Mapping Membrane Potential Transients in Crayfish (*Procambarus clarkii*) Optic Lobe Neuropils With Voltage-Sensitive Dyes

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1 Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, Maryland 21218; 2 Laboratory of Adaptive Systems, National Institutes of Health, Bethesda, Maryland 20892; and 3 The Babraham Institute Laboratory of Molecular Signalling, Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, United Kingdom

Yagodin, Sergey, Carlos Collin, Daniel L. Alkon, Norman F. Sheppard, Jr., and David B. Sattelle. Mapping membrane potential transients in crayfish (*Procambarus clarkii*) optic lobe neuropils with voltage-sensitive dyes. *J. Neurophysiol.* 81: 334–344, 1999. Voltage-sensitive dyes NK 2761 and RH 155 were employed (in conjunction with a 12 × 12 photodiode array) to study membrane potential transients in optic lobe neuropils in the eye stalk of the crayfish *Procambarus clarkii*. By this means we investigated a pathway linking deutocerebral projection neurons, via hemiellipsoid body local interneurons, to an unidentified target (most likely neurons processing visual information) in the medulla terminalis. Rapid (10- to 20-ms duration), transient changes in absorption with the characteristics of action potentials were recorded from the optic nerve and the region occupied by deutocerebral projection neurons after stimulation of the olfactory globular tract in the optic nerve and were blocked by 1 μM tetrodotoxin. Action potentials appeared to propagate to the glomerular layer of the hemiellipsoid body where synaptic responses were recorded from a restricted region of the hemiellipsoid body occupied by dendrites of hemiellipsoid body neurons. Action potentials were also recorded from processes of hemiellipsoid body neurons located in the medulla terminalis. Synaptic responses in the hemiellipsoid body and medulla terminalis were eliminated by addition to the saline of 500 μM Cd2+ or 20 mM Co2+, whereas the action potential attributed to branches of deutocerebral projection neurons in the hemiellipsoid body remained unaffected. Action potentials of hemiellipsoid body neurons in the medulla terminalis evoked postsynaptic potentials (50- to 200-ms duration) with an unidentified target in the medulla terminalis. Transient absorption signals were not detected in either the internal or external medulla nor were they recorded from other parts of the optic lobes in response to electrical stimulation of axons of the deutocerebral projection neurons. Functional maps of optical activity, together with electrophysiological and pharmacological findings, suggest that γ-aminobutyric acid affects synaptic transmission in glomeruli of the hemiellipsoid body. Synapses of the olfactory pathway located in the medulla terminalis may act as a “filter,” modifying visual information processing during olfactory stimulation.

**INTRODUCTION**

The optic lobes of stalk-eyed decapod crustaceans are organized for processing visual, olfactory, and tactile information. The isolated eye stalk offers a convenient preparation for the study of fundamental questions of neuronal integration including 1) afferent signal transduction and local feedback control in the visual and olfactory pathways, 2) convergence of sensory processing pathways, and 3) central feedback from the brain in the regulation of these pathways (Sandeman 1982; Strausfeld and Nassel 1980). The eye stalk contains three optic neuropils: 1) a peripherally situated lamina, 2) the external medulla, and 3) the internal medulla (Bullock and Horridge 1965). The medulla terminalis is a region of the lateral protocerebrum that receives and transforms signals from photoreceptors (Strausfeld and Nassel 1980). The glomerular neuropil of the hemiellipsoid body, the last station of the olfactory transduction pathway in decapods, is the most anterior of the protocerebral optic neuropils. Microglomeruli of the hemiellipsoid body contain extensive tracts of fine neuronal processes from deutocerebral projection neurons located in the olfactory midbrain and receive input from primary chemosensory antennal afferents (Hänström 1925; Sandeman 1982; Sandeman and Luff 1973). Thus the hemiellipsoid body and medulla terminalis are regions with potential for integration of optical and olfactory signals.

Electrophysiological studies (Mellon et al. 1992b) revealed that deutocerebral projection neurons synapse with dendrites of local hemiellipsoid body neurons in glomeruli of the hemiellipsoid body. These neurons, some of which are electrically coupled, generate periodic bursts of action potentials and can be driven synaptically in microglomeruli by stimulation of the olfactory–globular tract (Mellon et al. 1992b). Morphological studies (Mellon et al. 1992a) indicate that hemiellipsoid body neurons with their cell bodies located in the medulla terminalis send numerous processes into the glomeruli and somewhat fewer into the medulla terminalis. Physiological evidence concerning the output of hemiellipsoid body neurons is lacking. It also remains to be determined if stimulation of the olfactory–globular tract can elicit electrical responses in particular regions of the optic lobes other than the hemiellipsoid body and medulla terminalis.

Intracellular microelectrode recording from crayfish eye stalk and other preparations is limited by the difficulty of recording simultaneously from more than a small number of neurons. For functional mapping of transient membrane potentials in the optic lobes, we therefore utilized a photodiode array in combinations with voltage-sensitive dyes, an approach used successfully in other preparations for spatially resolved, optical measurements of action potentials and synaptic potentials in neural networks of vertebrates and inverte-

Here we used voltage-sensitive dyes to study pathways of deutocerebral projection neurons in the medulla terminalis of the crayfish eye stalk. Our studies illustrate the utility of voltage-sensitive dyes in the analysis of neuronal function and pharmacology in the arthropod brain. Some of the results have been published in abstract form (Yagodin et al. 1997).

MATERIALS AND METHODS

In vitro preparation

Juvenile crayfish (Procambarus clarkii) were maintained at room temperature in continuously aerated freshwater. Eye stalks were removed surgically and transferred rapidly to normal saline of the following composition (in mM): 205 NaCl, 5.3 KCl, 13.5 CaCl\_2, 2.5 MgCl\_2, 5.0 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, pH 7.4, adjusted with NaOH. The neural axis of the eye stalk was cleaned of surrounding muscle and connective tissue and transferred to an experimental chamber having a silicone rubber floor. The preparation was fixed in place by broken tips of glass micropipettes, and the severed end of the optic nerve was drawn into a glass suction electrode for electrical stimulation. For optical measurements in which dry objectives were used, a coverslip was placed over the preparation and fixed in place by wax. The preparation was perfused with saline except during optical recordings. All experiments were performed at room temperature (19–21°C).

Potential-sensitive dyes staining

Isolated preparations were incubated for 15–20 min in normal saline containing one of two potentiometric dyes, either RH155 (Molecular Probes, Eugene, OR) or NK 2761 (Nippon Kankoh-Shikiso Kenyusho, Okayama, Japan) at a concentration of 0.1–0.4 mg/ml. After staining, preparations were washed with at least three changes of normal saline.

Electrical stimulation and recording

Monophasic, rectangular voltage pulses (0.1- to 0.6-ms duration; 1- to 9-V amplitude) were applied to the optic nerve via the suction pipette. The stimulation frequency was 2 Hz for most experiments in which transient absorption signals were averaged. This frequency was found to be without effect on the amplitude of the optical signals recorded from the optic nerve and the hemielipsoid body. Extracellular recordings from the optic nerve and the hemielipsoid body were obtained with 100- to 200-μm inner diameter plastic suction pipettes in an en passant configuration.

Optical measurements

Multisite optical recording of transmembrane voltage was performed by imaging the dye-stained preparation on a photodiode array with the method first described by Grinvald and colleagues (1981). The optical recording system contained an array of 120 silicon photodiodes on a 12 × 12 square grid (MD-144-4PV; Centronic, Croydon, UK). This array was located in the image plane of a Zeiss UEM microscope (Carl Zeiss, Germany). Light from a tungsten-halogen lamp (100 W, 12 V) was passed through a water heat-filter and a widebandwidth interference filter (in most experiments 710 ± 35 nm). Light was collected with a ×1.6 intermediate lens and either a dry objective [Epiplan-HD, ×8, 0.20 numerical aperture (NA): Epiplan-HD, ×16, 0.35 NA; Epiplan-UD, ×40, 0.65 NA; all from Carl Zeiss, Germany] or a water-immersion (×30), high (0.9) NA objective (LOMO, Russia), forming an image on the photodiode array. The current from each photodiode was converted to a voltage (with an I/V converter with a 5 MΩ feedback resistance), amplified, and filtered through a high-pass filter (Physiology Department, Yale University) with a time constant of 500 ms. The 120 amplified signals from the photodiode array were multiplexed, digitized (12 bits) at a maximal rate of 2,880 frames/s, and stored in a computer (VME-bus Motorola 68030 with the Versados operating system). Typically, the responses of five consecutive stimulus pulses (2-Hz repetition rate) were averaged to reduce noise. Data acquisition and analysis software were those developed in the laboratory of Professor L. B. Cohen (Yale University, New Haven, CT).

Chemicals and statistics

All chemicals used in the preparation of salines and all pharmacological agents were obtained from Sigma Chemical (St. Louis, MO). All the results are expressed as means ± SE.

RESULTS

Types of transient absorption changes recorded from the optic lobes

For both dyes (RH 155 and NK 2761), three types of absorption signal were recorded from the eye stalk after orthodromic electrical stimulation of the optic nerve (Fig. 1): 1) non-dye-related light scattering, 2) dye-related passive membrane responses, i.e., electrotonically conducted stimulus artifacts, and 3) dye-related active membrane responses (action and synaptic potentials).

Non-dye-related changes in absorption were seen at high stimulus intensities (e.g., 0.1-mA, 9-V pulses). They were also recorded with white light or at a wavelength (800 nm) that is outside the action spectrum of the voltage-sensitive dyes. The amplitude of these dye-independent optical signals was greatest at the edges of the preparation, consistent with changes in light scattering (data not shown). Light scattering signals of this kind were described for other preparations (Grinvald et al. 1982; Kamino et al. 1990; Konnerth et al. 1987; London et al. 1987; Orbach and Cohen 1983;). These artificial signals, possibly related to tissue movements at the edge of the preparation, are quite distinct from the light scattering changes associated with peptide release from neuromuscular junctions that are of considerable physiological interest (Salzberg et al. 1985).

Dye-related passive (Fig. 1A) and active (Fig. 1, A and B) optical responses were observed at wavelengths within the triphasic action spectrum of the dye, between 520 and 735 nm, found previously in mammalian (Cinelli and Salzberg 1990; Grinvald et al. 1982; Morad and Salama 1979), elasmobranch (Konnerth et al. 1987), and invertebrate (Ross and Reichardt 1979; Senseman and Salzberg 1980; Yagodin et al. 1989) preparations. In contrast to active membrane optical responses, the passive membrane responses propagated decrementally from a stimulus application site reversed their polarity after inversion of stimulus polarity and were insensitive to 1 μM tetrodotoxin (TTX).

Two types of dye-related, TTX-sensitive absorption signals (fast, 10- to 20-ms duration, and slow, 50- to 200-ms
FIG. 1. Properties of transient absorption optical signals in crayfish eye stalk preparations stained with RH155 (A and C) and NK 2761 (B) elicited by orthodromic stimulation of the optic nerve. A: dye-related and voltage-dependent active and passive optical responses recorded from the optic nerve. Each trace represents changes in absorption from adjacent detectors (1–10) in the selected row of elements in the photodiode array, as indicated. Direction of signal propagation from the stimulation site is indicated by arrow. Compound optical action potentials (top row of traces) were abolished in the presence of 1 μM tetrodotoxin (TTX, middle row of traces), with only the electronic stimulus artifact remaining. These passive dye-related membrane responses disappeared when the transmission light was changed from 710 to 800 nm (bottom traces).

B: wavelength dependence of the absorption changes recorded from the optic nerve. There was no detectable optical signal at 430 nm (data not shown). The following interference filters were used: 430 {10 nm; 520 {15 nm; 710 {35 nm; and 735 {25 nm. For 800-nm light transmission a cutoff glass filter FS1; RG. 830 (Newport, Fountain Valley, CA) was utilized.

C: amplitudes of optical responses recorded in the hemiellipsoid body (left traces), and in the optic nerve (optical compound action potentials; right traces) were reduced after 20 min in Ca2+-free media [no ethylene glycol-bis(β-aminoethyl ether)-N,N',N,N'*-tetraacetic acid added], and the long-lasting component (N3) in the hemiellipsoid body optical response was abolished (2nd row of traces). Responses from control experiments are shown (top traces). After rebathing the preparation in normal saline responses were restored (traces in third row). The addition of 5 mM Cd2+ to the saline eliminated N2 and N3 peaks in the hemiellipsoid body optical response. No change in amplitude was observed for N1 peaks from the hemiellipsoid body. Similarly, the amplitude and shape of dye-detected action potential recorded from the optic nerve was unchanged (bottom traces). The point of application of the stimulus pulse (9 V; 0.08 ms) is indicated by arrows. In each of the optical traces presented, results of 25 (A and C) and 10 (B) sweeps were averaged.

Estimated conduction velocities in the optic nerve and within the hemiellipsoid body

A montage of pseudocolor images indicated that the wavefront of the transient absorption signal passed along the optic nerve resulting in a strong signal in the hemiellipsoid body (Fig. 2A). The wavefront of the optical action potential moved along the optic nerve at an estimated rate of about one pixel (64 μm per two frames (740 μs), corresponding to an average conduction velocity of approximately 0.1 m/s. This optical wave was observed to travel within the hemiellipsoid body at a similar velocity. This can be seen in Fig. 2B. The wave (peak N1) traversed a distance of 340 μm and reached detector 6 ≤4 ms, corresponding to a conduction velocity of 0.09 m/s.

Functional mapping

In an attempt to discover which regions of the eye stalk are involved in processing olfactory information, transient absorption changes were recorded from 40 preparations in duration) were recorded in the hemiellipsoid body, and only a fast component (10- to 20-ms duration) was measured in the optic nerve. In the absorption signals recorded from the hemiellipsoid body, a fast component (peak N1, Fig. 1C) was followed by a complex slow component on which was superimposed additional peaks N2 and N3. All the transients (peaks N1, N2, and N3) were sensitive to extracellular Ca2+, but the addition of either 5 mM Cd2+ (a concentration 10 × higher than the concentration necessary to block all types of plasma membrane Ca2+ channels) or 20 mM Co2+ (data not shown) inhibited only the later transients (peaks N2 and N3) in the hemiellipsoid body (Fig. 1C). The small reduction in amplitude of peak N1, observed only after 20 min in Ca2+-deficient saline, probably reflected the progressive depletion over prolonged period in low Ca2+-saline of [Ca2+] in deutocerebral projection neurons.

The TTX sensitivity of the initial (peak N1) and later (peaks N2 and N3) transient absorption signals together with the Cd2+/Co2+ sensitivity of peaks N2 and N3 indicated that initial and slow wave signals reflected respectively action potentials and synaptic potentials.
response to electrical stimulation of the optic nerve, which contains fibers of the olfactory globular tract as well as fibers processing visual information. As shown in Fig. 3A, after electrical stimulation of the optic nerve absorption transients with characteristics of polysynaptic potentials were observed in only two regions (~400-μm diam) of the optic lobes. The transient absorption signals from the hemiellipsoid body can be detected in a single trial, whereas to reveal signals from the medulla terminalis it was necessary to average several trials. No changes in absorption in any other areas of the crayfish optic lobes were detected, even after signal averaging 100 trials.

Analysis of the optical signals with respect to the morphology of the hemiellipsoid body and medulla terminalis revealed a distinct pattern of transient absorption responses that is similar to the distribution of processes of hemiellipsoid body neurons (Fig. 3, B and C, and Fig. 4). A bell-shaped distribution of signal amplitudes over the hemiellipsoid body is to be expected because of the rounded shape of the hemiellipsoid body itself, where the vertical track column exposed to a photodiode above the central part of the structure includes more synaptic arborizations relative to the track columns of the peripheral regions (Fig. 4B). Optically recorded action potentials appeared as a shoulder in the traces and were followed by postsynaptic potentials (PSPs) in dendrites of the hemiellipsoid body neurons (peaks N2 and N3), which were shown to be blocked by Cd²⁺ (Fig. 1C).

Transient absorption signals recorded from a region of the medulla terminalis also showed additional fast (peak N4;
FIG. 3. Multiple-site optical recording from the optic lobe of crayfish preparations stained with the voltage-sensitive dye RH 155 (200 μM). Changes in absorption in response to orthodromic electrical stimulation of the lateral protocerebral tract were detected with a 12 × 12 element photodiode array. Each trace represents membrane potential changes recorded by a single photodiode receiving light from 128 μm × 128 μm region of the preparation. Signals were recorded from 2 positions (I and II) on the preparation. Signals recorded in response to 25 consecutive stimulus pulses were averaged from each position in the optic lobes. A: overall view of the experiment. The recordings were obtained with 7-V, 0.2-ms voltage pulses applied to the optic nerve (on) with a suction pipette. The signals from a region restricted by a box were amplified and displayed in a location indicated by the arrow. Note that large optical signals were detected only in the hemiellipsoid body (he) and small signals could be distinguished in the medulla terminalis (mt) but not in other parts of the optic lobes; lamina (la), external medulla (em), and internal medulla (im). B: schematic representation of the cellular morphology of the he neuron (after Mellon et al. 1992a). Deutocerebral projection neurons (dpn) located in the lateral part of the deutocerebrum, form multiple synapses in the hemiellipsoid body. Positions of selected photodetectors, shaded on the scheme on A, were superimposed. C: recordings are from photodetectors identified in B, in response to a stimulus pulse occurring at the time indicated by the arrow below trace 6. The amplitudes of the traces were arbitrarily scaled to emphasize the latency of the responses. The optical signal propagated along a morphologically defined loop from the region 1 via loci 2, 3, 4, and 5 to region 6. There was no propagation of the signal from the region 1 directly to region 6.

10–20 ms in duration) and slow (peak N5) components (Fig. 3). Fast transient N1 with a similar duration of 10–20 mS, which appears as a shoulder in traces recorded from the hemiellipsoid body, and the fast transient N4, which appears as a separate peak in traces recorded from medulla terminalis, may indicate that there are action potentials generated by deutocerebral projection neurons and hemiellipsoid body neurons, respectively.

**Effects of stimulus strength on optically recorded activity in the hemiellipsoid body**

The amplitude of the signal detected by a single photodetector is proportional to the amount of membrane area activated, the amplitude of potential change in distinct parts of each neuron, and the amount of potential-sensitive dye bound to cellular membranes (Cohen and Lesher 1986; Kamino et al. 1989; Salzberg 1983). Thus increasing either the stimulus duration or the intensity of the applied current should activate more neurons, thereby increasing the area over which a voltage-dependent, dye-related signal is detected. An example of this effect was observed in chick embryonic brain stem after electrical stimulation of the vagal nerve (Kamino et al. 1990). It was of interest therefore that the amplitude of the signals recorded in the central or peripheral parts of the hemiellipsoid body increased in response to either enhanced stimulus duration (Fig. 5A) or higher stimulus intensity (Fig. 5B), but the boundaries of the locus remained unchanged, even when very high optical
patterns of activity in the hemiellipsoid body were imaged. Inactive detectors ionotropic GABA receptors (Sattelle 1990), inhibited electrically induced response recorded from the optic nerve and completely blocked transient absorption signals in the hemiellipsoid body (Fig. 6).

Synchronous optical and electrophysiological recordings were also obtained from the same region of the hemiellipsoid body in the absence and presence of 100 μM GABA (Fig. 7A). The filter time constants for both electrical and optical recordings were the same (500 ms). Before treatment of a preparation with GABA, the fast (10- to 20-ms duration), TXX-sensitive action potential was recorded with a suction electrode, along with the fast component of the optical signal (N1). The later transients (peaks N2 and N3) were detected in both electrical and optical recordings made from the hemiellipsoid body. After the addition of 100 μM GABA, all peaks disappeared from both the optical and electrical traces in 12 of the 15 preparations studied. A 10-min wash restored the responses. The typical action of GABA is shown in Fig. 6. In 2 of the 15 preparations studies, 100 μM GABA inhibited only the N1 peak (Fig. 7B), and in 3 of the 15 preparations studied the neurotransmitter had no effect. Extracellular Ca2+ removal [1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid] together with 500 μM Cd2+ eliminated the slow component of the optical response (n = 5; Fig. 7C). Addition of either 100 μM ACh or 100 μM Glu to the medium after recovery (n = 3) had no detectable effect on optically recorded synaptic signals (data not shown).

Treatment of the optic lobes with 30 μM muscimol (n = 5), an ionotropic GABA receptor agonist, also blocked reversibly transient absorption signals corresponding to compound action potentials in the optic nerve in a dose-dependent manner (Fig. 7D). The same concentration of muscimol also blocked (n = 5) optical response in the hemiellipsoid body completely, whereas 1 mM baclofen, a selective agonist of certain GABAγ receptors was without effect (Fig. 7E). Bicuculline (100 μM), a vertebrate GABAγ-type receptor antagonist and blocker of some but not all invertebrate ionotropic GABA receptors (Sattelle 1990), inhibited electrically evoked GABA-mediated response in the hemiellipsoid body only by 15% (n = 26; Fig. 7, F and G), and the addition of 100 μM picrotoxin, an effective blocker of GABA-related Cl− channels, only partially, 26% (n = 40), inhibited the electrically induced response recorded from the hemiellipsoid body (Fig. 7G).

**DISCUSSION**

**Mapping activity in the optic lobes**

Even by signal averaging 100 trials, optically recorded activity could only be detected in restricted loci of the hemiellipsoid body and medulla terminalis and not at all in other regions of the optic lobes (Fig. 3). These findings indicate that the hemiellipsoid body and medulla terminalis represent the last level of olfactory signal processing in the crayfish.
FIG. 5. Mapping of stimulus strength in the hemiellipsoid body stained with NK 2761 (A) and RH 155 (B). A: sequential pseudocolor images of electrically evoked optical activity patterns elicited by pulses of 9 V, 0.6 ms (top row) and of 9 V, 0.2 ms (bottom row). Position of the photodiode array with a background optical activity pseudocolor is depicted in the top left diagram. Optical traces above the montages represent the output of photodetector 46 in these 2 experiments. Subsequent frames indicated by the numbers follow at 2-ms time intervals. The image at the center of each row has the regions with highest optical activity outlined to stress the point that, in spite of the differences in amplitude and shape of the optical signals (see traces above the montage), the region of highest optical activity remains the same. B: 3-D representation of N2 peak amplitudes in the hemiellipsoid body in response to stimuli: 4 V, 0.2 ms (top plot and optical traces), 5 V, 0.2 ms (middle), and 7 V, 0.2 ms (bottom). The traces on the right depict the outputs of the 2 photodetectors registering respectively the optical signals from the central part of the hemiellipsoid body and from the edge of the structure. Time frames for 3-D plots correspond to the maximal amplitude of the N2 peak. The locus of the optical activity did not spread when the intensity of the electrical stimulus was increased.

The neuropils of the medulla terminalis and hemiellipsoid body are not geometrically structured, and each cell may be unique in its shape and projection (Sandeman 1982), but spherical microglomeruli where deutocerebral projection neurons synaptically interact with hemiellipsoid body neurons form a 20- to 30-μm thick layer at the surface of the structure (Mellon et al. 1992b). With the spatial resolution 34 μm × 34 μm used for our experiments we could not distinguish optical signals from a single glomerulus with a diameter of ~5 μm, but the resolution was sufficient to suggest that there are no aggregations of glomeruli into “barrels” >34 μm.

The failure to detect any spread of excitation from the hemiellipsoid body to neighboring regions when either the intensity or the duration of the stimulus was increased (Fig. 5) also indicates that the glomerular layer may be synaptically isolated from other areas of the optic lobes. Results similar to these were obtained on a vertebrate preparation, the olfactory bulb of skate Raja erinacea where the contour of the functional map of voltage-sensitive signals with the bulb remains unchanged when higher intensity pulse trains of electrical stimuli are applied to the olfactory nerve (Cinelli and Salzberg 1990), whereas an increase in stimulus intensity of the vagal nerve the spread of excitation in the embryonic brainstem has been shown (Kamino et al. 1989, 1990).

The TTX-sensitive peak N1 of the transient absorption signal from the hemiellipsoid body, which is attributed to deutocerebral projection neuron axons, spreads within this structure with a conduction velocity (0.1 m/s) similar to that of the compound action potential measured in the optic nerve (Figs. 2 and 3). This value is 10 times faster than the conduction velocity previously reported in Procambarus axons (Mellon et al. 1992b) and is close to a value of conduction velocities (0.32 ± 0.41 m/s) in the skate olfactory system (Cinelli and Salzberg 1990) but considerably lower than those observed in the turtle olfactory tract (Bliss and Rosenberg 1974; Waldow et al. 1981). The rate of rise and the amplitude of the optically recorded compound action potential declined at the entrance to the hemiellip-
FIG. 6. Ten micromolar γ-aminobutyric acid (GABA), but not 100 μM acetylcholine or 100 μM L-glutamate, blocked electrically evoked optical responses recorded from the hemiellipsoid body stained with RH 155. Five recordings were obtained from the same preparation; 25 trials were averaged in each experiment. Amplitudes of N2 peaks from these recordings are depicted in the histograms, where the signals from 30 regions of the preparation were analyzed. In the presence of 100 μM GABA, the amplitude of the compound action potential was reduced in the optic nerve region (the right part of the preparation) and was eliminated completely in the hemiellipsoid body (the main region where optical signals were recorded). The stimulus intensity in all experiments was 9 V, 0.6 ms.

soid body (frames 53 through 62, Figs. 2 and 4B), which may be due to 1) altered spatial separation of axons within the structure or 2) an increase in thickness of the preparation in which the number of neuronal processes remains constant.

Effects of GABA

Analysis of optical traces recorded from the optic lobes and the optic nerve revealed that 100 μM GABA inhibited optically recorded action potentials over the entire length of the nerve as well as suppressed long-lasting synaptic potentials in the hemiellipsoid body (Fig. 7). These complex and unexpected findings may be attributable to a direct presynaptic axonal inhibition for the following reasons. 1) High concentrations of GABA block optically recorded PSPs as well as action potentials in the hemiellipsoid body (Figs. 6 and 7A). 2) GABA (not shown) and the ionotropic GABA receptor agonist muscimol (Fig. 7D) inhibited transient absorption responses in both the optic nerve and the hemiellipsoid body in a dose-dependent manner.

Many invertebrate ionotropic GABA receptors are bicinephalen- and bicuculline-insensitive, picrotoxin-sensitive, GABA-gated chloride channels and differ from GABA C receptors in their sensitivity to certain allosteric modulators and GABA analogs, although bicuculline-sensitive ionotropic GABA receptors were also described (for review see Sattelle 1990). There are few studies on invertebrate
FIG. 7. Effects of GABA, putative agonists, and antagonists on the compound action potential and synaptic potentials recorded from the optic nerve and the hemiellipsoid body stained with RH-155. A: simultaneous optical (top trace) and extracellular electrical recordings (bottom trace) from the hemiellipsoid body in control conditions (left traces), in presence of 100 μM GABA (middle traces) and after 10 min wash in normal saline (right traces). The photodetector of the array received light from 34 μm × 34 μm area of the hemiellipsoid body. A suction pipette in en passant configuration was used for electrical recording from the hemiellipsoid body. The time constant of the amplifiers used for both recordings was 500 ms. The time at which the stimulus pulse (0.2 ms; 1 mA) was applied is indicated by an arrow under the left bottom trace. B: electrically evoked transient absorption changes in the optic nerve and the hemiellipsoid body in control conditions (top montage) and in the presence of 100 μM GABA (bottom montage). Stimulus intensity: 9 V, 0.4 ms. An inactive photodetector is indicated by an asterisk. GABA inhibited TTX-sensitive N1 peaks (indicated by arrows) and did not affect the long-lasting Ca²⁺/sensitive components of the optical response in the hemiellipsoid body. The photodetector received light from 34 μm × 34 μm square of tissue. GABA (100 μM) had no effect on the optical signal. D: muscimol dose dependence of optical signals recorded in the optic nerve and the hemiellipsoid body. Each photodetector received light from 128 μm² of tissue. E: baclofen (1 mM) did not affect electrically evoked optical potentials in the hemiellipsoid body but 10 μM muscimol did. The optical recordings were from 2 detectors receiving light from 34 μm × 34 μm regions of the preparation. F: effect of 100 μM bicuculline on electrically evoked optical responses recorded from the hemiellipsoid body. The time taken for recovery in normal saline was 15 min. G: effects of picrotoxin and bicuculline on the amplitude of optical responses recorded in the hemiellipsoid body. Histograms depict the percentage of a signal amplitude inhibition in comparison with control data. The results are shown as means ± SE. Signals from 40 and 26 regions, respectively, were analyzed in each experiment on picrotoxin and bicuculline.

GABAₐ-like receptors, and these include examples of receptors that are insensitive to a vertebrate GABAₐ receptor agonist, baclofen (Bai and Sattelle 1995; Sattelle 1990). Optical signals detected in the hemiellipsoid body in our experiments were baclofen insensitive (Fig. 7E) but muscimol (Fig. 7, D and E) and PTX (Fig. 7G) sensitive and were only slightly affected by bicuculline (Fig. 7F), resembling the ionotropic GABA receptor described for lobster olfactory projection neurons (Zhainazarov et al. 1997). GABA-mediated responses in the crayfish optical pathway were found to be sensitive to bicuculline (Pfieffer-Linn and Glantz 1989a, 1991), but several other crustacean GABA receptors are insensitive to this receptor antagonist (Marder and Paupardin-Tritsch 1978). This may account for our findings with bicuculline. The pharmacological results presented here indicate that ionotropic GABA receptors contribute to some of the observed effects of GABA.

The simplest explanation of insensitivity of optically re-
corded PSPs to GABA-induced reduction in optical action potential amplitude (Fig. 7B) is that GABA sensitivity does not reside in axons of deutocebral projection neurons. If such GABA receptors are located presynaptically, the question of the main neurotransmitter between deutocebral projection and hemiellipsoid body neurons remains open. Our findings appear to argue against a role for either Glu or ACh. However, possible problems of neurotransmitter access and/or breakdown in this preparation counsel caution in any such interpretation (Fig. 6). The sensitivity of optic lobe action potentials to GABA is of interest and parallels may be seen in the GABA_A-receptor mediated modulation of axonal conduction in developing rat optic nerve (Howd et al. 1996; Sakatani et al. 1992).

Negative feedback control in the optical pathway

There are several reasons local hemiellipsoid body neurons are important for optical and olfactory information processing. 1) They are the last neurons in the pathway for processing olfactory signals in crustacean nervous system (Seman 1982; Strausfeld and Nasser 1980); 2) they make synapses with unidentified neurons in the medulla terminalis (Mellon et al. 1992a). In support of the existence of an inhibitory loop in glomeruli is our finding that application of GABA blocked optically detected TTX-sensitive action potentials. The existence of a second inhibitory loop in the medulla terminalis is suggested by optical signals detected in this locus of the optic lobes where presynaptic boutons of hemiellipsoid body neurons with an unidentified target were found (Mellon et al. 1992a) and immunoreactivity to ACh was shown (Wang-Bennett et al. 1989). ACh is a major inhibitory neurotransmitter in the crustacean optical pathway (Pfeiffer-Linn and Glantz 1989a,b, 1991; Wang-Bennett et al. 1989). The results obtained in this study and elsewhere (Mellon et al. 1992a,b; Wang-Bennett et al. 1989) suggest two possible levels of negative feedback control of optical pathways by olfactory signals, 1) within glomeruli where GABA may act presynaptically to inhibit action potentials and 2) in the medulla terminalis where ACh may inhibit fibers of the optical pathway. Thus these two levels of inhibition could offer a basis for modulation of optical information flow by activation of a neuronal pathway processing olfactory information.

Prospects

Newly developed devices with photodiode arrays having 256 (Iijima et al. 1992), 448 (Nakashima et al. 1992), 464 (Falk et al. 1993), and 1,020 photodetectors (Hirota et al. 1995) offer significantly improved spatial resolution. Ultrafast (tens of microseconds time resolution) recording of electrical activity were now obtained (Rohr and Salzberg 1994). Nevertheless, even the relatively low spatial resolution (120 pixels of the photodiode array) used in our experiments was sufficient to address the questions posed.

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