ELECTROPHYSIOLOGICAL INVESTIGATION OF VISUAL NEURONS OF CEPHALOPODS

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PHYSIOLOGY

The authors describe a method of neurophysiological experiments on squid under urethane anesthesia. Responses of on-type elements are recorded from the surface of the optic lobe. Discharges of a rhythmic character were present throughout the entire illumination period. The receptive fields of these elements were 1.5-4°. Two types of elements were found in the deeper layers of the optic lobe. One responded with a short discharge when the light was turned on, the other revealed spontaneous activity in the dark which was suppressed by light.

Cephalopods, together with vertebrates and arthropods, are one of the three groups of animals with the highest stage of development of the sense organs, nervous system, and behavior. In the level of development of the visual apparatus cephalopods are, without doubt, far superior to arthropods, at least in such indicators as size of the eyes, magnitude of visual centers, and, probably, visual acuity. The completely independent origin of a perfect

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visual apparatus makes cephalopods an exceptionally interesting subject of study and one that is important for purposes of comparison. Much is known about the vision of vertebrates, and even arthropods, but the accounts on cephalopods in this area are very scanty. As far as microelectrode investigations of the impulses of visual neurons and their functional specialization are concerned, such works appear only sporadically, not because of a lack of interest or an understanding of the importance of such investigations, but rather because of the considerable methodological difficulties involved in experimenting on these animals (Boycott et al., 1965; Hartline and Lange, 1974).

The purpose of this article is to describe a method of electrophysiological microelectrode experiments on whole anesthetized cephalopods, in particular a method of recording single nervous elements from the optic lobes.

MATERIALS AND METHODS

The work was carried out at the Marine Experimental Station, Far East Science Center, Academy of Sciences of the USSR, on Popov Island. Most of the experiments were conducted on mature squid *Todarodes pacificus* in August-September. The animals were caught by fishing lines with several hooks and kept in traps with large-meshed netting, 2.5 × 1.5 × 1 m, at a depth of about 5 m for two weeks without extra bait.

The experiments were conducted under urethane anesthesia which proved to be the best in experiments with squid and octopus out of all the preparations tested (tubocurarine, myorelaxin, flaxedil, listenon). The animals were placed in seawater in a flat aquarium with a capacity of 10-20 liters which contained a 2% solution of urethane. The urethane concentration was raised gradually until active movement in response to painful irritation had disappeared (about 3%). The temperature was maintained at 13-18°C and the water was not changed during the experiment. Supplementary aeration of the water was provided by 1-2 microcompressors.

Electrophysiological experiments on the optic lobe were carried out as follows. Two clamps were placed at the dorsal anterior edge of the mantle and the skin removed from the head (Fig. 1). This operation neither interfered with respiration nor did it cause bleeding. The horizontally pointed wings were removed from the exposed interorbital cartilage; as a result the cartilage formed an adequately firm "runway" which was clamped. In this way the squid was fixed in the aquarium for the entire duration of the experiment. The optic lobe was exposed after accurate (in order not to cause bleeding by cutting the vessels) removal of the supraorbital cartilage.

We used metal microelectrodes (glass capillary tube filled with Wood's alloy) with a platinized tip (Gesteland et al., 1959) and transistor amplifier (passband 0.1-3 kHz, input resistance 5-10 MΩ, amplification factor 900) connected with an oscillograph and magnetic recorder.

RESULTS

With the proper dosage of urethane the animals maintained normal respiratory action for 6-10 h without changing the solution. Impulses recorded in the optic lobe remained unchanged in this time.

In the optic lobe we were able to record the reactions evoked by visual stimuli and spontaneous activity. The amplitude of impulses did not exceed 200-400 μV. We used a microlamp 3 mm in diameter, flashlight with energy of 36 J, and ordinary light as stimuli. Designed stimuli (contrasting disks, bands, and the like), which have proved so fruitful in experiments with amphibians and fishes, for example (Lettvin et al., 1959; Zenkin and Pigarev, 1969; Maksimova et al., 1971; Orlov and Kondrashev, 1978), has little effect. Except for the microlamp, not one stimulus elicited a sufficiently sharp reaction of recorded neurons.

The results given below were obtained in experiments on six squid, and in all the cases the character of the responses was the same. Two experiments were conducted on the octopus *Octopus dofleini*. Here the experiments were much more complicated due to the considerable softness of the head cartilge which produced a noticeable instability of response because of respiratory movements. Boycott and co-authors (1965) noted this among other difficulties. Respiratory movements may also be impediments in experiments with squid (Fig. 2a).

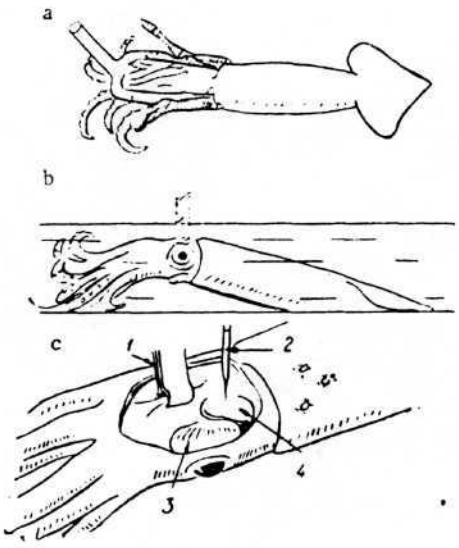

Fig. 1. Clamping of squid for neurophysiological experiments. a) Squid is clamped to edge of mantle (view from above); b) after exposure of optic lobe squid is fixed by clamp to the dorsal part of the head cartilage; c) operational field (skin and soft tissues removed): 1) clamp; 2) microelectrode, 3) eye, 4) visual field.

At the surface of the optic lobe we recorded only the responses of nerve elements represented by optic fibers, i.e., terminations of axons of eye photoreceptors (Young, 1971). They showed no spontaneous activity and responded to illumination with a volley of impulses which lasted as long as the light was on (Fig. 2a, b, d) and stopped immediately after the light was extinguished. The frequency of response in a number of cases reached 70-80 impulses/sec. The attention was caught by the small receptive fields which did not exceed 1.5-4°, a perfectly natural phenomenon for receptor cells. Scattered light, a large light spot, and contrasting bands, spots, etc. evoked no reaction.

Optic fibers form a very sharp retinotopical projection at the surface of the optic lobe. Projecting from the anterior end of the upper surface of the optic lobe are points of superficial space which lie nearly exactly behind the animal. At the posterior end of the upper surface of the lobe a lateral field of vision is projected and one point separated from the posterior end of the lobe for approximately 1/4 its length "stares out" almost exactly laterally.

While embedding the microelectrode in the depth of the optic lobe we recorded the reaction of elements with spontaneous activity in darkness which was suppressed by switching on the light (Fig. 2c, d). After switching off the light impulse frequency gradually increased and reached 35-40 impulses/sec. The initial density of impulses (frequency) was dependent on the brightness and duration of the preceding illumination: The density was smaller the greater the duration and brightness. In elements of this type with their comparatively large receptive fields (about 10°) one may observe some features of the functional organization of the receptive field. For instance, one of the elements on-center during the shift of the microlamp to one side of the center of the receptive field started to release a short discharge of impulses immediately the lamp was switched off (off-response) which alternated with the above described prolonged discharge (spontaneous impulse) (Fig. 2e).

A very unique reaction to the powerful short illumination of the eye by flashlight was recorded near the surface layers of the optic lobe. It was represented by a series of slow fluctuations of a potential organized in the form of "spindles" (Fig. 2f). Our amplifier was intended for the recording of rapid impulse reactions and hence it markedly distorted such a summary signal and made impossible the determination of its exact parameters. Nonetheless, we could see very clearly that the maximal amplitude of fluctuations in the sequential order of "spindles" during periodic stimulation showed a distinct tendency towards decrease. Moreover, in this order the number of periods of fluctuations in each of the "spindles" decreases.

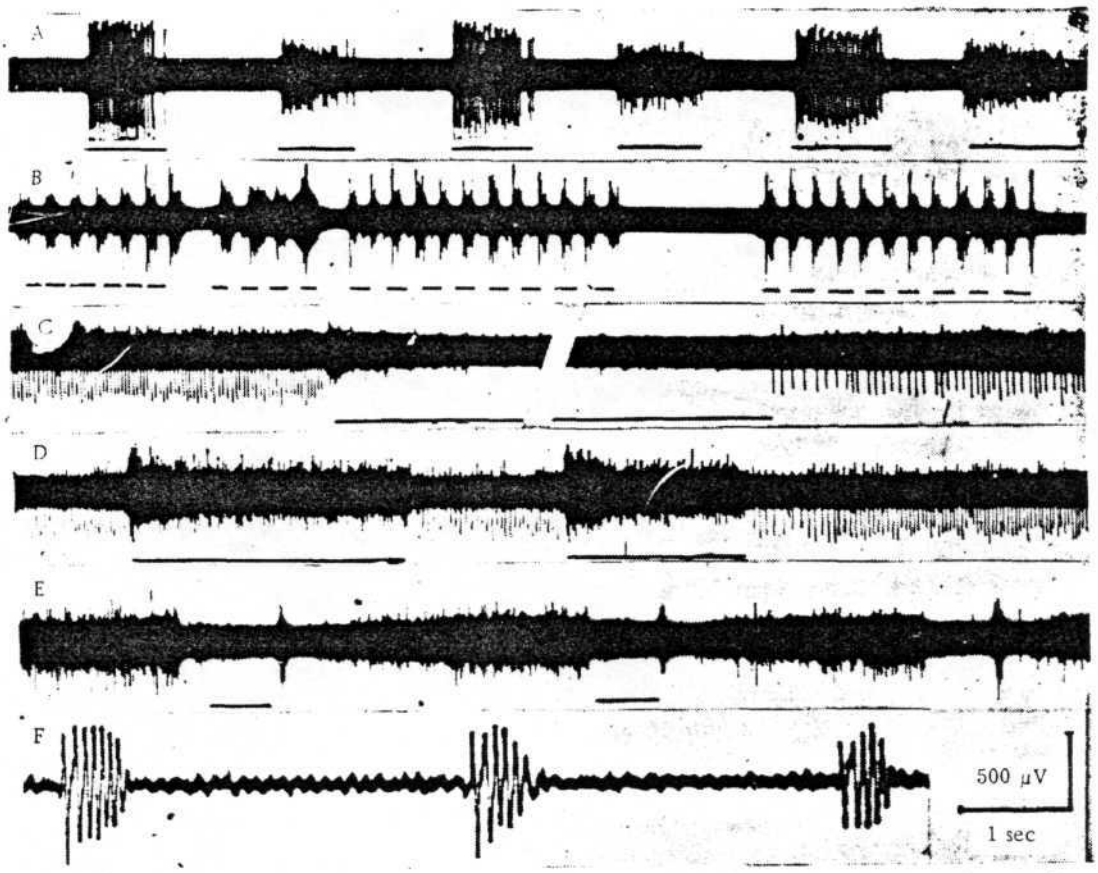

Fig. 2. Impulse and slow activity in the optic lobe of squid. The marker of stimulation corresponds to the switching on of light. A) Single optic fiber, the difference in amplitudes of the series of impulses is due to the shifting of the electrode during respiratory movement; B) type "A" fiber, frequency of on-burst made by hand; C) neuron with spontaneous activity in darkness and suppressed in light, switching on of light evokes a volley of fibers from another layer of the lobe; D) total response of "A" type fibers and "B" type neuron; E) response of "B" type neuron during eccentric position of light source in the receptive field; F) slow fluctuations during stimulation of the eye by flashlight.

Possibly, this slow activity represents a specific organized superposition of signals from many photoreceptors.

Our method makes it possible to record successfully the activity of optic neurons of several types from the optic lobe of squid while preserving a stable and fixed response for many hours. It is of interest to compare our results with those obtained by another method: preparation of an isolated eye and optic lobe.

Lange and Hartline (1974) described two types of optic elements recorded in the optic nerve of squid, the so-called "rapid" and "slow" afferent elements. Judging by the small size of the receptive fields, which did not exceed units of degrees, and a wide array of characteristics of response, the "rapid" afferents are fibers whose responses were recorded by us from the surface of the optic lobe, i.e., axons of photoreceptors. We found no elements similar in properties with "slow" afferents. At times elements differing in their latency were encountered, but it is fully possible that they were variations of one or the other types of "rapid" afferents. It may be that "slow" elements are more difficult to record by microelectrode because of their small number (Lange and Hartline, 1974).

"Rapid" elements and the superficial elements described by us do not have an antagonistic environment (only on-reaction in the receptive field is shown) and spontaneous activity to light and dark.

In contrast to superficial elements, neurons recorded in the deep regions of the optic lobe show brightly expressed spontaneous activity. It is not likely that they represent elements of the afferent chain which exerts its influence from the optic lobe on the retina (Young, 1971) but are close in properties to "slow" elements (Lange and Hartline, 1974). Moreover, a "sluggish" reaction and very distinct latency are characteristic for "slow" elements (Hartline and Lange, 1974), which cannot be said of the neurons described by us.

Slow fluctuations of the potential have been described more than once in works on squid and octopus. Many investigators have observed spontaneous and "optical" slow reactions in both the retina and the optic nerve of cephalopods (Byzov and Orlov, 1962; Boycott et al., 1965; Daw and Pearlman, 1974; Lange and Hartline, 1974). The slow responses from the superficial layers of the optic lobe of squid (Fig. 2f) are very similar to one of three types of slow fluctuations of the potential recorded in octopus (Tsukahara et al., 1973). Despite the fact that synchronous discharges of fibers of the optic nerve are regarded as sources of similar slow rhythmical responses, much of their formation remains unclear.

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