hz EOG oscillations. The signals remained highly synchronized even though there were no neural connections between the two recording sites through the digits (see I + II in Fig. 4 G). These results show that the spatially coherent 2.5-Hz EOG oscillations arise from the OE itself.

Rhythmic pattern of odor-evoked spike units in the TN

To examine the functional role of the EOG oscillations, we analyzed the firing patterns in the TN in the absence (0.10 ml/s) and presence (0.18 ml/s) of the 2.5-Hz EOG oscillations. Clean air did not activate any spike units in the TN at either flow rate (Fig. 5, A and B), although some increase in the units could often be seen at 1 s after the stimulus offset (off response), as previously reported in the land snail Achatina fulica (Chase 1981). All odorants tested evoked rhythmic bursts in the TN (Fig. 5 A). Spike-autocorrelations showed that the interburst frequency was constant across the odors, but changed for different flow rates (\(~1\) Hz at 0.10 ml/s and \(~2.3\) Hz at 0.18 ml/s; Fig. 5 C). The interburst frequency at 0.18 ml/s (2.3 Hz) was close to the frequency of the 2.5-Hz oscillations. This difference (0.2 Hz) was within the frequency resolution (0.25 Hz) of the spectrum analysis (4-s window). These results suggest that the EOG oscillations affect the spike pattern of olfactory outputs to the olfactory center.
Spike–LFP phase relationship in the absence and presence of the EOG oscillations

To further understand how the EOG oscillations modulate olfactory outputs, we analyzed the spike–LFP phase relationship. Each unit in the TN was assigned to the phases of the LFP oscillations in both the OE and TN (Fig. 6). First, we analyzed the spontaneous spikes. Spike phase histograms revealed that most spontaneous units appeared around the peaks of the TN oscillations, which occurred around the falling phases of the OE oscillations (Fig. 6B). To analyze the relationship between the oscillations in both the OE and TN, the phases of the spontaneous spikes relative to the OE oscillations were plotted against those relative to the TN oscillations (Fig. 6C). We found a short band parallel to the diagonal line (black arrow) in the two-dimensional plot, indicating that most spikes kept a constant phase difference and were loosely phase-locked to both oscillations.

Neither clean air nor rat chow odor at 0.10 ml/s increased the spike activity during stimulation (Fig. 5). Therefore the spike phase histograms for these two stimuli were similar to that for spontaneous spikes (Fig. 6, A1 and B). However, some trials with rat chow odor at 0.10 ml/s showed a phase shift during odor stimulation (Fig. 6A2). In these trials, the spikes were phase-locked to the rising phases of the OE oscillations and the peaks of the TN oscillations. The spike phase histogram also revealed one extra peak at the rising phase of the OE oscillations close to the zero crossing (2π/9266) that was absent from the spontaneous activity (Fig. 6B). Similar peaks at the rising phase were also seen for the other three odors at 0.10 ml/s (Fig. 6B). Therefore the appearance of this peak was caused by odor stimulation.

Next, we analyzed the spike-LFP phase relationship in the presence of the EOG oscillations in the OE (0.18 ml/s). As described above, entrainment of the TN oscillations by the 2.5-Hz EOG oscillations was often, but not always, observed.
Even though the pooled data included cases without the entrainment, the odor-evoked units were well phase-locked to the peaks of the TN oscillations and the troughs of the EOG oscillations (Fig. 6B, bottom). In other words, most spikes occurred when the OE and TN showed a particular phase relationship, namely the peak phases of the TN oscillations and trough phases of the EOG oscillations. Indeed, this phase relationship between the EOG and TN oscillations was typical when the EOG oscillations entrained the TN oscillations (Fig. 6A4). The two-dimensional plot also showed that more spikes were evoked around the peaks of the TN oscillations and the troughs of the EOG oscillations (Fig. 6C).

Furthermore, the spike number per burst around the troughs of the TN oscillations was smaller than that around the peaks of the TN oscillations (Fig. 6, A3 and A4). Even though the pooled data included cases without the entrainment, the odor-evoked units were well phase-locked to the peaks of the TN oscillations and the troughs of the EOG oscillations (Fig. 6B, bottom). In other words, most spikes occurred when the OE and TN showed a particular phase relationship, namely the peak phases of the TN oscillations and trough phases of the EOG oscillations. Indeed, this phase relationship between the EOG and TN oscillations was typical when the EOG oscillations entrained the TN oscillations (Fig. 6A4). The two-dimensional plot also showed that more spikes were evoked around the peaks of the TN oscillations and the troughs of the EOG oscillations (Fig. 6C).

Furthermore, the spike number per burst around the troughs of the TN oscillations was smaller than that around the peaks of the TN oscillations (Fig. 6, A3 and A4).

Next, we analyzed the ratio of the spike numbers between a preferred phase and its antiphase to estimate the strengths of the phase-locking for spontaneous, 0.10 and 0.18 ml/s stimulation. For the TN oscillation phase, the peaks ($\pi/3$ to $2\pi/3$) and troughs ($4\pi/3$ to $5\pi/3$) were compared for the three conditions, because the phase relationship was consistent within the three conditions. The numbers of spikes around the TN oscillation peaks were more than two times larger than those around the TN oscillation troughs (2.4 times, 168:70 spikes for spontaneous spikes; 2.2 times, 242:110 spikes for 0.10 ml/s; 2.7 times, 300:109 spikes for 0.18 ml/s). For the OE oscillation phase, the preferred phase differed for each condition. For spontaneous activity, the number of spikes around the zero crossing in the rising phase ($5\pi/3$ to $2\pi$) of the OE oscillations was >1.8 times larger than that around its antiphase (241:129). For 0.18 ml/s stimulation, the number of spikes around the trough ($4\pi/3$ to $5\pi/3$) of the OE oscillations was >2.1 times larger than that around its antiphase (269:124). These results indicate that spontaneous and odor-evoked spikes are phase-locked to the TN oscillation peaks and that entrainment of the TN oscillations by the EOG oscillations causes strong phase-locking (TN oscillation peaks and EOG oscillation troughs).

**DISCUSSION**

These results have defined the mechanical component of an odor stimulus as the key factor for inducing EOG oscillations in a slug. Furthermore, we revealed a local origin for EOG oscillations and a central origin for spontaneous oscillations using surgically isolated OE preparations. Correlation and spike phase analyses indicated that the EOG oscillations entrain slower oscillations in the TN (1–2 Hz) to the constant 2.5-Hz rhythm and regulate the patterning of olfactory output spikes. These results suggest a cross-modality interaction, such that the mechanical component of a wind-delivered odor plume induces EOG oscillations in the OE and these EOG oscillations then modulate the olfactory processing. Furthermore, this is the first demonstration of the presence of EOG oscillations in any invertebrate animal model.

**Origin of the LFP oscillations in the TN**

We previously found that the LFP oscillations in the whole tentacular ganglion and the TN are highly coherent (Ito et al. (Figs. 3 and 6, A3 and A4). Even though the pooled data included cases without the entrainment, the odor-evoked units were well phase-locked to the peaks of the TN oscillations and the troughs of the EOG oscillations (Fig. 6B, bottom). In other words, most spikes occurred when the OE and TN showed a particular phase relationship, namely the peak phases of the TN oscillations and trough phases of the EOG oscillations. Indeed, this phase relationship between the EOG and TN oscillations was typical when the EOG oscillations entrained the TN oscillations (Fig. 6A4). The two-dimensional plot also showed that more spikes were evoked around the peaks of the TN oscillations and the troughs of the EOG oscillations (Fig. 6C).
2003b). The well-characterized LFP oscillations recorded from the surface of the procerebral lobe are considered to arise from synchronized excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) in procerebral interneurons (Kleinfeld et al. 1994). Similar to this and many other systems, it is likely that these oscillations recorded from the surface of the neuropil and digits of the tentacular ganglion also arise from synchronized EPSPs and/or IPSPs. The oscillations of the cut end of the TN and the tentacular ganglion surface are antiphase when the TN activity is recorded at 400 μm away from the tentacular ganglion (Ito et al. 2003b), as carried out in this study. This opposite polarity suggests that they reflect the current sink and source of an open field type LFP.

Because of the long length of the TN, it might seem more likely that the LFP in the TN represents the unresolved bursts of small spikes in the TN rather than EPSPs and IPSPs. However, a subthreshold stimulation of the stratum oriens or stratum radiatum can evoke EPSPs in CA1 pyramidal cells, and corresponding field potentials can be recorded along the entire dendrosomatic axis of these CA1 pyramidal cells (500 μm) (Richardson et al. 1987). Therefore we suggest that the LFP in the TN reflects EPSPs and/or IPSPs in the oscillatory circuit in the tentacular ganglion.

**Generality of oscillations in regulating the spike timing**

The spontaneous and odor-evoked spikes were always phase-locked to the TN oscillations. This finding suggests that the oscillatory circuit in the tentacular ganglion that produces the TN oscillatory activity plays a crucial role in regulating the timing of olfactory outputs to the olfactory center. Odor-evoked fast oscillations in the first olfactory relay centers in vertebrates (Adrian 1942; Nikonov et al. 2002) and insects (Laurent and Davidowitz 1994) have been extensively studied and phase-locking of odor-evoked spikes to the oscillations is thought to be important for olfactory coding. These results suggest a generality of phase-locking to the global oscillations for olfactory coding, regardless of whether they are stimulus-evoked or intrinsic oscillations. Furthermore, our results show that, by entraining the TN oscillations, the EOG oscillations modulate the spike patterning of olfactory outputs to the slightly faster rhythm of 2.5 Hz, which is constant across odors.
However, it is important to point out that, because of the suction recording technique used in this study, our recording of the TN unit activity may be biased toward larger neurons with thicker axons running to the TN and may contain tactile modality and the activity of motor neurons. Therefore it will be important to record from individual neurons in the tentacular ganglion to further clarify the olfactory output in the TN.

Bidirectional interaction of oscillations between the OE and the tentacular ganglion

These results also show that spontaneous oscillatory activity in the OE originates from the tentacular ganglion, whereas the stimulus-evoked EOG oscillations in the OE entrain the oscillations in the tentacular ganglion (recorded from the TN in this study). A previous electron microscope study revealed that symmetrical synapses between horseradish peroxidase–labeled sensory neurons (unknown modality but most likely olfactory) and their postsynaptic neurons are common in the olfactory glomeruli in the digits (Chase and Tollloczko 1993). Therefore it is very likely that some synapses between the ORNs and postsynaptic neurons are symmetrical. This provides a possible neural pathway for transmitting the central oscillatory activity to the OE. Such a bidirectional interaction of oscillations has been reported between the OB and olfactory cortex of the rat (Kay and Freeman 1998). Our results are the first demonstration of such interactions between the OE and the first olfactory relay center. Taken together, these results suggest that olfactory processing is bidirectional in both vertebrates and invertebrates.

Mechanisms of the EOG oscillations

Ephaptic interactions among densely packed fascicles of nonmyelinated axons of ORNs have been suggested to play a role in the synchronization of ORNs in mammals (Boak et al. 2001). However, we found spatially coherent oscillations in our isolated OE preparations that lacked ORN axons, indicating that ephaptic interactions are not required for the generation of EOG oscillations in the slug. Presynaptic inhibition from postsynaptic neurons to ORNs has been reported in insects (Distler and Boeckh 1997), lobsters, turtles (Wachowiak and Cohen 1999), and rats (Aroniadou-Anderjaska et al. 2000). However, we can eliminate the contribution of possible neural connections through interneurons in the digits to the generation of the EOG oscillations through our surgical separation experiments. A possible remaining mechanism for the oscillations is the passive membrane property of ORNs, and possible mechanisms for the synchronization are gaseous transmitters and gap junctions. A previous computational model study showed that the intrinsic oscillatory properties of ORNs are caused by two types of voltage-gated ion channels that can produce EOG oscillations in a rainbow trout (Suzuki et al. 2004). NO has been suggested as one of the possible mechanisms of EOG oscillations, because it diffuses through neural tissues and increases the cGMP concentration through soluble guanylyl cyclase. Moreover, NO may directly activate olfactory cyclic nucleotide–gated channels independently of cGMP in the rat (Schmachtenberg et al. 2003). In L. marginatus, no NO-induced cGMP-like immunohistochemistry is detected in ORNs (Fujie et al. 2002), indicating that the effect of NO, if any, would occur through direct activation of olfactory cyclic nucleotide–gated channels rather than through soluble guanylyl cyclase. Olfactory dendrites and the surface of the cell bodies of mature rat ORNs show positive immunoreactivity for the gap junction subunit connexin 43 (Zhang et al. 2000). A computational model previously showed that gap junctions with an appropriate coupling strength between ORNs can propagate a wave-like activity in the OE (Simoes-de-Souza and Roque 2004). Another computational model study showed that weak coupling between a pair of neurons can slowly phase-lock the pair in anti-phase, whereas strong coupling quickly synchronizes the pair in phase (Sherman and Rinzel 1992). Because of the considerable delay in the appearance of the EOG oscillations (0.5–1 s in vertebrates), weak coupling between ORNs is one of the possible mechanisms for producing these oscillations. We observed EOG oscillations in isolated OE preparations that only retained the olfactory dendrites and the cell bodies of ORNs. These results support the contribution of gap junctions to synchronization.

Functional role of the EOG oscillations

In vertebrates, the EOG oscillations appear at a late phase of the odor response (0.5–1 s after the onset, corresponding to >10 cycles of fast oscillations). Therefore EOG oscillations may not be important for odor coding in vertebrates. These results show that 2.5-Hz EOG oscillations in the OE often appear at a relatively early phase (within 0.5 s) of the odor response in L. marginatus, which was shorter than one cycle of the TN oscillations (0.5–1 s). Therefore the EOG oscillations would affect the spatio-temporal coding of the odor stimulus in this slug.

The 2.5-Hz EOG oscillations affected the olfactory outputs (odor-evoked rhythmic bursts) by entraining the TN oscillations, suggesting a role in olfactory processing. This phase-locking of olfactory outputs to both the OE and TN oscillations after entrainment is consistent with previous observations in a catfish, in which some spike units in the OB become phase-locked to both the OE and OB oscillations after entrainment, suggesting that the EOG oscillations can enhance the synaptic transmission between ORNs and mitral/tufted cells (Nikonov et al. 2002). This effect would be useful for weak odor detection. In this study, we recorded multiunit activity from the cut end of the TN and provided further evidence that the majority of olfactory outputs become phase-locked after entrainment.

Slugs (Gelperin 1974) and snails (Chase and Croll 1981) can locate attractive odor sources by anemotaxis. Physiological data (Chase 1981) have indicated that the OE of the snail A. fulica is extremely sensitive to winds with a threshold of <25 mm/s. This study showed a similar result that air puffs at 0.10 ml/s (22 mm/s in velocity just after the exit from the Pasteur pipette) fail to evoke the EOG deflection, whereas air puffs at 0.14 ml/s (31 mm/s) evoke it. Although slugs often rhythmically turn their head to the left and right while they approach an odor source, it seems unlikely that they can actively create the velocity needed to evoke the EOG oscillations without a gentle wind (0.18 ml/s is ∼40 mm/s). Slugs have a tendency to move upwind if a very gentle wind is set up across the arena (Gelperin 1971). A. fulica also has a tendency to move upwind in airstreams at a flow rate of 100 mm/s (Chase and Croll 1981).
1981), which is fast enough to induce the 2.5-Hz EOG oscillations in this study. In a toad, the appearance of EOG oscillations is limited to the breeding migration period in which the toads show an anemotaxis behavior to the pond (Nakazawa et al. 2000). These behavioral and physiological data together with our results suggest that EOG oscillations may be related to anemotaxis behavior, probably by enhancing weak odor detection.

Possible contribution of mechanosensory neurons

The tentacles of slugs (Gelperin 1971) and snails (Chase 1981, 1985) are thought to be very sensitive to mechanical stimulation and olfactory stimulation. Because we did not record from individual olfactory receptor or mechanoreceptor neurons, the possibility that the EOG oscillations arose from mechanosensory neurons remains. However, because EOG oscillations have been described as an olfactory response in several vertebrates, it is more likely that the EOG oscillations arose from the ORNs in the slug. In either case, our results suggest that a gentle wind will evoke EOG oscillations and modulate olfactory processing in L. marginatus.

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