Coherent Network Oscillations by Olfactory Interneurons: Modulation by Endogenous Amines

A. GELPERIN, L. D. RHINES, J. FLORES, AND D. W. TANK

Biological Computation Research Department, AT&T Bell Laboratories, Murray Hill, New Jersey 07974

SUMMARY AND CONCLUSIONS

1. The procerebral (PC) lobe of the terrestrial mollusk Limax maximus contains a highly interconnected network of local olfactory interneurons that receives direct axonal projections from the two pairs of noses. This olfactory processing network generates a 0.7-Hz oscillation in its local field potential (LFP) that is coherent throughout the network. The oscillating LFP is modulated by natural odorants applied to the neuroepithelium of the superior nose.

2. Two amines known to be present in the PC lobe, dopamine and serotonin, increase the frequency of the PC lobe oscillation and alter its waveform.

3. Glutamate, another putative neurotransmitter known to be present in the lobe, suppresses the PC lobe oscillation by a quisqualate-type receptor and appears to be used by one of the two classes of neurons in the PC lobe to generate the basic LFP oscillation.

4. The known activation of second messengers in Limax PC lobe by dopamine and serotonin together with their effects on the oscillatory rhythm suggest the hypothesis that these amines augment mechanisms mediating synaptic plasticity in the olfactory network, similar to hypothesized effects of amines in vertebrate olfactory systems.

5. The use of a distributed network of interneurons showing coherent oscillations may relate to the highly developed odor recognition and odor learning ability of Limax.

INTRODUCTION

The dynamics of a neural circuit can be altered dramatically by modulatory neurotransmitters, particularly amines and small peptides (Harris-Warrick and Marder 1991; Kaczmarek and Levitan 1987). In invertebrate motor systems, neuromodulatory transmitters are capable of orchestrating pronounced changes in motor output (Kravitz 1990). Application of neuromodulatory transmitters to the stomatogastric ganglion of the lobster changes the basic pattern of the oscillatory rhythm. In one case an induced change of oscillatory rhythm corresponds to a behavioral change in the characteristics of chewing (Heinzel 1988a, b).

In addition to changing circuit dynamics, neuromodulatory transmitters are implicated in many forms of synaptic plasticity. Serotonin can produce both short-term and long-term effects on ion channels and synaptic facilitation in several molluscan preparations (Barzilai et al. 1989; Crow and Forrester 1991; Eskin et al. 1989). Repeated serotonin applications produce long-term alterations in synaptic efficacy in Aplysia sensory neurons by an adenosine 3',5'-cyclic monophosphate (cAMP)-dependent mechanism that requires protein synthesis (Castellucci et al. 1989), perhaps mediated by cAMP-inducible genes (Dash et al. 1990).

It is also possible that neuromodulators can have indirect effects on synaptic plasticity by acting on circuit dynamics in such a way that synaptic change is enabled. For example, in the mammalian hippocampus, long-term potentiation in region CA1 is most efficiently induced when synaptic inputs occur as repetitive short bursts at the theta frequency. A neuromodulator that enhances the afferent system dynamics to theta frequency bursting would enable synaptic modification without directly influencing the chemistry of synaptic transmission.

METHODS

The PC lobe was studied either connected to the cerebral ganglion or after complete isolation. Animals were first chilled on ice for 30 min, and then the brain and buccal mass were quickly removed and placed in cold (3–5°C) Limax saline (Delaney and Gelperin 1990). The cerebral ganglia were isolated and pinned to
the bottom of a silicone elastomer (Sylgard)-lined preparation dish. For intra-PC lobe measurements on lobes connected to cerebral ganglia, the PC lobe was desheathed by fine dissection, and a saline-filled patch electrode of 1- to 3-μm tip diameter was placed in the lobe, typically at the boundary between the apical cell body layer and the neuropil, for extracellular recording of LFP (Forda et al. 1982). A continuous perfusion system was used with a supply reservoir that could be switched between saline and drug solutions. For studies on the completely isolated PC lobe, the outer connective tissue was removed from the cerebral ganglion, leaving the thin inner connective tissue sheath intact. The PC lobe was isolated by making a transverse cut across the base of the lobe at its origin from the cerebral ganglion. The isolated lobe was then pinned by its inner connective tissue sheath to the Sylgard base of the recording chamber.

Each preparation was used for tests of a single neurotransmitter. In most cases two runs were made with each preparation, with a recovery time of 30 min between runs. All tests of neurotransmitter effects were done on PC lobes retaining their normal connections to the cerebral ganglia. The experimental chamber had an effective volume of 1 ml. The flow rate was 0.025 ml/s with a calculated exchange time of 40-60 s for complete solution exchange in the experimental chamber.

Intracellular recordings of PC neuron activity were obtained either by penetrating cells with finely beveled high-resistance (60-80 MΩ) microelectrodes filled with 2 M potassium acetate or by whole-cell patch recording with the use of nystatin to permeabilize the membrane patch (Horn and Marty 1988; Korn and Horn 1989). PC lobe field potentials were recorded with either a List model EPC 7 patch-clamp amplifier or a Getting model 5A DC amplifier, band-pass filtered between 0.03 and 3 Hz with the use of a PAR model 113 and recorded on a Gould 220 chart writer. Intracellular activity was recorded either with the patch-clamp amplifier or a Neurodata IR-283 intracellular amplifier. During some experiments, data were recorded on a NeuroCorder DR-484, digitized and stored on an Apple Macintosh II for later analysis.

Two tract tracing techniques were used to study connections to the PC lobe from the olfactory and medial lip nerves. Nickel chloride was applied to the cut central stump of the olfactory (ON) or medial lip (MLN) nerve and the preparation developed with dithiooxamide (Hackney and Altman 1982). Alternatively, a crystal of 1, 1',-didodecyl-3,3,3',3'-tetramethylindo carbocyanine perchlorate (DiI; Molecular Probes No. 383) was inserted into the neuropil of the freshly dissected PC, and after 6-18 h the preparation was examined with epifluorescent illumination (510- to 560-nm band-pass excitation filter, 580-nm dichroic mirror, 590-nm barrier filter) and photographed.

**RESULTS**

**PC lobe anatomy**

The PC lobe is composed of an apical region of neuronal somata and a basal region of synaptic neuropil (Fig. 1A). The basal PC neuropil has two major subdivisions, the terminal mass (TM) adjacent to the cell body layer and the internal mass (IM) located at the base of the PC where it originates from the metacephalobal lobe of the cerebral ganglion (Zs.-Nagy and Sakharov 1970). The major class of neuronal somata in the PC is 7-9 μm diam and has neurites that ramify widely and exclusively within the PC neuropil or among the neuronal somata of the PC (Veratti 1900). These intrinsic PC neurons are local interneurons in that their processes are confined to the PC lobe (Chase and Tolleczko 1989). The columnar appearance of the neuronal somata in some areas of the PC arises from the predomi-
of fibers connecting with the PC neuropil. Figure 1B is a brightfield photomicrograph of a DiI-stained PC lobe to show the position and size of the DiI crystal placed in the lobe 18 h previously. Figure 1C is a photomicrograph taken under epifluorescent illumination to show the prominent DiI-stained fiber tracts in the ON and MLN connecting with the PC neuropil. The fibers in the MLN all enter the branch of the MLN connecting to the ganglion and nose at the tip of the inferior tentacle (not shown). The nickel backfills of the ON showed fibers terminating in both the TM and IM. Similarly, nickel backfills of the branch of the MLN from the inferior nose also showed fibers terminating in both the TM and IM (data not shown). In some of these MLN preparations, the distribution of fibers in the TM and IM was not uniform. The lack of reaction product in any of the somata of the PC for either ON or MLN backfills provides further evidence that PC cells are exclusively local interneurons.

Evoked potentials

Further evidence for synaptic connectivity of superior and inferior olfactory fiber tracts with the intrinsic interneurons of the PC lobe is provided by evoked LFPs in response to shock of the ON or the MLN. Figure 2 shows a series of evoked LFPs in response to repetitive shocks of the ON at 1 Hz. These LFPs were recorded by a suction electrode positioned over the surface of the PC lobe. The evoked LFP has two components. The first peak remains stable at stimulus rates of >10 Hz and persists in low Ca\(^{2+}\), high Mg\(^{2+}\) medium (Gelperin et al. 1989) that blocks synaptic transmission in Limax (Delaney and Gelperin 1990). We therefore interpret this first peak to be produced by action-potential currents inafferent fibers entering the PC from the ON. The second peak decrements rapidly at modest stimulus rates (1 Hz, cf. Fig. 2) and is blocked by low Ca\(^{2+}\), high Mg\(^{2+}\) saline (Gelperin et al. 1989), indicating its dependence on synaptic activation. Intracellular recordings from PC lobe interneurons during trains of ON shocks suggest that the second peak of the evoked LFP is produced by currents in neurons postsynaptic to the ON fibers (data not shown). The decrement in the second peak during repetitive shocks results, at least partially, from the persistence of synaptic events produced by preceding ON shocks. Stimulation of the branch of the MLN that arises in the sensory ganglion at the end of the inferior tentacle elicits an LFP in the PC lobe that is qualitatively similar to that produced by ON stimulation (data not shown). These evoked LFPs produced by ON and MLN stimulation demonstrate functional synaptic connectivity between olfactory input and the PC lobe circuitry.

During study of the evoked LFPs, we found time-dependent variability of evoked LFP amplitude not associated with frequency-dependent depression of evoked responses. Further investigation revealed that the size of the synaptically dependent event evoked in the PC by ON or MLN shock was altered dramatically by an endogenous oscillation within the PC circuitry (Gelperin and Tank 1990).

LFP oscillation

Extracellular recordings from any location within the neuropil or cell body layer of the PC lobe reliably show rhythmic extracellular currents (Fig. 3A). Measurements of 100 cycles from each of 12 preparations yield an average LFP oscillation frequency of 0.69 ± 0.12 (SD) Hz. In the
absence of sensory input, the PC oscillation is very regular. The standard deviation of the mean frequency is 2.3 ± 0.8% of the mean for a set (n = 29) of 30-s samples taken from five preparations. As shown in Fig. 3A, each cycle of the oscillation is composed of an episode of extracellular current flow followed by a period during which little active current flow is measured. The duration of active current flow, measured as the ratio of the duration of active current flow (taken at half maximum amplitude) to the period of the oscillation (taken from peak N to peak N + 1 of the active current signal), was 15 and 19% of the period of the oscillation in two preparations (100 cycle samples).

A similar pattern of spontaneous activity is recorded from a PC lobe whether it is attached to or isolated from the cerebral ganglion (Gelperin and Tank 1990). Pieces of PC as small as one-fourth of the size of the intact structure still show an LFP oscillation (data not shown). The cell body layer, isolated so as to maintain the integrity of fiber connections within it, also continues to show an LFP oscillation (Fig. 3B). This isolated somata preparation (n = 3) was prepared by severing all fiber connections at the boundary between the inner edge of the cell body layer and the outer edge of the TM. The structural and functional integrity of the isolated cell body layer is presumably maintained by the highly branched neurites of PC neurons that form axosomatic synapses within it (Zs.-Nagy and Sakharov 1970).

The rhythmic variations in LFP are a coherent property of the PC lobe circuit, as shown by simultaneous recordings from two locations within the lobe. If the recordings are taken from the same layer of the lobe along the basal-apical axis, the waveforms recorded at the two sites are similar, and the peaks are in phase (Fig. 4A), although small phase gradients (<0.1 cycle) are occasionally observed. If the recordings are taken at different points along the apical-basal axis, the waveforms differ, although the peaks of the LFP signal remain phase locked. The most dramatic difference in waveform is seen between recordings from the cell body layer and the most basal neuropil, i.e., the IM. Pairs of recordings from these two sites show LFP changes of opposite polarity with small phase shifts (Fig. 4B). Recordings from the midregion of the PC, presumably in the TM, show biphasic LFP changes. Because the apical and basal regions of the PC lobe experience LFP changes of opposite polarity simultaneously, current will flow within and around the PC during each cycle of the LFP oscillation. This is presumably why recordings from the surface of the PC lobe, for example where the use of a suction electrode placed over the intact PC, also reveal the LFP oscillation (Gelperin and Tank 1990). The issue of odor-stimulated spatiotemporal variations in oscillatory activity within the PC lobe is being investigated with the use of a combination of electrical and optical recordings of regional variations in activity (Gelperin et al. 1992; K. R. Delaney, A. Gelperin, M. S. Fee, J. A. Flores, R. Gervais, D. W. Tank, and D. Kleinfeld, unpublished observations).

**Single-cell activity**

The most common activity pattern of individual PC neurons recorded in situ with nystatin-filled patch electrodes shows periodic bursts of action potentials. During the inter-
biphasic effect on LFP oscillation frequency and amplitude. An initial increase in frequency of 30–50% was seen after serotonin application, followed by a decrease that could be of equal magnitude (Fig. 9A). The effects of serotonin on oscillation waveform were variable. Full recovery to preserotonin frequency and waveform (Fig. 9B) often was not seen even after ≥1 h of washout, suggesting that the effects of serotonin action on PC neurons are long lasting. This is consistent with the demonstrated second-messenger mediated events triggered in Limax PC cells by serotonin (Yamane et al. 1989).

Although a change in oscillation frequency and waveform was consistently produced by serotonin application (n = 11; 9 preparations), multiphasic changes more complicated than the biphasic effect shown in Fig. 9 were often observed. These multiphasic responses rarely returned to preserotonin patterns. At present we do not fully understand the reason for the variability in serotonin’s effects.

**Glutamate**

Because some PC neurons are glutaminergic on the basis of immunocytochemical evidence (A. Gelperin and Huang, unpublished observations), we assayed the effects of exogenous glutamate application on the LFP oscillation. Glutamate (10^{-3} M) essentially eliminated the spontaneous oscillations of LFP (Fig. 10). The complete time course of the LFP waveform were more variable than dopamine’s effect on LFP oscillation frequency. The records in Fig. 6 show a change in waveform of the LFP as well as an increase in amplitude. The change in LFP amplitude during the entire course of the experiment shown in Fig. 6 is shown in Fig. 8A. A small decrease in amplitude is seen during the 50 s immediately after dopamine application, followed by a large increase in amplitude that outlasts dopamine application. In some preparations the initial decrease is more pronounced, and the later increase in amplitude more delayed. The average initial decline in amplitude, the secondary increase in amplitude, and postdopamine recovery are shown in Fig. 8B (n = 12; 6 preparations).

**Serotonin**

The effects of serotonin on the LFP oscillation were more complex than those produced by dopamine. As shown in Fig. 9, A and B, serotonin (10–100 µM) sometimes had a
FIG. 7. A: complete sequence of LFP oscillation frequency changes elicited by 100-μM dopamine application is shown. These data are from the experiment excerpted in Fig. 6. B: pooled data from 14 trials on 7 preparations show the increase in frequency of the LFP caused by 100 μM dopamine. Data were normalized by taking the control frequency before dopamine application as 100%.

FIG. 8. A: effect of 100-μM dopamine application on LFP amplitude. These data are from the experiment excerpted in Fig. 6. Note the small initial decrease followed by a much more prominent increase. B: pooled data from 12 trials on 6 preparations show the early and late effects of 100 μM dopamine on LFP amplitude.

DISCUSSION

The PC lobe of *Limax* and other terrestrial pulmonate gastropods (Bishop 1978; Van Mol 1967) is concerned with olfactory processing, on the basis of both its anatomy and physiology. The olfactory receptors and their associated ganglia at the tips of the superior and inferior tentacles project fiber tracts directly to the PC (Chase and Tolloczko 1993; Gelperin et al. 1989). The arrangement and density of synaptic contacts within the cell body and neuropil regions of the PC strongly suggest the importance of local circuit interactions (Chase and Tolloczko 1986, 1989; McCarragher and Chase 1985; Zs.-Nagy and Sakharov 1970). Shock of the ON or input to the PC derived from natural odorants applied to the sensory epithelium of the tentacle tips strongly modulates PC activity (Gelperin and Tank 1990).

Uptake of 2-deoxyglucose in the PC of the terrestrial snail *Achatina fulica* is greatly reduced if the superior nose connected to that PC is shielded from odor stimulation (Chase 1985).

The occurrence of rhythmic activity mediated by local circuit interactions within a second-order olfactory processing center is a remarkable functional analogy between molluscan and mammalian olfactory systems (Tank 1990).
A. GELLPERIN, L. D. RHINES, J. FLORES, AND D. W. TANK

The computational tasks of the olfactory system are to identify and categorize odors with exquisite sensitivity to weak or novel odors. Simulations of neural network models based on known anatomy and physiology of olfactory bulb (Li and Hopfield 1989) or PC lobe (Hopfield 1988) suggest that oscillatory dynamics can provide an effective way to achieve sensitivity to weak odor inputs.

*Limax* demonstrates a remarkable ability to modify its behavioral responses to odor cues by associative learning (Gelperin 1989; Sahley 1990). The learned modifications of *Limax*'s behavioral responses to odor cues require synaptic modification at some point in the odor-processing system, perhaps in the PC lobe. The function of the serotonergic or dopaminergic inputs to the PC lobe may be to enable synaptic modification, perhaps triggered by stimuli that have proven to be effective unconditioned stimuli in classical conditioning experiments with *Limax*. The mammalian olfactory bulb shows modifications of its oscillatory dynamics in response to odor cues, and the nature of the change in oscillatory dynamics is altered when the behavioral meaning of the odor cue is changed by a classical conditioning procedure (Freeman et al. 1988). Serotonin or dopamine may enable plasticity in the *Limax* PC lobe in a way analogous to the way norepinephrine enables learning-induced plasticity in the mammalian olfactory bulb (Gray et al. 1986).

Dopamine has a variety of synaptic actions in molluscan neurons (Walker 1986), mediated via receptors coupled either to classical, fast-acting ion channels (Berry and Cottrell 1975; Wieland and Gelperin 1983; Winlow et al. 1981) or to second-messenger systems (Cedar and Schwartz 1972; Holden-Dye and Walker 1989; Osborne 1977; Yamane et al. 1987). In *Limax* PC lobe cells, dopamine causes a transient, modest elevation in cAMP levels lasting only 2–3 min (Yamane et al. 1987). Dopamine application also lowers the phosphorylation level of several proteins of molecular weight ranging from 18 to 36 kD (Yamane et al. 1989).

The action of serotonin on the oscillating LFP of the PC lobe is both more long-lasting and more complex than that of dopamine. The LFP oscillation pattern following serotonin application often did not return to predrug baseline activity during washout, and some preparations showed one or more phases of postdrug activity changes. Serotonin application to PC lobe cells causes a large (20- to 30-fold) and rapid (within 30 s) increase in cAMP levels (Yamane et al. 1987) and alters the state of phosphorylation and synthesis of several proteins (Yamane et al. 1989). Serotonin's ability to induce long-term changes in gene expression and synaptic efficacy in the diploid neurons of the PC lobe

![Fig. 9](image-url)

**Fig. 9.** *A:* serotonin (5-HT) causes multiphasic changes in the frequency of the LFP oscillation. This is a plot of a complete experiment to illustrate one of the types of response to 10 μM 5-HT. The initial increase in frequency is followed by an equally dramatic decrease in frequency. *B:* changes in LFP amplitude caused by application of 10 μM 5-HT. These are data from the same experiment shown in *A.*

![Fig. 10](image-url)

**Fig. 10.** *A:* control record of the LFP oscillation obtained from an apical recording site in normal saline. *B:* application of 10^{-3} M L-glutamate inhibits generation of the LFP oscillation. Isolated events still evident in the field-potential record may be activity in small groups of cells or action potentials in cells very near the recording electrode. *C:* inhibitory effect of glutamate is fully reversible.
A M I N E S M O D U L A T E * L I M A X O L F A C T O R Y O S C I L L A T I O N S  1 9 3 7

Glu on

Glu off

FIG. 11. A: time course of glutamate inhibition of the procerebral LFP oscillation. Data shown are from the experiment excerpted in Fig. 10. B: data from 5 trials in 2 experiments demonstrate the ability of $10^{-3}$ M glutamate to reliably suppress the LFP oscillation.

(Chase and Tolloczko 1987) may underlie *Limax*’s ability to retain associative modifications of odor memories for >120 days (Delaney and Gelperin 1986).

A variety of glutamate receptors has been described on molluscan neurons (S.-Rozsa 1984; Watanabe et al. 1988). Hyperpolarizing responses are mediated by increased chloride or potassium conductances (Bolshakov et al. 1991; Walker 1986). The rhythmically active S2 subunit of the central pattern generator of *Helisoma trivolvis* is probably glutaminergic, because the IPSPs produced by S2 on its postsynaptic targets are mimicked by glutamate and quisqualate application (Quinlan and Murphy 1991). A quisqualate-sensitive potassium current has recently been characterized in identified buccal neurons B1 and B2 of *Aplysia* (Katz and Levitan 1993). Some fibers in the olfactory nerve of *Helix* are likely to be glutamatergic on the basis of the finding that stimulation of the ON in *Helix* elicits a hyperpolarization in the serotonergic metacerebral giant cell that is mimicked by glutamate application (Cottrell et al. 1972, Szczepaniak and Cottrell 1973). Glutamate may be the transmitter used to produce the periodic IPSP recorded in PC interneurons phase locked to the LFP oscillation.

The use of coherent oscillations shared between separate components of a neural circuit as a way to bind together features of a complex stimulus has been suggested to occur in the mammalian visual system (Eckhorn et al. 1988; Engel et al. 1990; Gray et al. 1989). This has stimulated great interest in the general issue of the computational role of oscillations in neural networks (Hopfield 1988; Sompolinsky et al. 1990; Tank 1990) and in several brain regions that generate rhythm bursts of neuronal activity at various frequencies, including the thalamus (Steriade and Llinás 1988), hippocampus (Traub and Miles 1990), suprachiasmatic nuclei (Moore 1983), and cortex (Llinás et al. 1991; Silva et al. 1991). These neuronal oscillators work over a variety of time scales and may depend on a combination of cellular and network properties for the expression of their oscillations. In the *Limax* PC lobe, natural olfactory stimulation has been shown to induce substantial changes in the timing of waves of electrical activity that span the width of the PC lobe and travel along its axis from the apex to the base (Gelperin et al., 1992).

We thank Dr. S. Curtis for Fig. 11.

Present address of L. D. Rhines: Harvard Medical School, Boston, MA.

Address for reprint requests: A. Gelperin, Rm lC464, AT&T Bell Laboratories, 600 Mountain Ave., Murray Hill, NJ 07974.

Received 26 May 1992: accepted in final form 17 December 1992.

REFERENCES


COTTRELL, G. A., MACON, J. B., AND SZCZEPAJNIK, A. C. Glutamic acid


AMINES MODULATE LIMAX OLFACTORY OSCILLATIONS


