Convergence of Multimodal Sensory Input Onto Higher-Level Neurons of the Crayfish Olfactory Pathway

DÉFOREST MELLON, JR.

Department of Biology, University of Virginia, Charlottesville, Virginia 22901

Received 24 May 2000; accepted in final form 9 August 2000

Mellon, DeForest, Jr. Convergence of multimodal sensory input onto higher-level neurons of the crayfish olfactory pathway. J Neurophysiol 84: 3043–3055, 2000. Intracellular electrophysiological studies of lateral protocerebral interneurons (LPIs) in the crayfish Procambarus clarkii have revealed convergence of multimodal sensory information onto these higher-level cells of the crustacean central olfactory pathway. Antennular stimulation by odors or electrical shocks generates excitatory-inhibitory sequences in some LPFs as does electrical or hydrodynamic stimulation of the antennae. Photic stimulation of the ipsilateral compound eye generates excitatory responses in LPFs, usually in the form of trains of impulse bursts that are timed to the peaks of the spontaneous oscillatory activity that characterizes these neurons. Focal electrical stimulation of the olfactory lobe, the termination point of antennular afferent input, or the accessory lobe, where higher-level visual and tactile inputs converge, also generates brief excitation and a delayed, prolonged inhibition in LPFs. Both phases of this activity are thought to be transmitted to the lateral protocerebrum via deutocerebral projection neurons, which have extensive dendritic arborizations in the olfactory lobe and the accessory lobe. The excitatory pathway is thought to synapse directly with target LPFs, whereas the inhibitory pathway is probably indirect and mediated through GABAergic interneurons within the lateral protocerebrum. There is evidence that both presynaptic and postsynaptic inhibition suppress activity in LPFs. Preliminary observations suggest that a small cluster of neurons adjacent to the hemi-ellipsoid body are inhibitory to LPI activity. Multimodal inhibitory and excitatory modulation of LPI activity may play a part in the contextual identification of odors in the crayfish olfactory system.

INTRODUCTION

Crustaceans and other arthropods detect odors through dendrites of bipolar olfactory receptor neurons (ORNs) associated with cuticular sensilla on their antennae from which axons project to olfactory lobes (OLs) within the midbrain (Boeckh and Tolbert 1993; Sandeman and Luff 1973). The OL neuropil is subdivided anatomically into 100 or more glomeruli, and it is thought that those ORNs responding to unique olfactory determinants converge within the same glomerulus. The ORNs synapse with output, or olfactory projection, neurons, whose cell bodies reside in cell cluster 10 (Sandeman et al. 1992) and whose axons course within the olfactory-globular tract (OGT) to the forebrain (Blaustein et al. 1988; Distler and Boeckh 1996; Mellon et al. 1992a; Sandeman and Luff 1973; Titova and Tsvilineva 1985). Responses of arthropod olfactory projection neurons to input from the ORNs are modified in the OL by inhibitory action from local intrinsic neurons (Christensen et al. 1996; Wachowiak and Ache 1994).

In crustaceans, an additional brain structure, the paired accessory lobes (ALs) lie adjacent to the OLs and are thought to be involved with olfactory processing. These structures are not present in insects or other arthropods, and they occur only in those crustaceans that have a burrowing or other quasi-territorial lifestyle (Sandeman et al. 1993, 1995). The ALs reach their maximum size in lobsters and crayfish, where their volume can be larger than that of the OLs. The functional significance of the ALs is not understood. Anatomically, they contain several thousand spherical or columnar glomeruli where synaptic connections occur between extrinsic and intrinsic interneurons. Unlike the OLs, the paired ALs are connected through a commissural pathway by local deutocerebral interneurons (Sandeman et al. 1995). The ALs receive no primary afferent input from any sense organ, but anatomical and electrophysiological findings in spiny lobsters (Wachowiak et al. 1996) and crayfish (Sandeman et al. 1995) indicate that interneurons convey odor information to the AL from the ipsilateral OL; furthermore, higher-order afferents from tactile and visual centers in other brain areas synapse in the AL glomeruli.

AL outputs, like those associated with the OL, are projection neurons, also with somata in cell cluster 10; their axons also run within the OGT to lateral forebrain centers (Blaustein et al. 1988; Mellon and Alones 1993; Tsvilineva and Titova 1985). A chiasm within the OGT, just rostral to the deutocerebral commissure (DC), permits some projection neuron axons to bifurcate to the lateral protocerebra on both sides of the brain (cf. Fig. 1). Other OL and AL projection axons ascend the OGT without branching (Blaustein et al. 1988; Tsvilineva and Titova 1985; Wachowiak et al. 1996). Projection neuron dendrite branching patterns can also be different; some dendritic arbors lie within either the OL or the AL, whereas others branch in both the OL and the AL (Mellon and Alones 1993; Mellon et al. 1992b). Projection neuron targets within the lateral protocerebrum include the hemi-ellipsoid bodies (HEB) and an area of the medulla terminalis referred to as the glomeruli centrales (Blaustein et al. 1988; Mellon et al. 1992a). The current picture of the projection pathways to the lateral protocerebrum of crustaceans, therefore, is of several parallel axonal populations, some of which originate solely in OL, others only within the AL, and some within both structures. It

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests: Dept. of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22903 (E-mail: dmf6d@virginia.edu).
is not yet understood how the different populations of projection neurons are subdivided among different target areas in the lateral protocerebrum.

Previously we have shown that one of the major projection neuron targets, the lateral protocerebral interneurons (LPIs) in the HEB, exhibits spontaneous oscillatory electrical activity, being driven by synchronous depolarizing synaptic input from other local neurons in the terminal medulla (Mellon and Alones 1997; Mellon and Wheeler 1999; Mellon et al. 1992b). The LPIs are interneurons intrinsic to the lateral protocerebrum; they have extensive dendritic branches (inputs) within the HEB and axon terminals (outputs) in the medulla terminalis lateral to the HEB. There, the LPIs synapse on other, unidentified neurons (Mellon et al. 1992a). Neither the specific role of the HEB in olfactory processing nor that of the LPI targets is currently understood. Here I report that OL and AL projection pathways generate short-latency direct excitation and long-latency indirect inhibition of the LPIs, something that has not previously been reported. In fact, although antennular input is known to excite the LPIs (e.g., Mellon and Alones 1997), no previous accounts of inhibitory suppression of LPI oscillations have been published. The present findings reveal prolonged feedforward inhibition of LPIs following focal electrical stimulation of projection neurons. Furthermore excitatory-inhibitory sequences were also seen in LPIs in response to stimulation of both the antennules and the antennae. Additionally, excitation of LPIs occurs in response to photic stimulation of the ipsilateral compound eye. Interactions were observed between these multimodal inputs. This study therefore identifies for the first time pathways through which different extra-olfactory sensory input can modify activity in the forebrain targets of the OLs.

METHODS

Southern red crayfish, Procambarus clarkii, were obtained from a dealer in Louisiana and housed in large (1,000 l) tanks of filtered, circulating fresh water; they were fed Elodea and other unidentified pond plants, maintained at a temperature of 25°C, and kept on a 12 h/12 h light/dark cycle.

Dissection

Animals were prepared for recording as described previously (Mellon and Alones 1995, 1997; Mellon and Wheeler 1999). Following decapitation, isolated crayfish heads were mounted on a Lucite recording chamber, and the cephalic arterial systems were cannulated and flushed with chilled, oxygenated crayfish saline. The antennules were inserted into an olfactometer, through which dechlorinated tap water flowed continuously and into which odorants could be injected at prescribed times and for specified durations. Saline that had passed through the blood system flowed into the recording chamber and then into a sump, from which both it and the discharge of waste water from the olfactometer were continuously aspirated. A pair of silver wire electrodes within the olfactometer contacted the antennules and was used to stimulate axons in the antennular nerve when required. A length of heat-shrink tubing was glued to the right eyestalk and, following exposure of the HEB by microdissection, afforded a semistable restraint during intracellular recordings from LPIs.

Electrical recording

We obtained intracellular recordings from 121 individual LPIs in the major lobe of the hemi-ellipsoid body during the course of the present study. Intracellular recordings from LPIs were obtained using sharp glass capillary electrodes filled with 2 M potassium acetate. Electrical activity was detected using an Axon Instruments Axoclamp IIA preamplifier in current-clamp mode. Signals from extracellular and intracellular electrodes were observed on a DC analog storage oscilloscope and also were recorded on VHS videotape recorder (Vetter Instrument). Extracellular records from the projection neuron axon tracts were made with glass capillary electrodes filled with 1 M NaCl and broken off to a diameter of 10–20 μm. The dorsal surface of the brain over the accessory lobe and olfactory lobe was desheathed, and the record-
ing electrodes were inserted to a depth between 200 and 500 μm to access the projection neuron fiber tracts. These tracts enter the accessory lobes in their rostral aspect, whereas they enter the olfactory lobes caudally (see Fig. 1B). Signals from extracellular electrodes were amplified (Grass Instrument, P-15 AC amplifiers) using a frequency band-pass of 0.1–10,000 Hz.

**Stimulation**

Timed pulses of odorant (0.02% tetramin solution), 0.1–1.0 s in duration, were delivered simultaneously to both antennules or both antennae via the olfactometer through a solenoid-activated valve, controlled by a pulse generator (World Precision Instruments). The wash water through the olfactometer was terminated as the odorant pulse was initiated and remained off for the duration of the pulse.

Electrical stimulation of the AL, OL, antenna II nerves, and lateral protocerebral cell clusters was by a focal glass suction electrode 100 – 300 μm in diameter. Stimuli were single shocks or brief trains of four to five shocks at 10 Hz, generated by Grass Instrument S88 and S8 stimulators and delivered through stimulus isolation units. Unless stated otherwise, the minimum stimulus durations and intensities required to obtain a response were used in obtaining the records presented in this paper.

Photic stimulation was supplied by a tungsten source and was delivered through a fiber optic cable that terminated within 5 mm of the affected compound eye. Light onset and duration were controlled by a shutter mechanism and were monitored by a second fiber optic cable directed at a photometer. Light levels at the compound eye were approximately 1.8 lm as measured by an optometer (United Detector Technology).

Current injection into LPIs was accomplished by passing depolarizing or hyperpolarizing current through the recording electrode with the Axoclamp preamplifier either in bridge or in discontinuous current-clamp mode. Stimulus triggering and duration were controlled by the Grass S88 stimulator.

**Pharmacological agents**

Drugs were obtained from Sigma Chemical and were made up to the stated concentrations in crayfish saline on the day of an experiment. They were applied via the brain cannulation system from a pressurized reservoir that could be substituted for the normal saline reservoir through a three-way valve. The drug solutions passed through the heat exchanger and thus were delivered at the same temperature (18°C) as the normal saline. Approximately 1 min (as measured by air-bubble transit time) was required for the drug solutions to reach the crayfish brain after the valve was opened. Persistence of the drug solutions in the saline bath surrounding the isolated head may have prolonged their action, but this was not usually a problem. Specific drug applications were performed with at least two isolated-head preparations, including at least one LPI from each of the different preparations.

All experiments were performed at a temperature of 18°C, as measured from the temperature of the saline that pooled in the head capsule.

**RESULTS**

**Organization of the olfactory system in the crayfish**

Diagrams of the brain of the crayfish P. clarkii are shown in dorsal view in Fig. 1, emphasizing the projection neuron pathways and their targets in the lateral protocerebrum. ORN axons enter the brain ventrally and are not included in Fig. 1A and B. Paired cell clusters 9, on the ventral aspect of the brain, in which the somata of some local olfactory interneurons reside, are not shown. Figure 1C is a diagram of the lateral protocerebrum, showing the terminal medulla and the bi-lobed hemi-ellipsoid body. Figure 2 is a diagram of the right hand side of the brain, incorporating the various experimental procedures discussed in this paper.
Stimulation of the antennules evokes delayed inhibition in LPIs

LPIs exhibit on-going, synchronous, depolarizing oscillations in membrane potential that apparently represent periodic synaptic events simultaneously imposed on the entire population of LPIs by an oscillatory center in the medulla terminalis (Mellon and Alones 1997; Mellon and Wheeler 1999). In response to odor pulses or electrical shocks applied to the antennules an LPI exhibits an initial excitatory synaptic potential (EPSP) and associated impulses and sometimes a delayed impulse burst (Mellon and Alones 1997). A more detailed analysis of these responses than was done previously reveals a silent period following the initial phases of excitation. The silent period appears to be the result of a stimulus-caused, transient reduction in amplitude of the periodic membrane potential fluctuations. Examples of this amplitude reduction—and the consequent failure of impulse generation—are shown in Fig. 3. In these examples, initial excitation of the LPI, evoked by an electrical or odorant stimulus, was clearly followed by a gap in the spiking activity generated by the periodic background depolarizations. Results of numerous presentations of odor pulses in another LPI are quantified in the peristimulus-time (PST) histogram of Fig. 4. Onset of an odor pulse, at time 0, was followed by transient increase in on-going impulse frequency, as noted previously by Mellon and Alones (1997), and a delayed impulse burst in each case. Following the impulse burst, indicated by the horizontal line in the histogram, the amplitude of the periodic background depolarizations was depressed, and, in consequence, the occurrence of single impulses declined for one or two oscillation cycles, revealing the delayed inhibitory action.

Projection neurons can inhibit LPI activity

Since projection neurons from both the OL and the AL ascend from the midbrain to the lateral protocerebrum in P. clarkii (Blaustein et al. 1988; Mellon et al. 1992a, 1993), they present an obvious possible source of the inhibition to the LPIs. We therefore used a focal electrode to stimulate either the OL or the AL while recording from LPIs in the HEB. Strong focal stimulation of both the OL and the AL generates excitatory-inhibitory response sequences in LPIs. Figure 5 shows typical responses to a single stimulus or brief stimulus trains delivered to the dorsal surface of either the ipsilateral OL or AL. Single focal stimuli to the AL, for example, evoked an impulse and a delayed, long-lasting inhibition of the periodic activity (Fig. 5A). Figure 5B shows the initial excitation at higher sweep speed. In Fig. 5C, in another preparation, a train of 5-Hz stimuli to the AL generated a plateau of EPSPs, superimposed impulses and, following termination of the stimulus, a long inhibitory phase, during which background activity was de-

![Fig. 3.](http://jn.physiology.org/)

![Fig. 4.](http://jn.physiology.org/)
evoking prolonged responses (i.e., the position in which the response threshold to stimulating current was lowest) was either the posterior margin of the OL or the anterior margin of the AL. As may be seen in the diagram of Fig. 1B, these are the areas in the two lobes that are closest to the major neuritic segments of the projection neurons, and it is assumed that, in these positions, the focal electrode stimulus recruited the largest number of projection neuron axons.

The prolonged inhibition seen following stimulus trains was accompanied by increased membrane conductance, measurements of which were obtained by injecting a series of hyperpolarizing current pulses (200 ms, 3 nA) across an LPI membrane during and following a 1-s, 10-Hz train of focal stimuli to the OL or AL. Figure 6 shows a record of one current-injection episode obtained from an LPI, while the inset summarizes the poststimulus recovery in the voltage change of this cell to the injected current following 30 identical current injection trains. Both figures have identical time bases. The conductance increase accompanying stimulation actually began prior to the onset of hyperpolarization, and it recovered to normal values in about 2.5 s following the end of the stimulus train, whereas the membrane remained hyperpolarized, and the periodic depolarizations were depressed, until about 5 s after stimulation.

Focal stimulation using single electrical stimuli at intensities and durations that were just threshold for excitatory and inhibitory responses in LPIs when applied to the AL usually produced no response when applied to the OL. Figure 7 shows PST histograms obtained from one preparation for 19 shocks delivered to the AL and for 17 shocks of identical strength and duration delivered to the OL. The focal electrode was moved from the AL to the OL and back again twice during the stimulating session. The reason for the difference between the OL and AL in sensitivity to focal stimulation is not understood. However, incidental observations in our laboratory suggest that there are at least twice as many projection neurons that arborize within the AL as there are in the OL, so the difference in threshold to focal stimuli may reflect a real difference in respective populations of excited projection neurons.

**Antenna II nerve stimulation generates responses in LPIs**

Stimulation of the antenna II nerves entering the tritocerebrum in *Procambarus* generated an initial excitation and strong secondary inhibition in both ipsilateral and contralateral LPIs. As shown in Fig. 8, 1-s trains of brief shocks delivered to the antenna II nerve branches and recurring at 10 Hz generated prolonged depression of LPI periodic activity (Fig. 8A). This inhibition was more extensive than that following a similar stimulus train delivered to the ipsilateral antennule (Fig. 8B), resembling the very effective inhibition of LPI activity seen following repetitive stimulation of the OL and AL. I next placed the antennae of two preparations into a modified olfactometer and stimulated them with 1% tetramin, 10^{-2} M-D-glucose, and brief electrical shocks. All of these stimuli were effective in generating an EPSP and superimposed action potential in the LPIs examined. As shown in Fig. 8C, however, a control stimulus, in which neither odorant was expelled into the olfactometer, was also effective in generating a spike response, suggesting that the mechanical stimulus from interrupting the flow of wash water over the antennae, prior to the
delivery of the odorant, may have been the causative agent. Phasic responses to water flow have been reported from receptors on the antennae of at least two other crayfish species (Sandeman 1989; Tautz et al. 1981). Projection neurons are the putative pathway for LPI inhibition

To confirm that the inhibition of LPIs following antennal and antennular stimulation occurs as a consequence to stimulating the olfactory midbrain projection neurons, we recorded simultaneous extracellular field potentials from the

OL and AL projection neuron fiber tracts following electrical stimulation of the antennule and the OGT. Figure 9 illustrates samples of these recordings. Maximal stimulation of the OGT, at a point near its entrance into the HEB, generated simultaneous antidromic compound action potentials in both the AL and OL recording electrodes following a delay of approximately 35 ms. In response to strong single electrical stimuli delivered to the antennules, complex field potentials were recorded in both AL and OL (Fig. 9, B and C). The form and time course of the OL response were similar to extracellular field potentials recorded by Sandeman and Sandeman (1998) in the OL of the Australian crayfish *Cherax destructor* following electrical stimulation of the antennules. The largest, sharply negative phase of the response represents compound action potential activity in both local OL interneurons and in OL projection neurons, since the potentials were similar in duration to the antidromic action potentials generated directly in the projection neurons (Fig. 9, A and D); the AL response was simpler and probably represented primarily projection neuron spike activity. Furthermore, the AL response was delayed by about 5 ms with respect to the OL response, a latency differential noted previously by both Sandeman et al. (1995) and Wachowiak et al. (1996).

Experimental evidence that projection neurons are the source of the large extracellular potentials recorded following antennular stimulation was obtained by employing refractoriness. As shown in Fig. 9D, electrical stimulation of the OGT generated antidromic (centripetal) compound action potentials that blocked appearance of the antennular nerve-generated compound action potentials in both OL and AL when the OGT stimulus fell within a critical time window prior to the orthodromic activation.

---

**FIG. 6.** Responses of an LPI (cell 021000) to injected current before, during, and following a 1-s train of 11 focal stimuli (10 Hz) applied to the ipsilateral AL. The electrotonic potential from the injected current was reduced, both during and for a short time following the stimulation. - - - - - - mean resting potential level. The histogram above the electrical record is aligned to the same time scale and indicates the recovery of amplitude of the membrane response to current injection.

**FIG. 7.** Peristimulus-time histograms showing spike frequencies in an LPI (cell 081299) in response to 17 individual low-intensity electrical shocks focally applied to the OL and 19 identical shocks applied to the AL. See text for discussion. Bin size: 0.1 s.
Photic stimulation generates excitation in ipsilateral LPIs

Previous methods used to stabilize the right-hand compound eye during dissection and recording shielded the retina from ambient illumination. Using our current techniques, the retina of the affected eye is exposed during all experimental procedures, and we have found that photic stimulation is a very effective means of exciting a majority of the LPIs tested, generating strong depolarizations, an increase in impulse frequency, and usually, multiple impulse bursts (Fig. 10). The presence of light also can increase the response of an LPI to other stimuli, as shown in Fig. 10, C–F. When electrical stimulation of the antennules occurred during a period of illumination, the response to the shock was enhanced compared with that occurring in the dark. This suggests the presence of neural integration of different sensory modalities in LPIs.

The proximity of the HEB to the three visual ganglia within the eyecup raised the possibility that the LPIs receive direct excitatory connections from the visual system. To examine this question, we surgically isolated the right-hand eyecup in two preparations by transecting the OGT at its emergence from the medial protocerebrum into the lateral protocerebral tract, following which LPIs were penetrated, and their response to illumination of the ipsilateral retina was recorded. This procedure abolished all responses of the ipsilateral LPIs to direct stimulation of the ALs as well as to electrical stimulation of the antennules and the antenna II nerves. It had no effect, however, on the response to photic stimulation of the ipsilateral compound eye. While not conclusive, due to the presence of other, non-OGT axons within the lateral protocerebral tract, these experiments indicate that the excitatory response of the LPIs to visual stimulation does not occur via the AL or OL projection neurons and may be through direct connections with neurons of the most proximal visual ganglion, the medulla interna.
Neural inhibition of LPI activity is mimicked by GABA and muscimol

To examine the identity of the transmitter responsible for inhibitory actions on the LPIs mediated by the projection neurons, the cephalic arterial system was perfused with the inhibitory transmitter GABA and two different GABA receptor agonists, baclofen and muscimol. Figure 11 shows the effects of these agents on the oscillatory activity normally observed in the LPIs. Both 100 μM GABA and 10 μM muscimol reversibly depressed the amplitude of the background depolarizations, while baclofen had no effect even at high concentrations.

Local cell cluster within the terminal medulla may mediate inhibition of the LPIs

Preliminary immunocytochemical findings from our laboratory (V. Alones and D. Mellon, unpublished observations) indicated that a group of neuronal somata just distal to the base of the HEB exhibits GABA-like immunoreactivity. Accordingly, I recorded from individual LPIs while simultaneously searching with a focal stimulating electrode for sensitive regions around the HEB that might mediate inhibition of the spontaneous LPI activity. One area, diagrammed in Fig. 12, inset, was consistently correlated with inhibition of LPI oscillations when stimulated with brief trains (4–5 pulses) of electrical stimuli at a 10-Hz frequency. PST histograms of the effects of such stimulation directed to this region on spontaneous impulses in LPIs from two different preparations are shown in Fig. 12. In each instance there was a clear and persistent inhibition of LPI spiking following approximately 50 individual repetitions of the stimulus train. These data are consistent with the results of our preliminary immunocytochemical observations and suggest that a region just distal to the base of the HEB may relay the inhibitory actions of the olfactory projection neurons.

DISCUSSION

Sensory relations of the olfactory and accessory lobes

The OLs receive direct input from aesthetasc sensilla on the lateral antennular filaments. ORNs associated with these sensilla are assumed to be olfactory receptors, as they respond, at very low concentrations, to amino acids, nucleotides, and amines in both clawed (Ache 1972; Johnson and Atema 1983) and spiny lobsters (Ache and Derby 1985; Thompson and Ache 1980). Other, nonaesthetasc chemoreceptors, as well as mechanoreceptors, are also believed to be present on both antennular filaments (Schmidt and Ache 1992; Schmidt et al. 1992). It is thought that the axons of these other receptors terminate in the lateral antennular neuropil and that some of them may make synaptic contact with local interneurons that communicate with the OL (Mellon 1996; Mellon and Alones 1995). Therefore the output of the OL projection neurons has been viewed as being primarily derived from antennular input pathways. While it is true that electrical stimulation of the antennules does not differentiate between mechanoreceptor axons and the much smaller and more numerous chemoreceptor axons, it is clear that both projection neurons and local interneurons associated with the olfactory lobes are excited in a dose-dependent manner by odorant stimuli (Mellon 1996; Mellon and Alones 1995, 1996). It is probable, therefore, that either chemo- or mechanoreceptor afferents in the antennules excite pathways that involve the olfactory lobes.

Our present understanding of the functional role played by...
the ALs is rudimentary, but previous experimental results in the crayfish *Cherax destructor* (Sandeman et al. 1995) and the lobster *Panulirus argus* (Wachowiak et al. 1996) suggest the presence of the pathways illustrated in Fig. 13. Local interneurons mediating OL input to the AL projection neurons have somata in cell cluster 9. Additional inputs to the AL projection neurons occur through neurons having synaptic contacts within the deutocerebral neuropil (DCN) and cell bodies in cluster 11, and they constitute the probable pathway through which AL projection neurons are excited by visual and tactile information from other processing regions in the protocerebrum.

Sandeman, Wachowiak, and their respective coworkers (referenced in the preceding text) suggested that the ALs process olfactory stimuli in the context of higher-order visual and tactile information, presumably through their connections with the OL and with nonchemoreceptor centers via the DCN. The current data suggest further that information about different sensory modalities is transmitted via projection neuron pathways to common targets within lateral forebrain, where our present findings show that multimodal sensory convergence occurs.

Central pathways mediating indirect olfactory lobe-accessory lobe influences on LPI activity

The existence of an afferent pathway from the OL to the AL projection neurons in *Procambarus* can be inferred from extracellular records of projection neuron activity (Fig. 9). Following electrical shocks to the antennules, the form and time course of compound action potentials recorded in the OL projection neuron tracts were similar in duration and amplitude to the antidromic action potentials generated in the projection neurons by electrical stimulation of the OGT (cf Fig. 9, A and D); furthermore, the AL response to the same antennular stimulation, which must have arisen via indirect connections through either the OL or the lateral antennular neuropil, was delayed 5–10 ms with respect to the OL response.

Data concerning the physiological properties of deutocerebral projection neurons derive from two studies in the spiny lobster (Wachowiak and Ache 1994; Wachowiak et al. 1996) and a preliminary study in *Procambarus* (Mellon and Alones 1996). Electrical stimulation of the antennular nerve, or of the antennules generated initial depolarizations with superimposed impulses in the projection neurons, followed in some cases by a prolonged inhibitory response. When present, responses of both OL and AL projection neurons to odorants applied to the antennules consisted of a brief phasic discharge of action potentials (Wachowiak and Ache 1994; Wachowiak et al. 1996). In OL projection neurons of *Panulirus*, refractoriness and/or secondary inhibition prevented a response to repetitive stimulation of the antennular nerve. Thus following stimulation of the afferent olfactory pathway in *Panulirus*, the neuronal circuitry of the OL and AL appear to pass only phasic information on to the lateral protocerebrum. Although the data from *Procambarus* are much more sparse, a similar conclusion can be drawn with respect to OL projection neurons. The functional implications of the periodic depolarizations and superimposed impulses that are so characteristic of LPIs (Mellon and Alones 1997; Mellon and Wheeler 1999; Mellon et al. 1992b) are not understood. Strong electrical or chemical stimulation of the antennules, via the projection neurons, generates brief EPSPs in an LPI that...
interact with the periodic depolarizations and can evoke impulse bursts. Because these bursts occur at the peaks of the periodic background depolarizations, and because these periodic depolarizations are synchronized in all of the LPIs in the major lobe of the HEB, we have suggested that they thereby synchronize the bursts in those LPIs that respond to a specific antennular stimulus (Mellon and Wheeler 1999). However, these synchronized responses must be truncated in time by the delayed inhibition following either antennular or antennal input.

The present data indicate that long-lasting inhibition occurs following trains of focal stimuli to either the AL or the OL; this unphysiological mode of projection neuron excitation must effectively bypass local circuitry within the OL and AL, emphasizing both the direct and the indirect effects of the projection neurons on the LPIs, including long-lasting inhibition in response to repetitive focal stimulation. Whereas antennule stimulation evokes responses in both OL and AL projection neurons, antenna II stimulation presumably generates responses only in AL projection neurons, mediated by DC neurons that have inputs within the DCN from other brain areas (Sandeman et al. 1995; Wachowiak et al. 1996). Following repetitive stimulation of antenna II, this pathway is capable of evoking inhibitory responses in LPIs that can last for many seconds. Thus it is possible that the antenna II afferent pathway, in contradistinction to that from the antennules, is capable of generating prolonged trains of impulses in those projection neuron pathways that mediate (indirectly) the inhibition of LPIs.

Actions of GABA and muscimol

The preliminary pharmacological studies described in the preceding text show that LPIs are inhibited by GABA and muscimol but not by baclofen. These data are consistent with findings by Zhainazarov et al. (1997) for properties of ionotropic GABA receptors found on projection neurons of Panulirus. They are also commensurate with the effects of GABA and muscimol on responses recorded by voltage-sensitive dye techniques from the isolated Procambarus HEB by Yagodin et al. (1999). I conclude that inhibition of LPI activity following stimulation of projection neurons is mediated by GABAergic neurons within the lateral protocerebrum; furthermore, our findings suggest that LPIs possess postsynaptic ionotropic receptors similar or identical to those identified elsewhere in the crustacean olfactory pathway.
Electrical stimulation of the projection neuron pathway and application of both GABA and muscimol dramatically decreased the amplitude of the periodic background depolarizing events that underlie the spontaneous activity in LPIs. This is presumably partially the result of an increased conductance of the LPI membrane in response to ligand binding, as is suggested by the initial reduction of electrotonic potentials generated by injected inward membrane current during trains of focal stimuli to the OL and AL. The timing of the conductance and membrane potential changes that occur following trains of stimuli to the projection neurons, and their interpretation, are complex, however. Almost all types of GABA receptors so far studied either directly activate or indirectly regulate membrane chloride channels. While it is clear that the initial response of LPIs to either single stimuli or to stimulus trains is membrane depolarization, this could be the result of GABA-induced chloride currents, since $E_{Cl}$ is close to the resting membrane potential in nearly all neurons (Hille 1984), including those in crustaceans. For example, the estimated $E_{Cl}$ in spiny lobster projection neurons is $-73.5$ mV (Zhaninazarov et al. 1997). While we have not made systematic measurements of resting membrane potentials in crustacean LPIs, incidental observations indicate that they are in the range of $-70$ to $-75$ mV. Thus it would not be surprising if repetitive stimulation of GABAergic inhibitory neurons generated modest depolarizing membrane potential changes in the LPIs. In fact, in the records of Fig. 6 it is clear that the largest membrane conductance increases occurred during the depolarizing phase of the response to the stimulus train. Furthermore, recovery of the membrane conductance to normal values following stimulation consistently occurred prior to the decay of-and in some cases even before the maximum value was attained of-membrane hyperpolarization. This sequence of events is difficult to reconcile simply with an increase in chloride conductance alone, for if the initial depolarization is due to outward (synaptic) chloride current, the hyperpolarization that follows cannot have the same biophysical basis. On the other hand, the record of Fig. 12 indicates that direct electrical stimulation of the (putative) inhibitory interneurons in the MT produces only hyperpolarizing responses in the LPIs, rendering less plausible the possibility that the initial depolarizing response to projection neuron stimulation is GABA-mediated. If the initial depolarizing synaptic potential involves a nonselective cation channel, membrane potential changes effected by a secondary GABA-induced increase in chloride conductance could be undetectable if the level of depolarization produced by the initial synaptic input were close to $E_{Cl}$. In that case, the delayed hyperpolarizing response to stimulus trains again must be the result of some other synaptic action. An obvious possibility to consider in crustaceans is the presence of presynaptic inhibition of excitatory synapses, in particular, those responsible for the periodic membrane depolarizations that are characteristic of the LPIs. Examination of the records in Fig. 5, C and D, for example, indicates that the amplitude of these depolarizing events is indeed reduced during the membrane hyperpolarization, a period during which membrane conductance is either normal or close to recovery. Co-operative pre- and postsynaptic GABA-mediated inhibition is well known at crustacean neuromuscular junctions (Dudel 1962; Dudel and Kuffler 1961). Furthermore it is important in preventing reafference during tailflip escape responses in the crayfish (Bryan and Krasne 1977), where, as a presumably GABA-mediated mechanism, it outlasts postsynaptic inhibition by up to hundreds of milliseconds (Kennedy et al. 1980). In agreement with these observations in other crustacean systems, our data show that LPI inhibition consists of two phases: a brief phase in which the LPI membrane conductance is greatly increased and a secondary phase lasting many times as long and during which the amplitude of the periodic EPSPs that normally excite the LPIs is greatly depressed.

Theoretical considerations

Stimulation of the three sense organs that influence activity in the LPIs generates an initial excitation and, in two cases (antennule and antenna II) a delayed inhibition in these hemiellipsoid cells. The net response of any LPI to olfactory input will depend, therefore, upon the sensory context in which the odor stimulus is presented to the antennules. Furthermore even though the spontaneous oscillatory activity of the entire population of ca 200 LPIs is synchronized (Mellon and Wheeler 1999), the extent to which any one LPI is modulated by olfactory and electrical stimuli to the antennules (Mellon and Wheeler 1999) and the responses of different individual LPIs to odorant and photic stimuli in the same preparation. The LPI population response to different stimulus regimes will, therefore, be difficult to predict.

Excitatory-inhibitory sequences as a response to olfactory nerve volleys seem to be characteristic of neurons in central olfactory pathways across phyla, presumably reflecting the presence of extensive neural mechanisms that narrow the focus of projection neuron responses through differentially targeted feed-forward or lateral inhibitory pathways (Christensen et al. 1995; Hamilton and Kauer 1988; Mori et al. 1981; Wachowiak and Ache 1994). These response features can also be seen in higher-level vertebrate olfactory centers. Mitral/tufted cell axons ascend the lateral olfactory tract to the piriform cortex and there make synaptic connections with superficial and deep pyramidal cells, respectively, in cortical layers II and III, and also with intrinsic neurons (Biedenbach and Stevens 1969; Haberly 1973; Haberly and Bower 1984; Nemitz and Goldberg 1983; Wouterlood et al. 1985). Among the general findings obtained by these authors is that pyramidal cells receive direct excitation from mitral/tufted cell axons following stimulation by odors. However, electrical stimulation of the lateral olfactory tract generates excitatory-inhibitory sequences within pyramidal neurons, apparently through both convergent feedback and, possibly, feedforward pathways mediated by intrinsic GABAergic neurons. There are also extensive interactions among the pyramidal neurons in the different cortical regions as well as centrifugal pyramidal fibers that descend the lateral olfactory tract to mediate inhibition in the bulb. Importantly, because sensory context plays a critical role in olfactory-mediated behaviors, the piriform cortex may be involved in the evaluation of odors in terms of their immediate relevance. This is suggested by findings in the rat that neurons in both the piriform cortex and the orbito-frontal cortex, with which it has extensive interconnections, are selectively active during odor discrimination tasks in response to temporally relevant, asso-
associated, nonodor environmental features (Schoenbaum and Eichenbaum 1995).

In crustaceans, it is presently not understood how LPIs are implicated in olfactory evoked behavior; however, the demonstration that separate, parallel multimodal pathways mediating both excitation and inhibition converge on the HEB from other brain regions provides a rich basis for informed speculation. The sensory context in which odors are perceived must be important in all animals. In female rodents, for example, an olfactory memory is formed when accessory olfactory bulb mitral cells that have been activated by male pheromones during mating become hyperexcited through the release from granule cell-mediated feedback inhibition. This occurs within the accessory olfactory bulb, through the action of metabotropic glutamate receptors (mGluR2) on the granule cells which activate a presynaptic suppression of the GABA release that normally occurs following mitral cell activity (Hayashi et al. 1993; Kaba et al. 1994). Poorly understood aspects of the sensory context associated with mating behavior are believed to prime the mGluR2 receptors involved in this memory acquisition. In this example, therefore a context-specific suppression of olfactory-induced feedback inhibition serves as the mechanism for that acquisition. In the crustacean lateral protocerebrum, different sensory modalities may enhance or inhibit the response of local interneurons to olfactory stimuli. Future studies of the projection neuron pathways in crustaceans must therefore be directed at understanding the contextual environment in which olfactory-driven activity in the HEB is modified by parallel multimodal sensory inputs.

The author is grateful to Dr. David Sandeman for useful discussions of experiments, Dr. Peter Brunjes for help with the vertebrate literature, and K. Dame for editorial assistance.

This study was supported in part by National Institute on Deafness and Other Communication Disorders Grant DC-02376 and by National Science Foundation Grant IBN-9727753.

REFERENCES


