Oscillations and Gaseous Oxides in Invertebrate Olfaction

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SUMMARY

Olfactory systems combine an extraordinary molecular sensitivity with robust synaptic plasticity. Central neuronal circuits that perform pattern recognition in olfaction typically discriminate between hundreds of molecular species and form associations between odor onsets and behavioral contingencies that can last a lifetime. Two design features in the olfactory system of the terrestrial mollusk *Limax maximus* may be common elements of olfactory systems that display the twin features of broad molecular sensitivity and rapid odor learning: spatially coherent oscillations in the second-order circuitry that receives sensory input; and involvement of the interneuronal messengers nitric oxide (NO) and carbon monoxide (CO) in sensory responses and circuit dynamics of the oscillating olfactory network. The principal odor processing center in *Limax*, the procerebrum (PC) of the cerebral ganglion, contains on the order of 10^5 local interneurons and receives both direct and processed input from olfactory receptors. Field potential recordings in the PC show an oscillation at approximately 0.7 Hz that is altered by odor input. Optical recordings of voltage changes in local regions of the PC show waves of depolarization that originate at the distal pole and propagate to the base of the PC. Weak odor stimulation transiently switches PC activity from a propagating mode to a spatially uniform mode. The field potential oscillation in the PC lobe depends on intercellular communication via NO, based on opposing effects of reagents that decrease or increase NO levels in the PC. Inhibition of NO synthase slows the field potential oscillation, while application of exogenous NO increases the oscillation frequency. A role for CO in PC dynamics is suggested by experiments in which CO liberation increases the PC oscillation frequency. These design features of the *Limax* PC lobe odor processing circuitry may relate to synaptic plasticity that subserves both connection of new receptors throughout the life of the slug and its highly developed odor learning ability. © 1996 John Wiley & Sons, Inc.

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INTRODUCTION

We wish to understand the computational principles that endow olfactory systems with their exquisite molecular sensitivity and robust synaptic plasticity. To this end, it seems adaptive to identify those design features common to the phyletically widest array of olfactory systems possessing the twin features of sensitivity and plasticity. Analysis based on this broad comparative approach suggests that network oscillations mediated by monoxides of nitrogen and carbon may be general design features of olfactory systems.

Coherent oscillations are a nearly ubiquitous feature of vertebrate olfactory bulb and cortex. Lord Adrian first described olfactory oscillations more than 50 years ago as "induced waves" of 50-Hz activity in the olfactory bulb of the hedgehog,

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induced by odorant stimulation of the olfactory mucosa (Adrian, 1942). Subsequent work revealed similar bulbar oscillations in olfactory systems from a large number of vertebrate species, for example frogs (Delaney and Hall, 1995; Delaney and Kleinfeld, 1995; Gerard and Young, 1937), fishes (Satou, 1990), and a variety of mammalian species, including humans (Freeman, 1991; Gray, 1994; Ketchum and Haberly, 1991). Recent work demonstrating odor-modulated or odor-triggered oscillations in mollusks (Delaney et al., 1994; Gelperin and Tank, 1990; Kawahara et al., 1995; Kleinfeld et al., 1994; Schütt and Basar, 1994) and arthropods (Laurent and Davidowitz, 1994; Laurent and Naraghi, 1994; Mellon et al., 1992; Wu et al., 1995) lends further support to the idea that the timing function provided by oscillations may be fundamental to the computations performed during odor recognition and categorization.

The roles of nitric oxide (NO) and carbon monoxide (CO) in olfactory processing and synaptic plasticity may also be clarified using a comparative approach. NO is involved in olfactory processing at both the sensory periphery (Breer and Shepherd, 1993; Broillet and Firestein, 1996) and in central structures (Vincent and Kimura, 1992). The role of NO in central olfactory processing is suggested by dense staining in the olfactory bulb with an antibody directed at neuronal nitric oxide synthase (nNOS) and dense staining for nicotinamide adenine dinucleotide phosphate (NADPH), an electron donor that serves as a cosubstrate for NOS (Bredt et al., 1991). The C-terminal sequence of NOS can transfer electrons from NADPH to chromogenic substrates such as nitroblue tetrazolium, a reaction that forms the basis for NADPH-diaphorase (NADPH-d) staining. The NADPH-d activity of NOS provides a presumptive marker for NOS-containing cells localized in the olfactory epithelium and bulb (Croul–Ottmann and Brunjes, 1988; Davis, 1991; Spessert and Layes, 1994; Zhao et al., 1994). NO mediates olfactory oscillations in a terrestrial mollusk (Gelperin, 1994a,b) and likely plays a similar role in insects, based on NADPH-d staining in antennal lobes of several species (Müller, 1994; Müller and Bicker, 1994).

CO was recently suggested as a putative interneuronal messenger (Maines, 1993) involved in olfactory processing (Leinders–Zufall et al., 1995; Verma et al., 1993; Zufall et al., 1995; Ingi and Ronnett, 1995) and synaptic plasticity (Dawson and Snyder, 1994; Hawkins et al., 1994; Shinomura et al., 1994; Stevens and Wang, 1993; Zhuo et al., 1993). In vertebrate brain constitutive heme oxygenase-2 (HO2) degrades heme to biliverdin and CO (Maines, 1988). HO2 displays selective localization in a number of brain regions, with highest concentrations in the olfactory epithelium and bulb (Ewing and Maines, 1992; Sun et al., 1990; Verma et al., 1993; Vincent et al., 1994). Physiological analysis of the role of CO is greatly aided by use of caged CO compounds (Kao et al., 1995), which allow precise temporal and spatial application of CO to olfactory circuits while monitoring circuit dynamics. Pharmacological depletion of CO depends on the use of metalloporphyrin inhibitors of heme oxygenase that have variable specificity for HO2 versus NOS (Meffert et al., 1994; Zakhary et al., 1995).

The relation between olfactory oscillations and neuronal communication via rapidly diffusing membrane permeant gases may reside in the differing spatial scales of diffusive interactions versus classical neurotransmition. Diffusive neurotransmission from a point source of NO can produce a sphere of influence 50–100 μm in diameter (Gally et al., 1990; Lancaster, 1994; Wood and Garthwaite, 1994), while a single presynaptic release site liberating a classical transmitter acts over a few microns. If central representations of odors in olfactory bulb or procerebral (PC) lobe involve distributed spatial patterns of excitation with local size scales in tens of microns, perhaps gaseous neurotransmission sets the length scale for the local regions of excitation.

To examine the role of olfactory oscillations mediated by NO and CO in an animal with highly developed olfactory acuity and plasticity, we study a macrosmatic (Shepherd, 1993) mollusk, *Limax maximus,* for which odors provide the only source of information about objects at a distance (South, 1992). Striking parallels emerged in several aspects of odor learning behavior (Sahley, 1990) and odor processing circuitry (Gelperin, 1989) between *Limax* and the canonical macrosmatic mammal, *Rattus norvegicus* (Chase and Tolloczko, 1993; Holley, 1991).

FIELD POTENTIAL OSCILLATION IN PC LOBE

The PC lobe of the *Limax* cerebral ganglion contains on the order of 10^5 local interneurons (Gelperin et al., 1986) and receives direct input

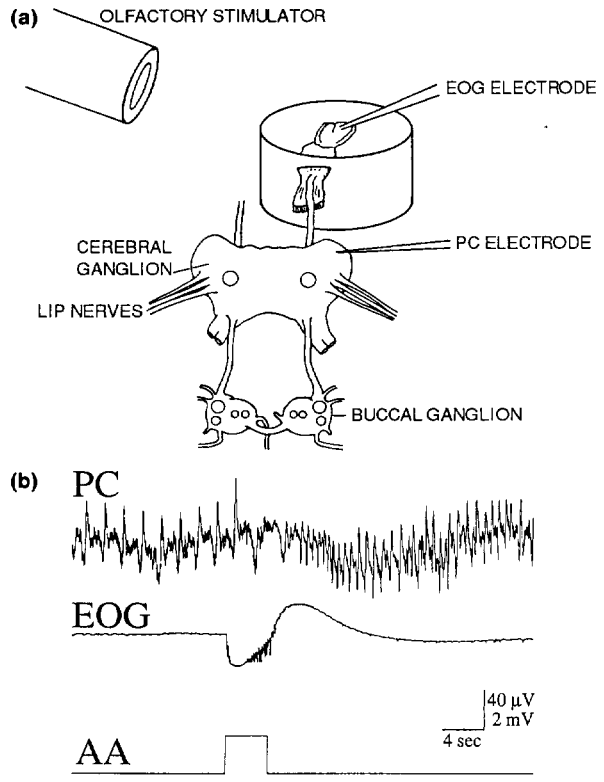

Figure 1 Odor input alters the PC oscillation. (a) A 4-s puff of amyl acetate (AA) to the olfactory epithelium of a nose–brain preparation activates receptors as monitored by the electro-olfactogram (EOG) and (b) produces a biphasic effect on the frequency of the PC oscillation as monitored with a field potential electrode in the PC neuropil.

from olfactory receptors (Chase, 1985; Chase and Tolloczko, 1993). Field potential recordings in the PC with nose attached show a 0.7-Hz oscillation that is altered by odor input (Delaney et al., 1994; Gelperin and Tank, 1990; Gervais et al., 1996) [Fig. 1(A,B)]. A similar oscillation in local field potential in the PC was found in *Helix* (R. Chase, pers. commun.; Schütt and Basar, 1994) and in *Limax flavus* (Kimura et al., 1993). To determine if the PC oscillation occurs *in vivo,* Balaban and colleagues adapted a technique used previously for implanting fine wire stimulating electrodes in various regions of the *Helix* cerebral ganglion (Balaban and Chase, 1989; Balaban and Maksimova, 1993) to implant a fine wire recording electrode on the surface of the *Limax* PC (Gelperin et al., 1994). PC activity was recorded from slugs between bouts of feeding or locomotion after complete recovery from anesthetic. A prominent 0.7-Hz field potential event was recorded from the surface of the PC lobe *in situ* ($n = 3$) (*cf.* fig. 2 in Gelperin, 1994b).

With a pair of wires implanted in the PC, it is possible to measure field potential events during behavior by recording differentially between the pair of implanted electrodes. A recording from a slug implanted the previous day with a pair of 25-μm stainless steel wires in the cell body layer of its right PC is shown in Figure 2. Activity was recorded from the freely moving animal before and after exposing it to odor stimuli using a small piece of odorant-bearing filter paper placed approximately 5 cm in front of its head. The PC field potential exhibits a noisy oscillation in the range of 0.5–1 Hz prior to odor stimulation. The strong odor stimulus of amyl acetate caused a substantial increase in the amplitude of the oscillation, presumably reflecting an increased coherence. Further work will determine if the different patterns of oscillation frequency shifts seen with potato and garlic odors *in vitro* (Gervais et al., 1996) are seen *in vivo.* Using the implanted electrode pairs it should be possible to perturb PC dynamics with stimulation applied between the pair of electrodes in the PC to directly test the idea that PC processing is essential for odor identification and perhaps odor learning.

OPTICAL RECORDING OF PC LOBE ACTIVITY

We wished to look for spatial patterns as well as temporal patterns of PC lobe activity in response to odor stimulation. Optical recordings of voltage changes in a grid of small regions of the PC were made using a cooled CCD camera after staining the PC with the voltage sensitive dye di-4-ANEPPS (Fluhler et al., 1985; Loew et al., 1992). The relatively large fluorescence signal recorded from each small region of the PC, each of which included approximately 1000 cells, obviated the need for signal averaging. To study odor responses, the PC lobe was left attached to the superior nose via the cerebral ganglion with the nose available for odor stimulation using a multichannel puffer (Egan and Gelperin, 1981). CCD images of the PC lobe were magnified so that 100×100 pixels covered the entire PC. Images were obtained at 10/s. The change in fluorescent signal for each pixel was expressed as a fraction of the average fluorescence in the image (Fig. 3). Two major results emerged from these studies (Delaney et al., 1994; Kleinfeld et al.,

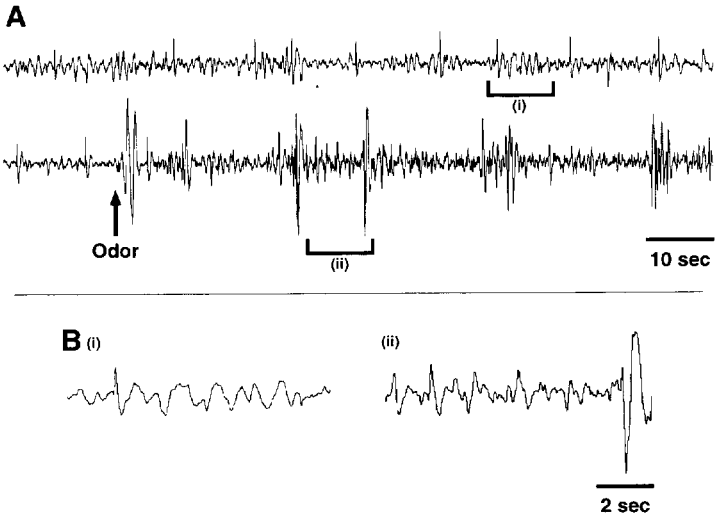

Figure 2 (a) Recording of activity in the procerebral lobe of a freely moving slug in the absence of strong odor (upper trace) and following presentation of a strong odor stimulus (lower trace) at the time shown by the arrow. The odor continued for the remainder of the lower trace following the arrow. The upper and lower traces are continuous. Note that the field potential recorded from the procerebral lobe oscillates in the range of 0.5–1 Hz in the absence of a specific strong odor stimulus, although the amplitude and frequency of the oscillation vary in time. Presentation of a strong odor (amyl acetate) caused a substantial increase in the amplitude of the oscillation. The underlined regions (i) and (ii) mark portions of the record shown in (b). (b) Expanded portions of the record in (a) showing the oscillation of the field potential (i) before and (ii) after presentation of the odor stimulus.

1994). First, the temporal pattern of oscillating activity seen in both field potential and intracellular measurements corresponds to a spatial pattern of wavelike activity that starts with depolarization at the distal tip of the PC and propagates in about 1.4 s to the base of the PC. The wavelike propagation normally always proceeds from distal tip to base, although ionic or pharmacological manipulations can transiently reverse the direction of propagation (Kleinfeld et al., 1994). This suggests that the direction of wave propagation results from the dynamics of the interactions among neurons respon-

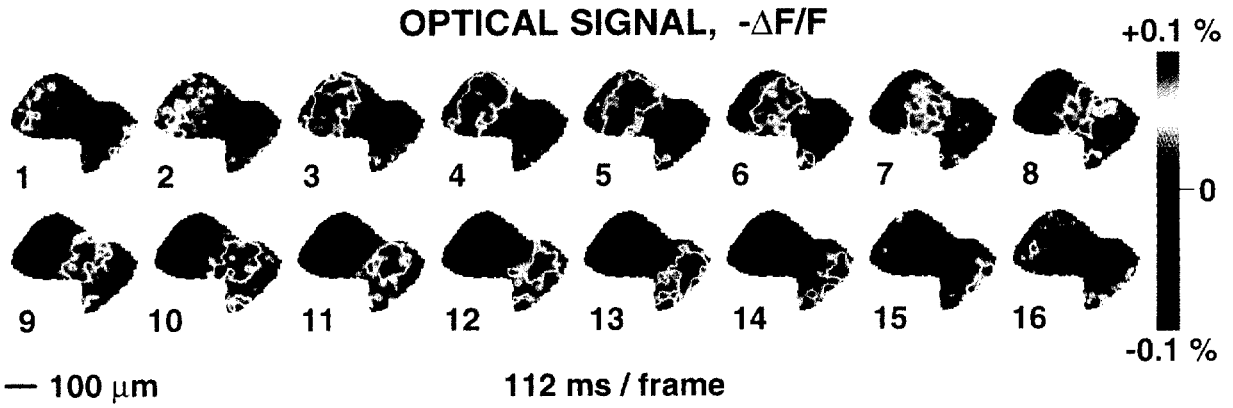

Figure 3 Optical recordings from a PC lobe stained with the voltage sensitive dye Di-4-ANEPPS. A series of CCD images of a PC lobe are shown. Activity in the absence of sensory stimulation is characterized by depolarization (red) developing at the distal tip of the PC, expansion of the area of depolarization, and then propagation of the wave of depolarization to the base of the PC. Blue indicates hyperpolarization from the average level.

sible for the wave rather than from hard-wired directional connections between these neurons.

The second major result of the imaging work showed that odor stimulation causes a transient collapse of the phase gradient along the distal–proximal axis of the PC lobe, so that for a few cycles the entire PC depolarizes in synchrony (Delaney et al., 1994; Gervais et al., 1996). This collapse of the phase gradient was caused by both behaviorally attractive odors, for example, 2-ethyl-3-methoxypyrazine (potato odor) and 1-octen-3-ol (mushroom odor) (Hopfield and Gelperin, 1989), and behaviorally repellent odors, for example, amyl acetate and garlic odor (Sahley, 1990). Therefore the change in wave propagation measured in these optical studies can signal the presence of an odor but not the identity of the odor. Future optical recordings will look for spatial patterns of PC responses to odor on a finer spatial scale, particularly because odor-specific patterns are reported in honeybee antennal lobe (Küttner et al., 1995) and salamander olfactory bulb (Cinelli et al., 1995a,b; Cinelli and Kauer, 1995).

As a step in the direction of fine scale imaging, we applied two-photon laser scanning microscopy (Denk et al., 1990, 1994, 1995) to preparations of the isolated PC lobe previously labeled with calcium green-10-kDa dextran (Gelperin et al., 1995). Two-photon laser scanning microscopy has several advantages relative to confocal scanning microscopy. A tightly focused laser beam is used for excitation during the scan. Because of the quadratic dependence of absorption on the local intensity, which accounts for the optical sectioning properties, phototoxicity and photobleaching are virtually absent outside the focal slice. Also, because optical resolution and sectioning are achieved during excitation, fluorescent photons can contribute to the signal even if they are scattered, because they do not have to pass through a pinhole in the optical path, as in confocal microscopy. Finally, in two-photon excitation the excitation wavelength is roughly doubled from the UV or blue/green into the red or infrared, where scattering is reduced and hence optical penetration improved.

The calcium green label was applied in the cell body layer where it was internalized and transported by processes of both bursting (B) and nonbursting (NB) cells (Kleinfeld et al., 1994). A field potential electrode monitored the oscillation while two-photon images were obtained from selected cells and cell processes. A camera-lucida-like image constructed from a series of optical sections collected with 0.6-μm spacing between sections is shown in Figure 4. The Golgi-like quality of the calcium green labeling is evident. To obtain calcium signals on the time scale of single action potentials, a series of line scans was obtained (6 ms/scan) from a line of pixels intersecting pro-

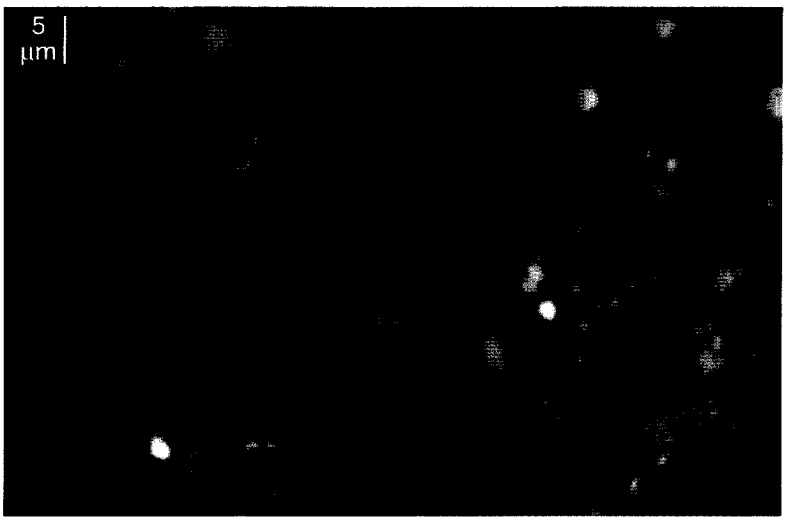

Figure 4 Image derived from a stack of two-photon full-field scans of a PC previously labeled with calcium green-10-kDa dextran placed extracellularly in the cell body layer. Scattered well-stained somata are seen despite being embedded in large numbers of unlabeled somata. Numerous labeled processes belonging to cells outside the scanned field are seen, often with varicosities.

cesses of B cells [Fig. 5(b)]. The stack of line scans is shown in Figure 5(c). The calcium signals from the time sequence represented by the stack of line scans is shown in Figure 5(a), where the red line corresponds to the right-hand B cell process and the blue line corresponds to the left-hand process. The simultaneously recorded local field potential (LFP) oscillation is shown in green. Calcium transients due to single action potentials in the B cell processes can be seen clearly in some of the optical records (stars). It will be very interesting to determine spiking patterns in B and NB cells that respond to odor-elicited inputs to the PC.

The change in mode of PC oscillation from wave propagation to spatially uniform excitation could have dramatic consequences for excitation of PC output cells. PC output neurons with somata in the pedal ganglia have been identified in *Helix* (Chase and Tolloczko, 1989; Ratte and Chase, 1995) and in *Limax* (Gelperin and Flores, 1996; Gelperin et al., 1994). If the dendrites of these PC output cells are arrayed along the distal–proximal

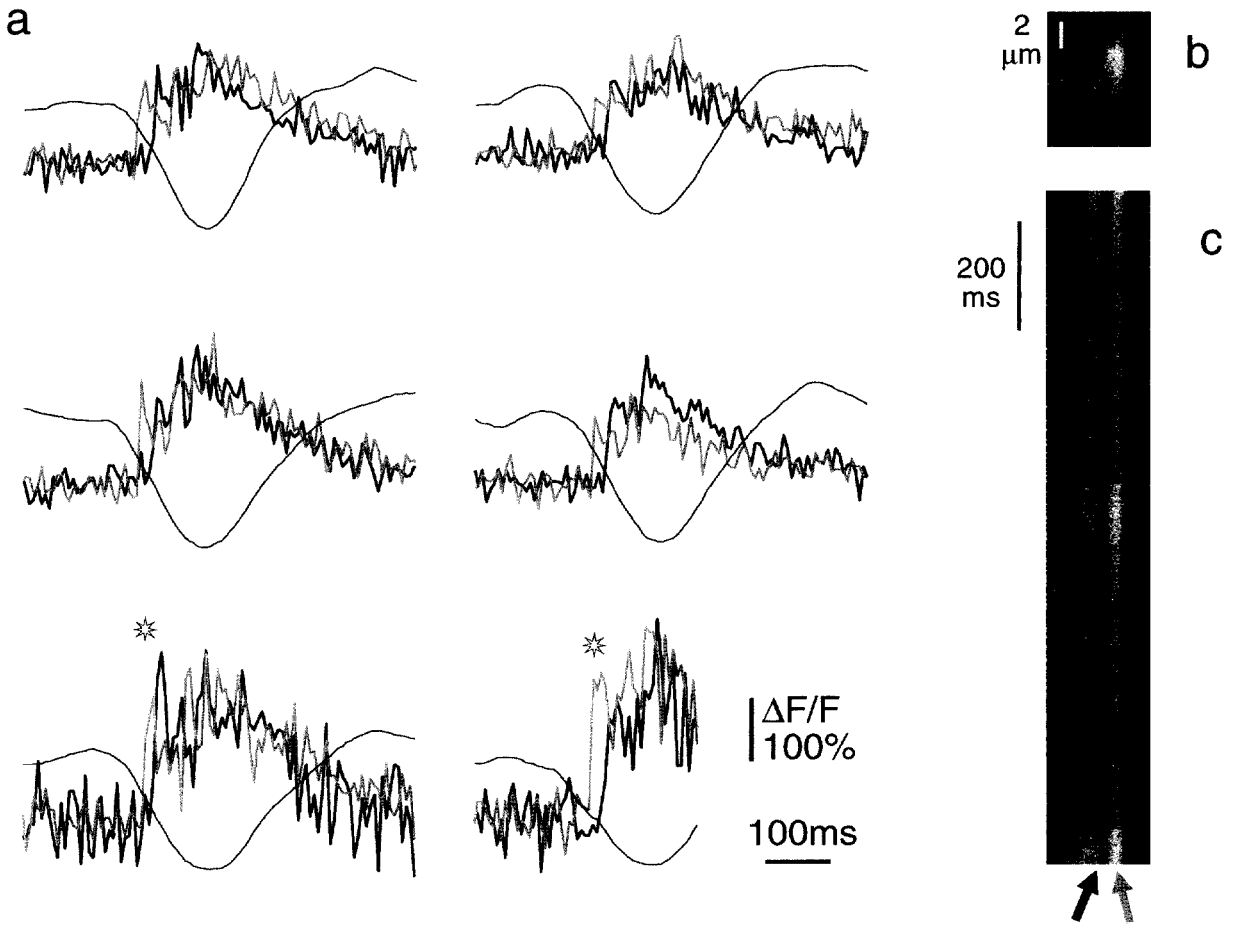

Figure 5 (a) Calcium green signals in B cell processes recorded optically with simultaneous recording of the oscillating local field potential. (b) Repetitive line scans intersecting the two B cell processes shown were made at 167 Hz. (c) A stack of 210 line scans is shown. A time series of calcium green fluorescence values in each B cell process is derived from the stack of line scans by reading the pixel values from a vertical line through the stack of line scans centered in the signal from each process. The time series of fluorescence values for the two processes are shown in (a) with the simultaneously recorded field potential, shown as a smooth downward deflecting trace. The black trace of fluorescence values is derived from the part of the stack indicated with a black arrow while the grey trace is derived from the signals indicated with a grey arrow. Calcium transients due to single action potentials are indicated by stars above the fluorescence traces.

axis of the PC lobe, the change from wave propagating mode to simultaneous depolarization mode could produce a very large change in the synaptic input to the dendrites. Labeling of the pedal output cells with the carbocyanine dye DiI in living preparations (Gelperin and Flores, 1996) allows this idea to be tested.

GASEOUS OXIDES AND OLFACTORY OSCILLATIONS

NO is a small and reactive gaseous molecule that mediates a variety of communicative and cytotoxic roles (Moncada et al., 1991; Nathan, 1992). NO synthesizing neurons are widely distributed in the mammalian brain (Bredt et al., 1991; Egberongbe et al., 1994; Schmidt et al., 1992; Valtschanoff et al., 1993; Vincent and Kimura, 1992) with olfactory bulb second only to cerebellum in prevalence of NO synthesizing neurons.

The first indications that NO might play a role in invertebrate nervous systems came from biochemical measurements of arginine conversion to citrulline, presumably via NO synthase, by hemocytes of the horseshoe crab *Limulus* (Radomski et al., 1991). Subsequent biochemical work indicating activity of an NO synthase in central nervous system (CNS) extracts from locust, and histochemical demonstration of NADPH-d enriched neurons in locust CNS strongly suggested a role for NO in intercellular communication in arthropod brains (Elphick et al., 1993). In the molluscan CNS, demonstrations of NADPH-d staining (Chichery and Chichery, 1994; Cooke et al., 1994; Elofsson et al., 1993; Jacklet and Gruhn, 1994; Moroz et al., 1994; Sánchez-Alvarez et al., 1994) and measurements of NO effects on central neural networks (Moroz et al., 1993), membrane conductances (Gilly et al., 1995; Sawada et al., 1995), synaptic transmission (Jacklet, 1995; Meulemans et al., 1995), and learning (Robertson et al., 1994, 1995; Teyke, 1995a,b) make it clear that NO is involved in a variety of integrative functions in mollusks.

Given the demonstration of intense diaphorase staining in the olfactory pathway of *Helix* (Cooke et al., 1994; Sánchez-Alvarez et al., 1994) and *Limax* (Gelperin, 1994a) and the robust field potential oscillation in the PC lobe of *Limax,* it seemed logical to use the *Limax* PC oscillation to test a role for NO in central olfactory processing. A few percent of PC cells show NADPH-d staining and hence are presumptive NO secreting cells. Reduction of NO communication among PC cells slows or stops the oscillation while application of exogenous NO increases the PC oscillation frequency.

Two methods were used to reduce NO communication among PC cells. First, oxyhemeproteins such as oxymyolobin or oxyhemoglobin were applied to the PC while the oscillation was monitored with a field potential electrode. Oxyhemeproteins bind NO tightly and rapidly and therefore greatly reduce extracellular levels of NO. Applying a bolus of oxyhemeprotein in the PC perfusion stream transiently slowed the oscillation frequency. Bathing the PC in 5–10 m*M* oxyhemeprotein stopped the oscillation reversibly. Control solutions of Methemeprotein had little or no effect. A second method used to decrement NO communication was to inhibit NO synthase with a false substrate, for example, an arginine with a methyl ester substituent such as L-N^G-nitroarginine methyl ester (L-NAME). The false arginine binds to NO synthase and blocks its activity. Application of L-NAME to the PC lobe at 10 m*M* greatly slowed the oscillation rate while application of 20 m*M* L-NAME stopped the PC oscillation reversibly (Fig. 6). The inhibitory effect of 10 m*M* L-NAME on the PC oscillation frequency can be overcome by addition of 5 m*M* L-arginine (Gelperin, 1994a).

Application of exogenous NO increases the rate of the PC oscillation. An NO source with defined kinetics of NO evolution is diethylamine/NO (DEA/NO) (Hrabie et al., 1993). Applica-

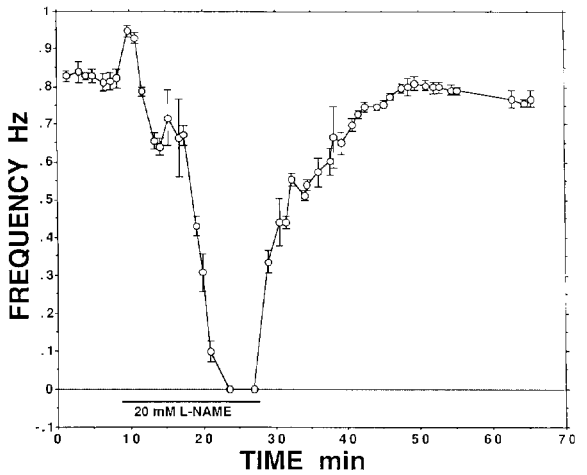

Figure 6 The PC oscillation is stopped, reversibly, by the NO synthase inhibitor L-NAME at 20 m*M*, indicating that NO generation by PC cells is necessary for expression of the PC oscillation.

tion of DEA/NO solution to the PC increases the oscillation frequency by 20% (Gelperin, 1994a,b). Caged NO, in the form of nitrosylpentachlororuthenate (NPR) (Carter et al., 1993; Murphy et al., 1994; see also Makings and Tsien, 1994), allows generation of NO in tissue with temporal and spatial control. A brief flash of near UV (360 ± 10 nm) to a PC bathed in NPR increased the burst rate of a B neuron and increased the PC oscillation frequency (Fig. 7). Note in Figure 7 that each burst of the B cell is accompanied by a field potential event. The field potential events most likely result from extracellular flow of synaptic currents set up in NB neurons throughout the PC lobe by synaptic input from the B cells.

How might NO communication in the PC lobe enhance odor recognition? Perhaps odor recognition involves readout of a spatial pattern of microdomains within the PC set up by the subset of afferent fibers activated by a particular odor. Afferent input may lead to spatially localized increments in NO levels with consequent uncoupling of local areas from the overall PC oscillation. A subset of the olfactory receptors shows diaphorase staining as do fibers in the olfactory nerve (Gelperin et al., 1996). Experimental tests of this idea will use imaging studies at high spatial resolution and very localized uncaging of NO from NPR.

Odor learning is very highly developed in *Limax,* displaying higher order phenomena such as second-order conditioning, blocking, and inhibitory conditioning (Sahley, 1990; Sahley et al., 1992), and multiple phases of memory consolidation differentially susceptible to disruption by cooling (Sekiguchi et al., 1991, 1994; Yamada et al., 1992). If synaptic plasticity in the PC lobe plays a role in odor learning, NO communication among PC cells could be relevant to implementing learning-dependent synaptic plasticity, a role suggested for NO in the mammalian brain (review in Schuman and Madison, 1994). Given that odor memories in *Limax* can last more than 120 days (Delaney and Gelperin, 1986), NO action via augmented cGMP synthesis may lead to long-term changes in gene expression in PC cells, which are remarkable compared to other neurons in the CNS for being strictly diploid (Chase and Tolloczko, 1987) and undergoing postembryonic neurogenesis of at least a month (Zakharov and Ierusalimskii, 1995). Inhibition of NOS *in vivo* impairs associative olfactory memory in the honeybee (Müller, 1995) and snail (Teyke, 1995a,b), consistent with a role for NO in *Limax* odor learning.

CO may also play a role in mediating olfactory oscillations in *Limax.* If CO is applied to the isolated PC lobe by photolytic release from NV-CO/Na (Kao et al., 1995), the frequency of the LFP oscillation is increased (Fig. 8). The oscillation frequency increased from 0.8 ± 0.01 to 12.1 ± 0.2 Hz (mean ± S.D.) during a series of 400-ms uncaging pulses of 360 ± 10 nm light (n = 30). Increasing the light intensity, exposure duration,

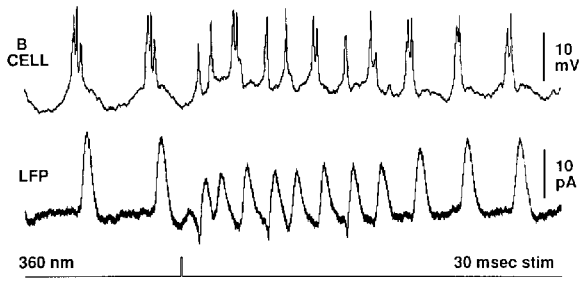

Figure 7 Bursting (B) PC cells are stimulated to burst at higher frequency by a pulse of NO liberated by a brief flash of near-UV delivered to a preparation bathed in NPR (50 μM). Intracellular recordings from the B cell were obtained using the nystatin perforated-patch technique (Horn and Marty, 1988; Walz, 1995). The local field potential (LFP) recording shows that each burst in the B cell was accompanied by a field potential event.

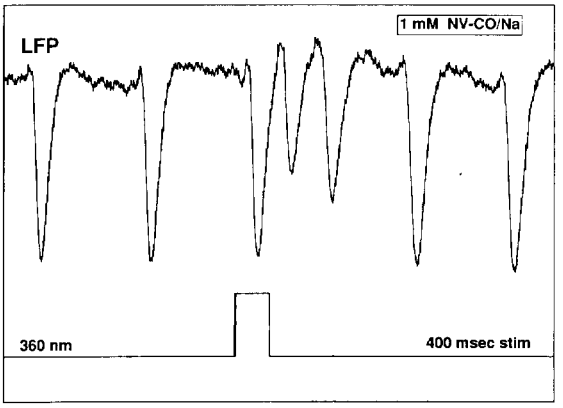

Figure 8 CO application to the PC increases the frequency of the field potential oscillation. The preparation was bathed in 1-mM caged CO in the form of NV-CO/Na while a saline-filled patch electrode monitored the field potential oscillation. As indicated by the lower trace, a 400-ms uncaging pulse of 360 ± 10 nm light was applied to the PC, which increased the frequency of the oscillation. Similar light pulses in the absence of caged CO had no effect.

or fraction of the PC illuminated by the uncaging flash all increased the magnitude of the frequency increase ($n = 4$ experiments). It will be interesting to see if the effects of CO on LFP oscillation frequency result from excitation of burster cells in the PC, as seen for NO.

CONCLUSIONS

Essentially all olfactory systems are known to oscillate in response to odor. The work with the *Limax* olfactory system demonstrated that oscillatory dynamics have a nontrivial structure that involves both space and time. In particular:

1. Oscillations are the resting state of the olfactory analyzer; in the absence of odor there is sustained periodic output from a class of neuronal bursters.
2. The resting state is also characterized by a spatial gradient of activity; the periodic output appears as propagating waves.
3. Odor-elicited input changes the frequency and collapses the spatial gradient; only the frequency change appears to be odor specific.
4. Nitric oxide is necessary for the olfactory oscillation.
5. Carbon monoxide may be involved in the oscillation.

A model that reproduces the known dynamics based on the anatomy and chemical features of the PC lobe remains to be constructed. Understanding the computational role of olfactory oscillations and gaseous neurotransmission in olfactory processing will depend critically on further development of representational network models of the *Limax* PC (Gelperin et al., 1989), as well as the vertebrate olfactory bulb (Li and Hopfield, 1989; Rall and Shepherd, 1968; White et al., 1992) and its constituent neurons (Bhalla and Bower, 1993).

The phenomenology seen in *Limax* may be general. Recent work has shown that the olfactory bulb of the frog, either *in vivo* or *in vitro,* exhibits partially coherent waves of activity in the absence of odor (Delaney and Hall, 1995; Delaney and Kleinfeld, 1995). The application of odor leads to a dramatic increase in the spatial coherence, in addition to an increase in the frequency of the oscillations. With regard to mammals, a recently developed slice preparation from rat olfactory bulb displays oscillatory field potential epochs *in vitro* (Elaagouby et al., 1995), which suggests an avenue for the exploration of ground state oscillations in the bulb that is complimentary to that of *in vivo* recording (Wilson and McNaughton, 1994).

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