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Octopus Optic Responses

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A study of responses in *Octopus vulgaris* visual system was carried out. Gross recordings were made in the free swimming animal responding to brief flashes of light. A simple negative electroretinogram was recorded within the eye beginning at 8 msec peaking at 20–25 msec. With the animal resting, the amplitude of the ERG could be increased by gentle tactile stimuli which produce no movement or pupil change. Immediately behind the retina, an oscillating potential was recorded beginning 15 msec after the flash and lasting for about 40 msec with a frequency of about 150 per second. This was believed to be produced by synchronized volleys in the optic nerve fibers travelling at about 1 m per sec. In the optic lobe, one could record the ERG, the arriving oscillatory volley and a gross response starting at 30 msec after the flash and peaking 20 msec later.

Introduction

Octopus' eyes are the largest of any invertebrate and are optically similar to those of vertebrates. There is an anterior chamber, a pupil with a horizontal slit, a lens and a posterior chamber. There are eye lids and there are external ocular muscles which, together with the statocysts, maintain the pupil slit horizontal whatever position the body takes (8). The retina is unlike that of vertebrates. It consists of a single layer of receptor cells with the light-sensitive ends facing toward the lens and with the optic nerve fibers running directly centrally through the sclera. These receptors are packed together in a rectangular array. The outer segments of the long retinular cells are convoluted by microvilli possibly comparable to the rhabdomes of the arthropod eye. Each receptor narrows at its base, loses the pigment and passes through the basement membrane of the retina.

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Then it expands again into a cell body with a nucleus from which the axon emerges. The beginning of the axon has a number of short side branches on which other nerve fibers end which are believed to be efferents to the retina (9). This site could also provide for interconnection between retinal elements. The optic nerve fibers which emerge in bundles from the deep side of the sclera run into an optic lobe situated immediately medial to each eye. The fiber bundles carry out a dorsal ventral decussation so that the dorsal part of the retina projects to the ventral part of the optic lobe (10). The optic lobe is unusual among invertebrate ganglia in that although it has a peripheral cortex of perikarya with processes projecting centrally, it also has a center consisting of many multipolar cells forming islands amongst ingoing and outgoing fibers. Fibers leave the optic lobe in the optic tract and project to the opposite optic lobe and to most of the supraesophageal parts of the brain.

The large amount of behavioral and neuroanatomical data now available for octopus (for summary: 10) present an attractive opportunity for electrophysiological analysis of its visual mechanisms. Unfortunately, there are a number of technical problems which have restrained electrophysiological studies. Octopus has no skeleton except for a cartilaginous cranium. This is distortable and fragile so that it cannot be used for restraint or to stabilize heavy electrodes with respect to the nervous system. Furthermore, the dexterity of octopus arms and the elaborate skin, body and eye musculature constantly tend to move electrodes and there is no known curarelike agent for invertebrates. It is, therefore, necessary to develop other methods of stabilization. Another problem is related to the blood supply. The blood contains no clotting mechanism and therefore even minor damage is counteracted by local vascular spasms which may shut off all activity in the region under observation. The third problem is an anatomical one. The cell bodies, unlike vertebrate nerve cells, are not interposed between a dendronal input region and an axonal output. Instead, a single extension of the cell body branches richly in a neuropil region containing both afferents and efferents so that there are slim chances of observing the details of the origins of the cell's activity. The tracts of fibers running between the cell body neuropil regions are made up mainly of small diameter fibers.

Faced with these problems we decided to examine first the gross responses from the visual system in unrestrained animals with minimal operative damage so that we could judge the normality of unit responses recorded in isolated and immobilized preparation. For this purpose we used brief flashes of light which synchronize large numbers of cells to produce easily

recorded potential changes. Readers of previous work by these authors need not be encouraged that we have changed our views on the artifact-generating nature of brief massive stimuli. They are used here only to produce a large easily recorded response which provides a first indication of the location and time of responses to visual stimuli.

Methods

Octopus vulgaris were caught and kept as described by Boycott (2). Anesthesia was by immersion in 2.0% Urethane in sea water. A single midline incision was made over the cranium and the subcutaneous space opened by blunt dissection to expose the membranes which cover the orbit. A single incision through these membranes exposes the white body. This was gently deflected laterally, thus keeping most of the blood supply intact. This maneuver exposes the back of the eye, the optic lobe, and the upper part of the wall of the orbit. The electrodes to be used were 100-μ insulated copper wire (General Electric Formex). It was found that kinks placed in this wire by the animals' moving were enough to break its insulation but that baking the wire at about 160 C for 12 hours hardened the coat and prevented breaks. A length of the wire was threaded by needle through the dorso-lateral part of the skull. Up to four electrodes were used simultaneously in the eye and optic lobe. Next a 0.5-mm sphere of dental impression cement was fused to the wire which had passed through the skull: This prevented any further withdrawal. The wire now cut off at a suitable length and thrust into the structure from which we wished to record. Incisions into the orbital membranes were not closed by sutures since this produced irritation and exploration of the wound by the arms. Allowing the skin and its musculature to slip back over the skull was sufficient protection. The skin incision was lightly sutured. Electrodes within the eye could be observed ophthalmoscopically. At the end of the experiment, the animal was killed and the location of the tip was inspected and insulation checked. Since we could obtain consistent recordings for hour-long periods, it was evident that the electrodes were not moving during the recording periods. Animals allowed to survive for several days showed a slight whitening of regions in the optic lobe around the electrode position. The visual-motor reactions of these surviving animals seemed grossly normal.

After implantation of electrodes, the animals were resuscitated by massage until spontaneous breathing movements resumed and then placed in a tank of cooled, 20 C, aerated sea water. About 1.5 m of wire led from the animal to conventional amplifying and recording equipment. The animal

could swim freely within the confines of the tank trailing the light wires. Brief, less than 0.1-msec, light flashes were generated by a xenon discharge in a General Radio Strobotac and directed at the animal through the side of the tank.

Results of Gross Recording of Responses

Within the Eye. An electroretinogram is recorded between a single electrode within the posterior chamber of the eye and the sea water. It is a simple negative monophasic wave opposite in sign to the vertebrate ERG which perhaps fits in with the fact that the photoreceptive elements are also oriented in the opposite direction (Fig. 1). This response has been extensively studied by many authors (1, 3, 4, 6, 7). Historically it was the first ERG to be recorded. When evoked by brief flashes in the intact animal, its latency is less than 8 msec with a peak at 20–25 msec. Its sign is always negative irrespective of which part of the retina is illuminated and we have never observed oscillations within it either during brief flashes or continuous illumination. During steady illumination with the optic nerves cut there is a steady response but in the intact animal there are transients during the on and off of the light. The lower trace in Fig. 1 shows an example of this during steady illumination by a 60-watt bulb at 3-m distance. Furthermore, during the illumination there are irregular variations. The transients and the irregular variations are at least partially explained by variations of pupil diameter.

The most interesting variations of amplitude of the flash evoked ERG were seen when the animal had settled down and was sitting quite stationary while being illuminated once a second by the flashes. There was a steady background illumination of the animal sufficient to allow close observation of eye, pupil, and body movements. Under these quiet conditions, the amplitude of the flash-evoked ERG would settle down to an extremely steady height for 10–15 min periods. Now if an arm was gently touched or there was a stamp on the floor, there were no signs of head, eye or pupil movements but the amplitude of the ERG would increase by as much as 100%. Further stimuli would arouse the animal to move but it would soon settle back to the still position and the ERG could again be increased by gentle stimuli without motor signs. This observation strongly suggests that in this animal the receptor cells themselves are subject to efferent control since there is ample evidence that they are the generators of the negative wave.

Behind the Eye. Monopolar recording immediately behind the eye produces an ERG similar in shape to that seen within the eye. If the electrode

is behind the illuminated part of the retina, the sign of the potential is the opposite that within the eye. If the electrode is behind a dark part of the retina, the sign is reversed and therefore bipolar recording behind the eye can give biphasic recordings of the electroretinogram. As the animal continues to recover from the anesthesia, a new phenomenon appears: Fast oscillations ride on top of the ERG (Fig. 2). They are never seen in front

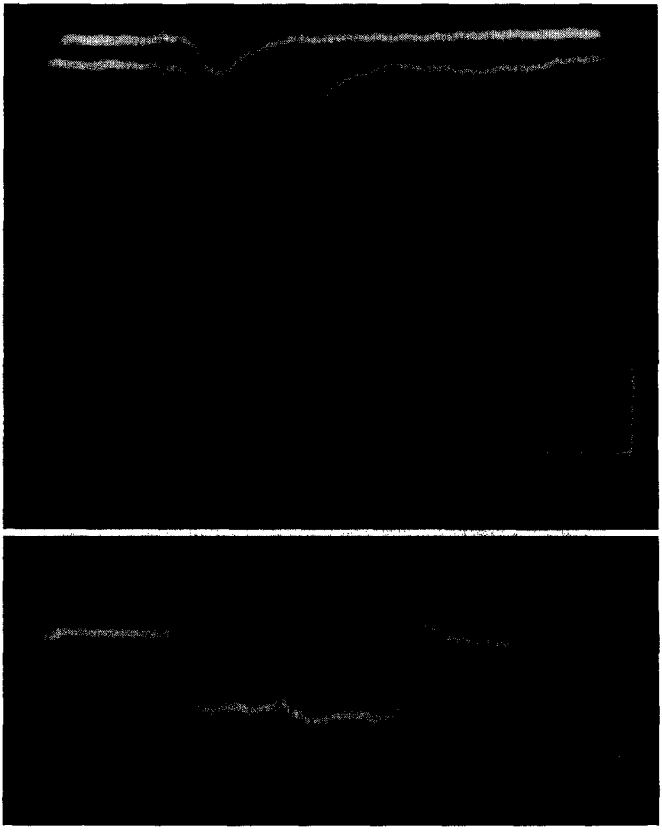

FIG. 1. Upper picture: Electroretinogram from octopus eye after section of all optic nerves behind the eye. Stimulus was a brief flash. The lower trace was recorded between an electrode in the posterior chamber of the eye in front of the retina and an electrode in sea water. The upper trace shows the field spread of the ERG into the deafferented optic lobe and was recorded from a monopolar electrode within the optic lobe. Time, 40 msec. Voltage, 1 mv, negative down. Lower picture: Monopolar recording of ERG with intact eye. Response to steady illumination by 60-watt bulb at 3-m distance. Time 1 sec. Voltage, 1 mv.

of the retina but can be recorded on the back side of the retina when the eye is intact. They begin about 15 msec after the flash, last for about 40 msec and have a period of about 7 msec. They can be recorded on the back of the eye when all optic nerve fibers are cut and there is no response in the optic lobe. As we shall see, they are also recorded by bipolar recording at some locations in the optic lobe. If we compared the oscillations recorded by close bipolars behind the eye with those seen at some distance in the

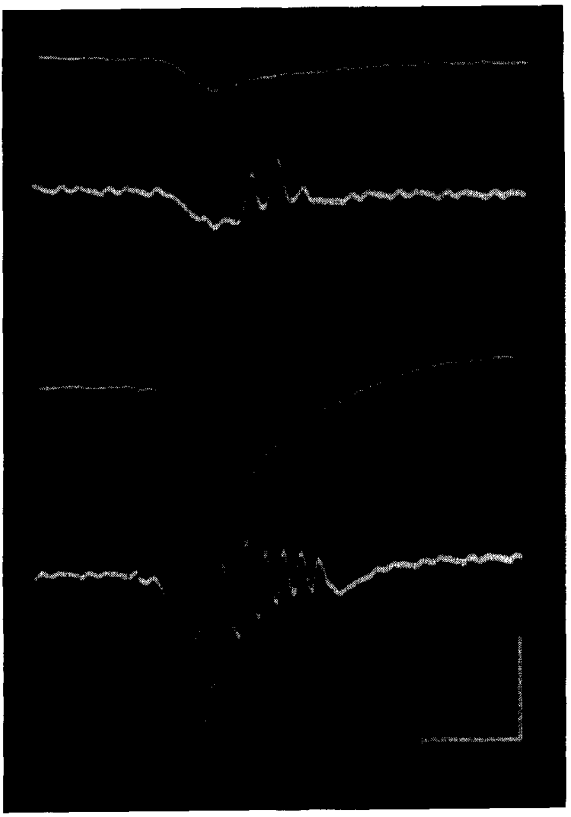

FIG. 2. Upper pair: Response to dim brief flash. The upper trace is the ERG recorded within the eye. In the lower trace the monopolar recording electrode was placed immediately behind the eye on the optic nerves and shows the brief burst of oscillations in addition to the smooth ERG. Lower pair: Response to intense flash. ERG within the eye recorded on the upper trace and ERG and oscillations behind the eye recorded on the lower trace. Time 40 msec. Voltage 1 mv for electrode within the eye, and 500 μv for electrode behind the eye.

optice lobe, a phase lag was seen. We suggest that these oscillations are signs of bursts of synchronized impulses within the optic nerves. Measuring the distance between the pairs of electrodes behind the eye and in the optic lobe and measuring the phase lag suggested that the conduction velocity of these impulses is of the order of 1 m per sec.

Within the Optic Lobe. Three types of potential can be recorded within the optic lobe. The first is the field of the ERG generated within the retina. This can be partially eliminated by bipolar recording but, because of its large size and uneven distribution on the back of the eye, it is often impossible to eliminate it entirely (Figs. 3, 4). The second type is the

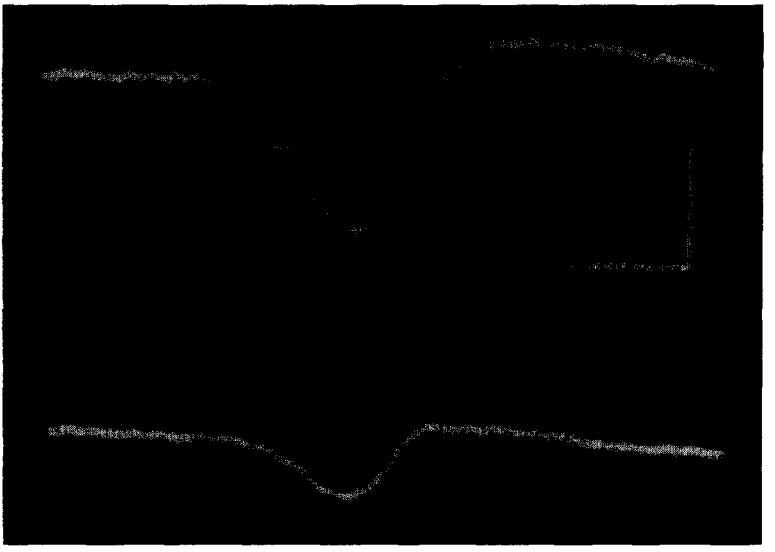

FIG. 3. Bipolar recording of slow waves evoked in the optic lobe by a brief flash to the eye. In the upper trace, the earliest component is the field spread of electroretinogram. The second later wave is a response localized in the optic lobe. In the lower trace another pair of bipolar electrodes in the same optic lobe at a different locus, record little if any field spread of the ERG but the optice lobe wave appears. Time 40 msec. Voltage 400 μv.

oscillating potential which we attribute to conducted impulses within the entering optic nerve fibers. These have highly localized distributions within the lobe. The third is a slow wave which we attribute to the activity of cells within the optic lobe. It begins about 30 msec after the flash and peaks about 20 msec later. Monopolar recordings may show all three

components while close bipolar recording often shows only this third component. A striking feature of the third component, the optic lobe wave, is its extreme lability. Even during the rest state when the ERG and optic nerve oscillations have achieved a steady amplitude, this wave is constantly varying. Simultaneous examination of four electrodes within the optic lobe showed that the variations were not uniform and suggested that the loca-

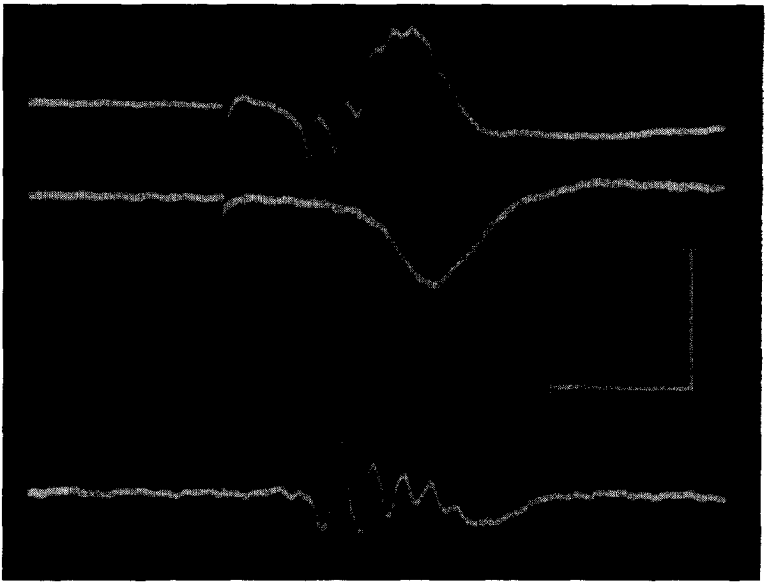

FIG. 4. Three bipolar recordings from different locations in the same optic lobe following a brief flash to the eye. In the upper trace both the fast oscillations, believed to be a sign of arriving impulses, and the late slow wave can be seen. In the middle trace, only the late slow wave is seen. In the lower trace the main response is the fast oscillation. Time 40 msec. Voltage 400 μv.

tion of activity was meandering. The gentle arousal stimuli described above produced dramatic shifts in the location, amplitude and sign of the evoked response even in the absence of any apparent motor movement by the animal. It is quite apparent even by this crude stimulus and recording method that the optic lobe of the brain is not a simple relay station.

Other Regions. No electrical signs were seen of projections from one optic lobe to the other. This was tested by cutting the optic nerves on one side and recording from both optic lobes. When the eye whose optic nerves were cut was illuminated, we recorded only signs of field spread of the ERG in its optic lobe. When the intact eye was stimulated, we recorded

the usual responses in its optic lobe but nothing in the contralateral optic lobe. This does not mean that there are no projections from one optic lobe to the other. A more likely interpretation is that the crossing volley is too desynchronized to evoke a large enough wave to be recorded by these methods.

Similar negative results were obtained from many recording sites within the vertical and frontal lobes. On two occasions just discernible 20-μv waves at about 100 msec after the flash were seen in or near the vertical lobes for a brief period. Stimulation of the region through the recording electrodes suggested that the area was far from dead. Again the most likely explanation for the failure is desynchronization of the afferent volley by the time it arrives in these structures.

Results of Recording of Unit Responses

In a series of experiments to be reported in detail elsewhere, Lettvin and Maturana succeeded in dissecting small strands from the optic nerves and were able to record both efferents and afferent units. The efferent axons to the retina were continually active and increased their firing rate when small arousing stimuli were applied. These stimuli were of the type shown to increase the height of the ERG. The much smaller axons which come from the retina respond to light in restricted areas of the visual field and there appears to be a lateral interaction between retinal nerve cells.

Discussion

The recent detailed studies of cephalopod ERG by Tasaki, Oikawa and Norton (7) and Hagins, Zonana and Adams (4) have been done on isolated retina. Because the axons of the retina emerge from the scleral surface, the dissection strips the axons off at the point of their emergence from the cell body. This factor makes it likely that the isolated cephalopod retina is more damaged than the equivalent isolated vertebrate retina where the ganglion cell axons are cut at some distance from the ganglion cells.

Damage to the point of origin of the optic nerve impulses seems the most likely reason why these authors have not seen the rapid oscillations which we recorded from the back of the retina. Furthermore such a dissection may disrupt lateral connections within the retina and current pathways outside the retina which would explain why Tasaki *et al.* (7) report no lateral reversal of the ERG potential sign although we believed we saw clear signs of this. Since we have presented evidence that efferents affect the size and distribution of the ERG, it is clear that its nature will be changed in an eye connected to the brain even if local damage to the retina is avoided.

Very slow oscillations of the ERG were reported by Frohlich (3) in cooled isolated eyes. MacNichol and Love (5) reported that they recorded in the squid a brief burst of oscillations from the optic nerves after a light was turned on. They refer to these as Frohlich oscillations but it should be pointed out that their frequency is more than 10 times faster and they are not sustained. It is true that Frohlich's oscillations were recorded under cool conditions but we never saw any variation of frequency between animals whose temperature varied by 5 C and we never saw sustained oscillations and therefore the fast oscillations are not extremely temperature sensitive. It seems likely therefore that the initial burst of oscillations which we and MacNichol and Love recorded are different from the slow sustained Frohlich waves which we never observed in the intact animal. It is difficult to rule out damage produced around the recording sites as a possible cause of the oscillations. It is well known for example that slight damage to cat dorsal roots will produce synchronized bursting in afferent fibers. However, we observed the oscillatory potentials when electrodes were lying on the surface of the optic lobe and had not penetrated and were distant from the eye and it seems unlikely that damage had been produced either in the retina or in the optic nerve fibers.

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