Brain Research, 105 (1976) 525–532

MOTOR AND BEHAVIOURAL RESPONSES OBTAINED BY STIMULATION WITH CHRONIC ELECTRODES OF THE OPTIC LOBE OF *SEPIA OFFICINALIS*

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(Accepted September 1st, 1975)

SUMMARY

A new technique using a stimulating chronically-implanted electrode has allowed us to study the motor responses induced by electrical stimulation of the optic lobe in a freely swimming *Sepia*.

Electrical stimulation of the cortex of the optic lobe produces no motor response; this is in agreement with the results of preceding authors. The stimulation of the neuropil of the optic lobe by monopolar electrode produces many different motor responses, in support of Boycott's results[2] obtained by the same type of excitation in acute experiments. However, the field of stimulation of these electrodes could not always have been the same and it is possible that we were sometimes stimulating nervous structures close to the optic lobe. Stimulation by a bipolar electrode, however, which does not have this disadvantage, induces only two very different motor responses: an ipsilateral rotation and an 'alarm reaction', so called because of its similarities to the 'attentive immobilization' of higher vertebrates. These two reactions are very complex and their different components are linked together as in a behavioural response from an intact animal. These reactions present very different characteristics of excitability. They are obtained from many areas in the neuropil of the optic lobe, within which there does not seem to be any preferential localization.

These results emphasize the importance of the optic lobe in motor control.

INTRODUCTION

The functional organization of the central nervous system of Cephalopods has been studied by various authors. Among the older studies, Bert's[1], Von Uexküll's[14] and Polimanti's[12] can be mentioned. In 1961, Boycott[2] resolved the often contradic-

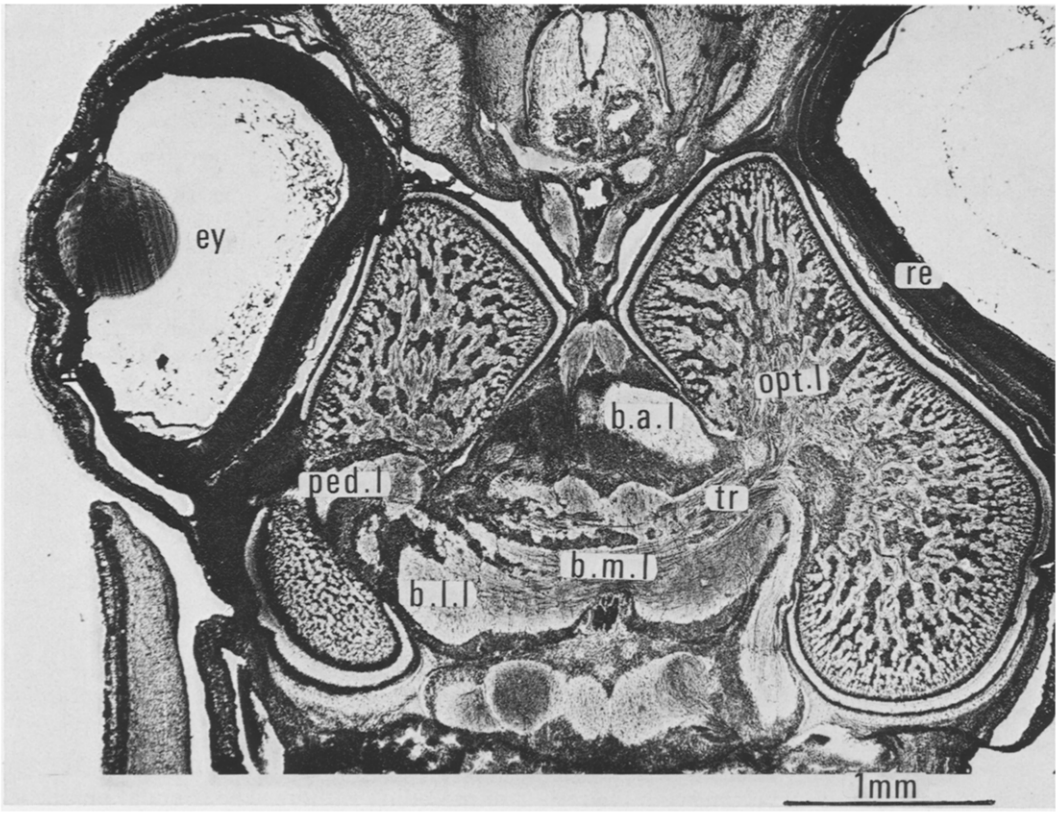

Fig. 1. Asymmetrical horizontal section through the head of a juvenile *Sepia*. Cajal silver stain. Abbreviations: b.a.l., basal anterior lobe; b.l.l., basal lateral lobe; b.m.l., basal median lobe; ey, eye; opt.l., optic lobe; ped.l., peduncle lobe; re, retina; tr, tracts to the basal lobes. (Photograph by courtesy of Dr. J. B. Messenger.)

tory results obtained by these authors and set out the results he himself obtained by electrical stimulation of the different lobes of the central nervous system of the cuttlefish. In 1967, Messenger[10] by the same technique analyzed the effects caused by stimulation of the peduncle lobe in *Octopus*. In these acute-type experiments, however, the animals survive hardly more than 20 min and the stimulation of the deeper regions of the central nervous system has to be carried out after removal of the overlying nervous structures. This is why it seemed interesting to us to re-examine the experiment using a stimulating electrode chronically implanted in an unrestrained animal.

MATERIAL AND METHODS

The experiments were performed either with 50–100 g animals bred in the laboratory of Luc-sur-Mer or with 200–1000 g individuals caught by local fishermen.

In this preliminary study, we shall only describe the results obtained by stimulation of the optic lobes. The optic lobes represent important nervous masses[2] laterally situated between the eye and the supraoesophageal mass (Fig. 1).

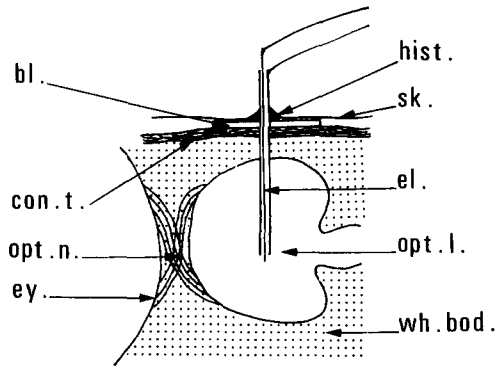

Fig. 2.

Fig. 2. Diagram illustrating the setting of the electrode. Abbreviations: bl., plate of plastic; con.t., connective tissue; el., electrode; ey., eye; hist., histoacryl; opt.l., optic lobe; opt. n., optic nerves; sk., skin; wh. bod., white body.

The animal is first quickly anaesthetized by immersion in 0.5% urethane in seawater. The water temperature is normally about 16 °C. The animal is placed in a tank so that it lies on an inclined cork-plate. It is sufficiently held in position by a 'cradle' made with a series of pins stuck into the cork, around the animal, in order not to damage the fins. A U-tube is introduced into the pallial cavity to ensure irrigation of the gills. The tank contains seawater with 0.2% urethane, which provides sufficient anaesthesia for the operation. Recording the electrocardiogram (by a bipolar electrode made of two joined enamelled silver wires slipped under the cuttle-bone) suggests that an animal placed in these conditions remains in a good physiological state[6].

The stimulating electrodes may be monopolar or bipolar. The monopolar electrode consists of 0.08 mm enamelled steel wire, maintained with Araldite in the steel body of a hypodermic needle 0.3 mm in diameter (Inopiq) between 10 and 15 mm long. The steel wire is welded to a very fine and supple insulated cable, which is connected to the cathode of the stimulator. A varnish coat (Alvar) insulates the welding and areas that might have been stripped during the operation. The bipolar electrode is also made of 0.08 mm wire in the steel body of a 0.3 mm needle but its outer surface is insulated with varnish except at the end. The steel wire is about 1 mm longer than the bare end of the needle. It is welded to a thin and supple cable connected to the cathode of the stimulator, whereas the body of the needle is connected to the earth of the pulse generator. The electrodes are maintained by a small oval plate of inert plastic (about 8 mm × 5 mm). The part of the electrode that is longer than the plastic plate is adjusted according to the size of the animal and to the depth desired.

The dorsal skin of the head is cut along an arc (8–10 mm long) parallel to the wall of the orbit, exposing the underlying tight connective tissue. The latter is cut along a few millimetres to locate the white body cushioning the optic lobe on all sides. The electrode is implanted through the white body and the plastic plate, which has been coated beforehand with tissue adhesive (Braun, Melsungen: histoacryl), adheres

strongly to the connective tissue in less than 20 sec. The electrode therefore rests on the only rigid tissue present in this area. The plastic blade is then re-covered with the skin and the whole is maintained by histoacryl. Fig. 2 is a diagram illustrating the setting of the electrode.

It is preferable to implant the electrode through the white body rather than to move this aside because it is highly vascularized and its lesion would be followed by serious haemorrhage.

The exact site of implantation of the electrode end can only be determined *a posteriori*; after the experiment a slight electrocoagulation is performed (1 mA for 20 sec), which can easily be detected on sections made with a cryostat.

Once the electrode has been implanted, the animal is placed in a large tank with transparent sides containing fresh circulating seawater. The animal quickly recovers (5–10 min) and appears to be behaving normally after 15–20 min. Sometimes the cuttlefish pulls at the wires with its arms but they are so very light that the animal quickly gets used to them. When stimulating by monopolar electrode a silver electrode is placed in the tank and connected to the earth of the stimulator. The bipolar electrodes have an average impedance of 30 kΩ with little dispersion. The electrical stimulation has been realized by square waves with various frequencies and time duration.

Forty animals were used for monopolar stimulation and 80 for bipolar stimulation. The success rate is approximately 80%.

This whole procedure appears to be only slightly traumatic to the animals and they survive excellently. Nevertheless the experiments cannot last more than 4–6 days, as the histoacryl tends to separate from the tissues and the electrode can then be easily pulled off by the animal. The wound heals after a few days.

RESULTS

Firstly we are able to confirm that the stimulation of the cortex of the optic lobe produces no motor response. Only stimulation of the central neuropil produces motor responses.

Monopolar stimulation

Many different motor responses can be observed on stimulating the neuropil of the optic lobe. The most frequently obtained are as follows: ocular movements, pupillary dilatation, retraction of the head, rotation of the head towards the side stimulated, colour changes, lifting up of the first pair of arms, the appearance of black dorsal 'eye-spots' on the mantle, stretching and flattening of the whole animal, complex movements of the fins and short movements of the whole animal. These responses are very distinct presumably because the stimulation field of the monopolar electrodes must differ so greatly in different preparations and from moment to moment; this is why we have preferred the use of bipolar electrodes.

Bipolar stimulation

Only two very distinct categories of responses were obtained.

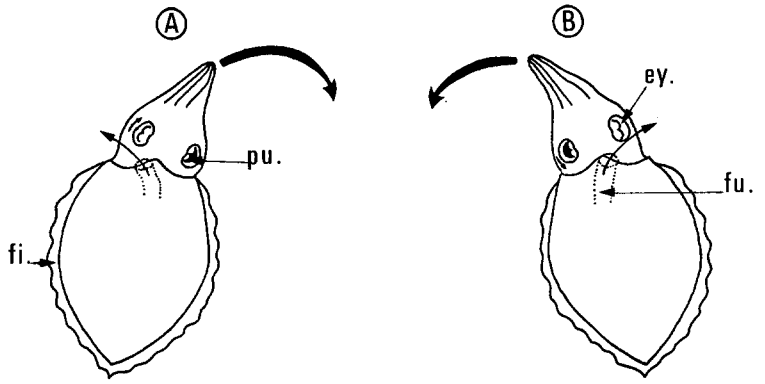

Fig.3.

Fig. 3. Ipsilateral rotations. A: stimulation of right optic lobe. B: stimulation of left optic lobe. Abbreviations: ey., eye; fi., fin; fu., funnel; pu., pupil.

(1) Ipsilateral rotation movements. This reaction begins with a rotation of the whole eyeballs, the contralateral eye turning forwards and the ipsilateral eye backwards (Fig. 3). The head then rotates towards the side stimulated; the funnel turns in the opposite direction and through a combined action of the fins and the funnel the animal swings round towards the source of the stimulus. At the same time there is darkening and ipsilateral pupil dilates. This response appears especially after trains of impulses (frequency of train, 20–30 cycles/sec; duration of pulses, 2–3 msec. The threshold may vary considerably (from 0.4–0.8 mA). Only trains of sufficient duration (1500 msec on average) induce a complete rotation of the animal. With 500 msec trains only ocular movements are obtained; with 1000 msec trains a turning of the head is also observed and sometimes a 90° rotation of the whole animal. The resting state is quickly resumed after stimulation has ceased. A first train of impulses greatly facilitates a second response if two consecutive trains are separated by less than 5 sec.

(2) 'Alarm reaction'. This behavioural reaction is very complex. In many ways it is similar to the 'attentive immobilization' shown by higher vertebrates. Its main features may be summarized as follows: eyes look up, bilateral pupil dilates, the respiratory movements stop, then reappear, the head retracts, the whole animal flattens and the fins spread out, there is expansion of chromatophores around the eyes and the edges of the fins and the two black 'eye-spots' appear on the dorsal mantle.

This reaction is maintained throughout stimulation and with prolonged trains (2000 msec) it can last for about 10 sec after stimulation. With a longer periods of excitation it can be followed by escape movements of the animal: strong contractions of the mantle and ink ejection[2]. The 'alarm reaction' occurs especially with following conditions: frequency of train, 40–60 cycles/sec; duration of pulses, 5 msec. The threshold varies as before. Only long trains can induce this reaction with all its components (1000 msec on average). This 'alarm reaction' has already been mentioned by Holmes[9] under the name 'flattened posture'. It is easily obtained from a normal cuttlefish by the sudden presentation of an object in the visual field of the animal.

The complete refractory period of this reaction is near to 20 sec, so that it is very different from the cycle of the preceding reaction.

These two types of responses are obtained from many areas in the neuropil of the optic lobe and there does not seem to be any preferential localization. Generally for a given area of excitation, we have obtained only one type of response. The maximum stimulation time is around 10 h; afterwards responses become less clear and an important lysis can subsequently be seen around the end of the electrode.

Direct current stimulation induces no response, except at high thresholds, above 1.5 mA. The animal then shows violent mantle contractions accompanied by ink ejection. It seems that this reaction is not very specific but rather a response to excessive stimulation. Stimulation at low frequency (1 cycle/sec) or by short pulses (less than 0.8–1 msec for 2 min) produces no motor response.

DISCUSSION

The technique described here allows a much longer experimentation time than does Boycott's acute method[2]. True the duration of the experiment is limited (by rejection of the electrode) to 6 days but there is no reason to suppose that longer implantation would give any new information. The animals remain, moreover, in good physiological condition and can live quite normally after the rejection of the electode.

Stimulation by monopolar electrode induces many motor responses and in general our results support those of Boycott[2]. It is clear, however, that the field of stimulation of these electrodes is important, and it is not impossible that we were sometimes stimulating some other nervous structures close to the optic lobe (*e.g.* peduncle lobe, see ref. 10). On the other hand, the use of bipolar electrodes must have elicited the optic lobe's own responses.

It will be noticed that not only are the responses obtained very complex but they often appear together in sequence as in the case of intact behavioural responses. This finding again completely agrees with Boycott's results[2]. Thus, the ipsilateral rotation can be compared to that performed by the animal when it detects a prey in its lateral visual field[11]. In the same way, the 'alarm reaction' obtained is at first sight similar to the one observed when an animal is surprised by the presence of an object in its visual field. This reaction has been classified by Young as a 'dymantic' reaction[15] and is presumed to be a response to deter a predator. This reaction will be analyzed in a later behavioural study[5].

In contrast to Polimanti[12] but in support of Boycott[3] we have found no precise localization of different functional areas within the optic lobe. The cortex is unexcitable and only the stimulation of the neuropil causes motor responses. This is in agreement with the findings of Sanders and Young[13] and of Boycott[2]. The different stimulus parameters needed in order to obtain the two types of responses along with the differences of excitability cycles might indicate the existence of several categories of motor neurones. In this connection, it seems interesting to note that, histologically, the centre of the optic lobe is very complex. It shows many cell islands separated by tracts of fibres. These cell islands are made up of at least two classes of neurones:

some comparatively large ones with a conspicuous dendritic trunk and an axon passing through the optic tracts; others much smaller and multipolar, their branches running in all directions. It does not seem impossible that these two types of neurones might have different excitability characteristics. However the physiological significance and the precise connections of these cells are not known at present[4,17,18].

On the other hand, the various connections of the optic lobe are well known[17,18] and it is clear that the lobe is much more than a mere visual relay station. The principal afferents come from the retina, the peduncle lobes, the subvertical lobe, the basal lobes and from the magnocellular lobes. The principal efferents go to the retina, the peduncle lobes, the superior frontal lobe, the basal lobes (anterior, posterior and lateral) and to the magnocellular lobes. These numerous efferents may account for the variety of the effects reported here. It must also be remembered that the studies of Young and his co-workers suggested very strongly that the optic lobe is involved in memory and conditioning processes[3,16].

Electrical stimulation, which presumably produces a rather localized excitation, causes extensive and well-defined reactions that cannot be compared to the motor activities induced by injected drugs[7,8]. Drugs that modify cholinergic transmitters only cause more or less isolated motor reactions, which are never coordinated into a typical behavioural sequence. It is hoped that further investigations of the *Sepia* central nervous system by this electrophysiological method, alone or in association with pharmacological methods, may allow us to further elaborate the functions of the different lobes in the integration of complex motor behaviour.

ACKNOWLEDGMENTS

We are grateful to Professor J. Z. Young (The Wellcome Institute for the History of Medicine, London, Great Britain) and Dr. J. B. Messenger (Department of Zoology, University of Sheffield, Sheffield, Great Britain) for their critical review of the manuscript and Dr. D. Richard (Laboratoire de Neurophysiologie comparée, Université de Paris) for his advice relating to electrical stimulating techniques.

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