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MOBILIZATION OF A COORDINATED ESCAPE RESPONSE BY GIANT AXONS IN THE OPHIUROID, *OPHIOPTERIS PAPILLOSA*

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SUMMARY

1. The escape response of *Ophiopteris papillosa* to contact by a predatory asteroid consists of a fast withdrawal of the stimulated arm, completed in less than 1 s, followed by rapid locomotion carried out by coordinated rowing of the two arms opposite the one stimulated.

2. Electrical activity recorded from the radial nerve cord (RNC) during the locomotory phase consists of small-amplitude spikes ($<5\,\mu V$).

3. The initial arm jerk response is mediated by sequential activation of segmental intervertebral muscles, and the onset of activation progresses centrally at a rate of $<10\,cm\,s^{-1}$ commencing 100–200 ms after stimulation.

4. Electrical activity recorded from the RNC immediately after tubefoot stimulation consists of a burst of large-amplitude spikes ($50–100\,\mu V$) that propagate centrally at approximately $50\,cm\,s^{-1}$.

5. Electrical activity in the RNC with the lowest threshold to direct electrical stimulation consists of large spikes propagating at a mean velocity of $55\,cm\,s^{-1}$ at 13°C, and this activity persists in a Ca^{2+}-free medium.

6. Electrical and tubefoot stimulation of the arm tip in the same preparation both trigger a burst of large action potentials that propagate at approximately $50\,cm\,s^{-1}$.

7. The identity of the giant axons activated by tubefoot stimulation, the pathways they follow and their role in mobilizing the coordinated escape response are discussed.

INTRODUCTION

Giant axons occur in the nervous systems of many animals. By definition these axons are abnormally large in diameter compared to other fibres in the same animal and are specialized for rapid transmission of action potentials over relatively long distances. As discussed by Dorsett (1980), in nearly every known case the giant fibres form a critically important link in the chain of neuronal elements responsible for rapid integration of sensory inputs and coordination of an appropriate behavioural escape response to noxious stimuli or dangerous conditions. Giant fibres can be terminal motor elements (e.g. third-order giant axons in stellar nerves of squid;

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Young, 1939) or interneurones (e.g. Mauthner fibres in the spinal cord of fishes; Faber & Korn, 1978; Rovainen, 1979). The formation of the giant fibres (often from many smaller neurones) can follow several different morphological plans (see Dorsett, 1980).

Giant fibre systems in the Echinodermata have received little attention. Primarily this is because most of these creatures are very slow moving and earlier histological studies have shown the great majority of axons to be less than 1 μm in diameter (Smith, 1937, 1965). Technical difficulties inherent in both physiological and anatomical approaches to the study of such small neuronal elements have impeded advancement of our understanding of the most basic neurobiological principles of echinoderms (see reviews by Pentreath & Cobb, 1972, 1982). Parallels between echinoderms and other phyla, particularly the closely related chordates, would be valuable, but they remain to be established.

Of the echinoderms, only the ophiuroids (serpent or brittle stars) have 'giant' axons, up to 10 μm or more in diameter, as first shown anatomically by Pentreath & Cottrell (1971) and physiologically by Brehm (1977). Giant axons have now been described in a number of species (Cobb & Stubbs, 1981; Stubbs & Cobb, 1981; Pentreath & Cobb, 1982; Tuft & Gilly, 1984), and giant fibres thus appear to be a unique and perhaps universal feature of this class of echinoderms. It is noteworthy that Smith (1965) predicted the existence of giant axons in ophiuroids largely on the basis of behavioural observations.

Although giant axons are clearly present in the ophiuroid radial nerve cord (RNC) which runs the full length of each arm, anatomical details of the pathways and circuitry are only beginning to become established. Physiological studies of the function of ophiuroid giant axons have been few, and giant fibres have been implicated in the control of bioluminescence (Brehm, 1977) and as the basis for photosensitivity (Stubbs, 1982).

In this paper we describe the behavioural escape response of an ophiuroid to contact by a predatory asteroid and the underlying role played by the largest giant axons in the RNC. Following the terminology of Tuft & Gilly (1984), we refer to the most rapidly conducting axons in *Ophiopteris* as 'type 1' fibres. Action potentials in type 1 giant axons are triggered by appropriate sensory inputs and can spread from arm tip to arm tip in a large ophiuroid within a few hundred milliseconds. Functionally these giant fibres enable the rapid execution and coordination of the escape response.

MATERIALS AND METHODS

Large specimens of *Ophiopteris papillosa* were collected subtidally from Monterey Bay. Animals were maintained in running sea water at 15 °C. Exposure of the radial nerve cord was carried out as previously described (Tuft & Gilly, 1984), but in the present study animals were never exposed to $MgCl_2$-based anaesthetizing solutions.

Experiments were carried out in natural sea water (NSW) or Ca^{2+}-free sea water (for Fig. 9) which contained (in mmol l^{-1}): NaCl, 470; KCl, 10; $MgCl_2$, 60

Tris, 10; Na^+-EGTA, 10. pH was adjusted to 7·8, and osmolality was 980 mosmol kg^{-1} H_2O. To change solutions during electrophysiological experiments without disturbing the recording conditions, the bathing medium was slowly drained and new medium was added to cover the entire animal to the previous level.

Electrophysiological experiments

Suction electrodes for recording were either polyethylene or glass with final tip diameters of approximately 150 μm. Voltages sensed by one Ag/AgCl wire inside the NSW-filled electrode and another one outside near the tip were amplified by a Tektronix Type 122 differential preamplifier with low/high-pass filters set at 1 kHz/8 Hz. Records were photographed from a storage oscilloscope.

The bipolar stimulating electrode was made of 70 μm platinum wires spaced 150 μm apart and insulated except for the tips. The electrode was placed on top of the RNC, generally straddling a ganglionic swelling exposed near the tip of an arm. Stimulating pulses were 0·5–2 ms in duration.

Conduction velocity of action potentials was estimated as the time from the beginning of the stimulating pulse to the first peak of the spike divided by the inter-electrode distance (0·45–2·7 cm). Values determined with closer electrode spacings are thus subject to a higher degree of uncertainty due to possible variations in latency for spike generation. The temperature for these experiments was 13 °C.

Most recordings were made from the oral surface of ganglionic swellings in the ectoneural portion of the RNC. Very similar recordings were obtained from the aboral surface of the RNC after cutting the hyponeural motor nerves passing into the ossicles and reflecting the RNC to one side to allow access with the electrode. The state of the thin layer of aboral hyponeural tissue under these conditions is not known.

Experiments were also carried out in which the tip of an *Ophiopteris* arm was stimulated with an asteroid tubefoot secured to a hypodermic needle with fine thread. The needle was attached *via* a plastic rod to the movable breaker of a d.c. relay that could be activated with a toggle switch. The closing relay simultaneously moved the tubefoot several millimetres to hit the ophiuroid arm and triggered a sweep on the oscilloscope.

Force measurements

Contractile responses of detached, but otherwise 'intact', arms (i.e. no windows exposing the RNC) were measured in response to electrical stimuli (1 ms) applied to the oral surface of the arm near the base of the spines in the region where the tubefeet emerge. The arm was mounted in a way similar to that used for electrical recordings, but a loop of fine thread was tied around the arm just proximal to the delicate terminal portion (see Fig. 5). The force transducer (Gilly & Hui, 1980) was attached to this thread, and the output signal was recorded on a Brush 280 pen oscillograph (Gould, Inc., Cleveland, OH). Experiments were carried out at 18–20 °C.

Behavioural experiments

For experiments examining the time course of behavioural responses, the tip of one arm was stimulated with a hand-held tubefoot, and the ophiuroid's reaction was videotaped or photographed with a motor-driven 35 mm camera. The temperature was approximately 16 °C.

RESULTS

Behavioural escape response of Ophiopteris *to a predatory asteroid*

Ophiopteris exhibits a vigorous escape response if it comes into contact with the predatory asteroid *Pycnopodia helianthoides*. A complete response is shown by the time lapse series in Fig. 1 (exposures every 0·4 s). When the tip of an arm is touched by the asteroid tubefoot (time 0), *Ophiopteris* rapidly pulls away from the stimulus by withdrawing the arm in a snake-like recoiling motion. This 'arm jerk' response is very fast and is largely completed by the 0·4 s frame. Its time course will be analysed more completely below. A second stimulus was delivered near the 1·6 s frame with a similar result. Although the arm tips are the most sensitive spots on *Ophiopteris*, contact of other parts of the arm with *Pycnopodia* can elicit similar responses. Similarly, stimulating an arm tip with a bare needle sometimes yields the response, but the asteroid tubefoot is more reliable. Habituation of this phase of the escape response was observed.

Immediately after the arm jerk response, the ophiuroid initiates a stereotyped locomotory response (0·4–3·6 s frames). The tips of the two arms opposite to the stimulated one are planted on the substrate, and the more proximal portions of the arms produce a coordinated power stroke that propels the animal away from the stimulus. These two legs may then be re-extended and the stroking repeated for one or more cycles. Following this power stroke phase, the animal changes to a more conventional form of locomotion using a single leading arm with no specific pattern of arm movements (3·6–5·4 s frames). Again, the asteroid tubefoot appears to be more effective than a bare needle in producing the full escape response, but this point was not systematically studied.

RNC activity following contact of an arm tip by Pycnopodia

In our initial attempts to record electrical activity in the RNC underlying the escape response, we recorded from restrained animals following manual application of stimuli. As an example, Fig. 2 shows recordings made when a homogenate of minced *Pycnopodia* tubefeet was dripped onto the tip of an *Ophiopteris* arm (trial 1). A long period of heightened electrical activity ensued during which an unrestrained animal would be moving. After 20 s the activity subsided, and a second stimulus (trial 2) again yielded heightened electrical activity.

RNC activity recorded 1–20 s after stimulation is probably due to the locomotory phase of the escape response. Amplitudes of spikes thus recorded are much smaller than the spontaneous unitary potentials in type 1 axons (20–50 μV) recorded unde

nearly identical conditions by Tuft & Gilly (1984). Thus it is unlikely that these 'giant' axons are directly involved with locomotion, for example as final motor fibres.

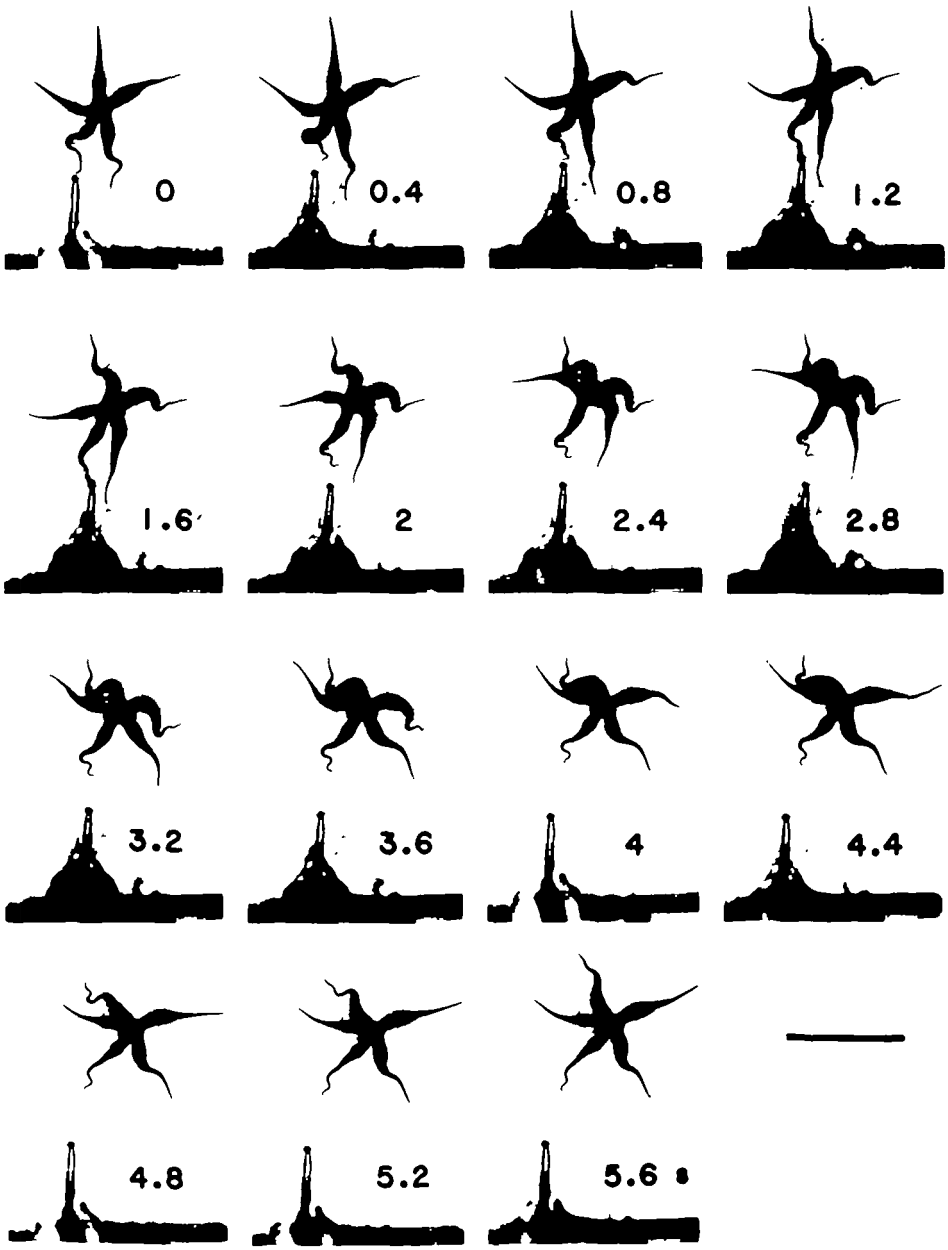

Fig. 1. Complete escape response of *Ophiopteris*. Time lapse sequence with exposures taken every 400 ms as indicated. The '7 o'clock' arm tip was touched first near time 0 by tubefeet from the predatory asteroid, *Pycnopodia helianthoides*, and the escape response was initiated. A second contact was made near the time of the 1·6 s exposure. Scale bar, 10 cm.

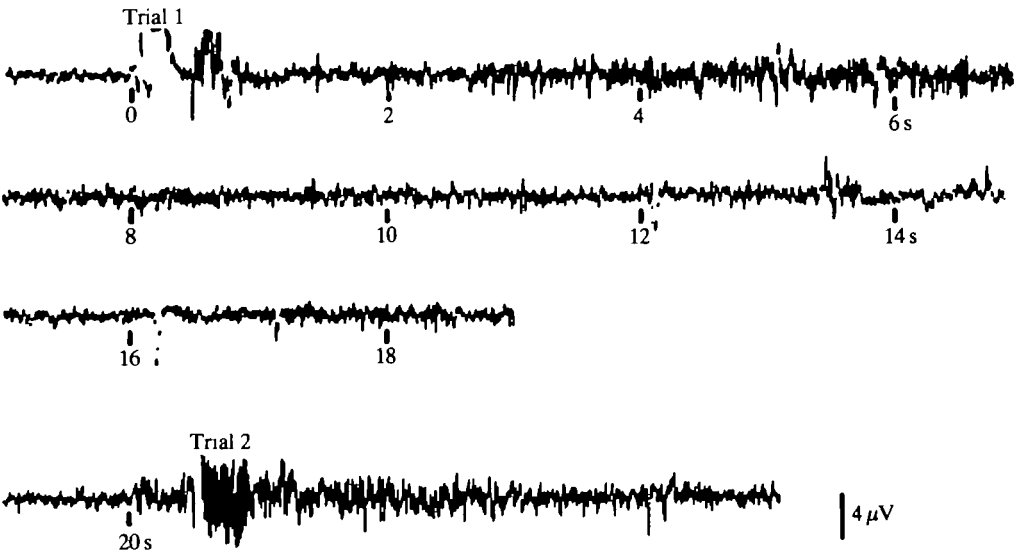

Fig. 2. Radial nerve cord electrical activity triggered by stimulation of an *Ophiopteris* arm tip by a homogenate made from *Pycnopodia* tubefeet. One drop was applied at time 0 (trial 1) and a second one as indicated (trial 2). The recording is continuous.

The remainder of this paper will concentrate on the initial arm jerk phase of the behavioural sequence described above and on fast electrical activity in the RNC that precedes the motor output.

Time course of the arm jerk response

Fig. 3 shows the time course of a videotaped arm jerk response. At time 0 (first panel), the arm tip was stimulated by a *Pycnopodia* tubefoot, and successive panels show both the position of the arm at indicated times and the former position at time 0 (lighter outline).

In 20 such experiments the first detectable arm tip movement occurred at 242 ± 94 ms (mean $\pm$ S.D.), and in Fig. 3 the arm tip moved before 211 ms. The large standard deviation reflects both real variability in the response and the uncertainty in determining the exact time at which the stimulus was applied. As can be estimated visually from Fig. 3, the snake-like recoiling motion characterizing the arm jerk was almost completed in less than 1 s.

Fig. 4 shows graphically the pattern of motion for the data in Fig. 3. Solid circles represent displacement of the arm tip from its time 0 position. Since the arm tip is progressively withdrawn by sequential activation of the segmental intervertebral muscles, this measure gives a rough indication of the rate at which the 'wave' of muscle activation progresses centrally. The rate is given by the slope of the solid line (fitted by eye) and is $7{\cdot}7\,\mathrm{cm\,s^{-1}}$.

A similar measurement can be obtained by estimating the position of the most centrally located segment in which shortening of intervertebral muscles must have occurred at each time. These positions are indicated by the arrows in Fig. 3, and

their sequential displacement from the time zero position of the arm tip is plotted as open symbols in Fig. 4. Again, the rate at which the wave of muscular activity propagates centrally is about $7{\cdot}7\,cm\,s^{-1}$ (dashed line) after an initial delay of approximately 100 ms.

Mechanical measurements of intervertebral muscle activity

The time course of contraction for intervertebral muscles in an intact arm following electrical stimulation was also determined using a force transducer (see

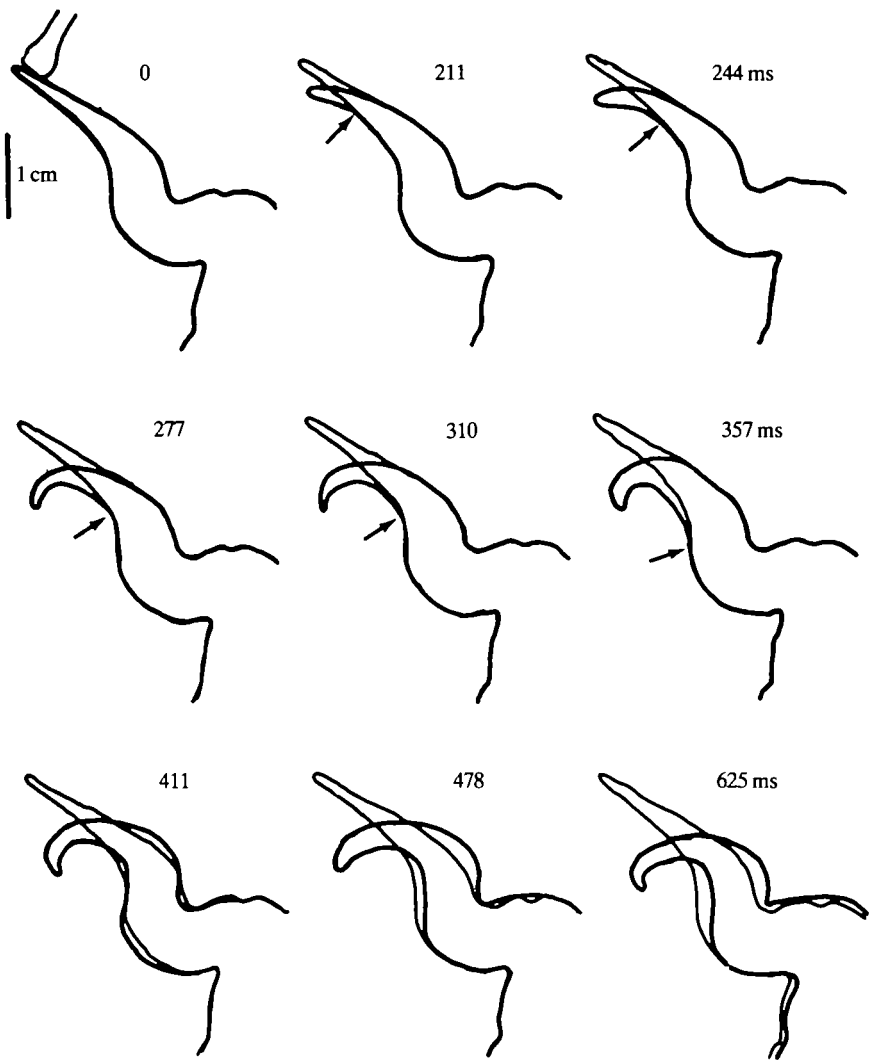

Fig. 3. Time course of the rapid arm jerk phase of the escape response. The positions of the *Ophiopteris* arm at time 0 when tubefoot stimulation occurred and at the subsequent indicated times are illustrated (heavier outlines). The time 0 position is also repeated in each panel (lighter outlines). Arrows indicate the most central sites where activation of intervertebral muscles causing flexing of the arm is estimated to occur.

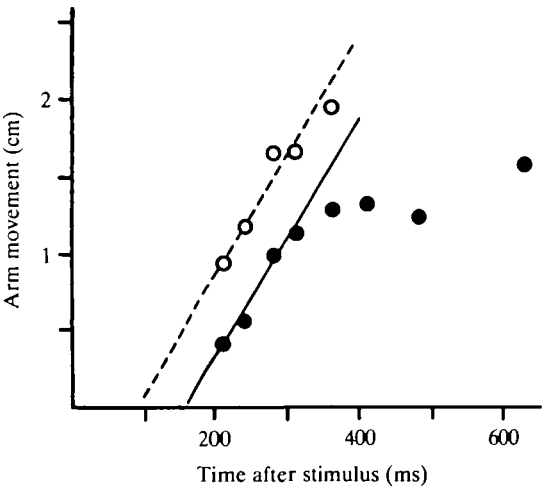

Fig. 4. Time course of the spread of mechanical activation of intervertebral muscles underlying the arm jerk response. Filled circles represent displacement of the arm tip from its time 0 position in Fig. 3. Solid line was fitted to the initial rate by eye. Empty circles represent the most distant site of intervertebral muscle activation as estimated by the arrows in Fig. 3. Dashed line with a slope of 7·7 cm s^{-1} and a delay of approximately 90 ms was fitted by eye.

Materials and Methods). Fig. 5A shows a typical response to a 1-ms shock delivered at a site 5·4 mm from the arm tip (see inset). Force rises briskly and then relaxes slowly, resulting in a slow twitch-like response whose amplitude can be graded with stimulus intensity (not illustrated). At a higher sweep speed (Fig. 5B), the latency for force development following a brief shock can be demonstrated to be very short, typically 20 ms or less. These responses probably result from direct activation of the muscle fibres and do not involve neuromuscular transmission. Similar contractions with brief latencies can be evoked by stimulation at sites further down the arm (see Fig. 5C,D obtained with stimuli applied at 9·1 and 14·4 mm, respectively). Properties of the intervertebral muscles thus determined appear to be fairly constant from segment to segment along the arm.

Application of a brief electrical stimulus to the sensitive arm tip also results in a measurable twitch. Two examples are shown in Fig. 5E,F, in which the latencies for force development are approximately 60 and 80 ms, respectively, or roughly 3–4 times longer than that seen with stimuli applied more centrally. The added delay is not simply due to the mechanical arrangement of the arm and force transducer. Frame by frame analysis of videotapes of these experiments confirmed a delay of approximately 80 ms for the first detectable movement of the arm tip away from the stimulating electrode. These longer delays observed with arm tip stimulation probably reflect involvement of the nervous system.

Fast mechanical responses to tubefoot stimulation of an arm tip

Arm jerk responses could also be generated by stimulation of the sensitive arm tip with the relay-driven tubefoot (see Materials and Methods). Fig. 6A shows a rapid

response of a restrained, isolated arm preparation to a relay-driven tubefoot. The downward arrow indicates the time when the tubefoot hit the ophiuroid arm tip; the upward arrow shows when the relay was switched off. The brief period of high-frequency noise accompanying relay movement is a mechanical artifact of the force transducer apparatus. Upon contact of the arm tip by the tubefoot, force develops rapidly after a delay of about 80 ms, and the overall pattern of the response is very similar to that evoked by electrical stimulation of the arm tip (Fig. 5E,F).

Although rapid responses like that in Fig. 6A were typically generated by this method of stimulation, longer latencies (up to 250 ms) and weaker responses were

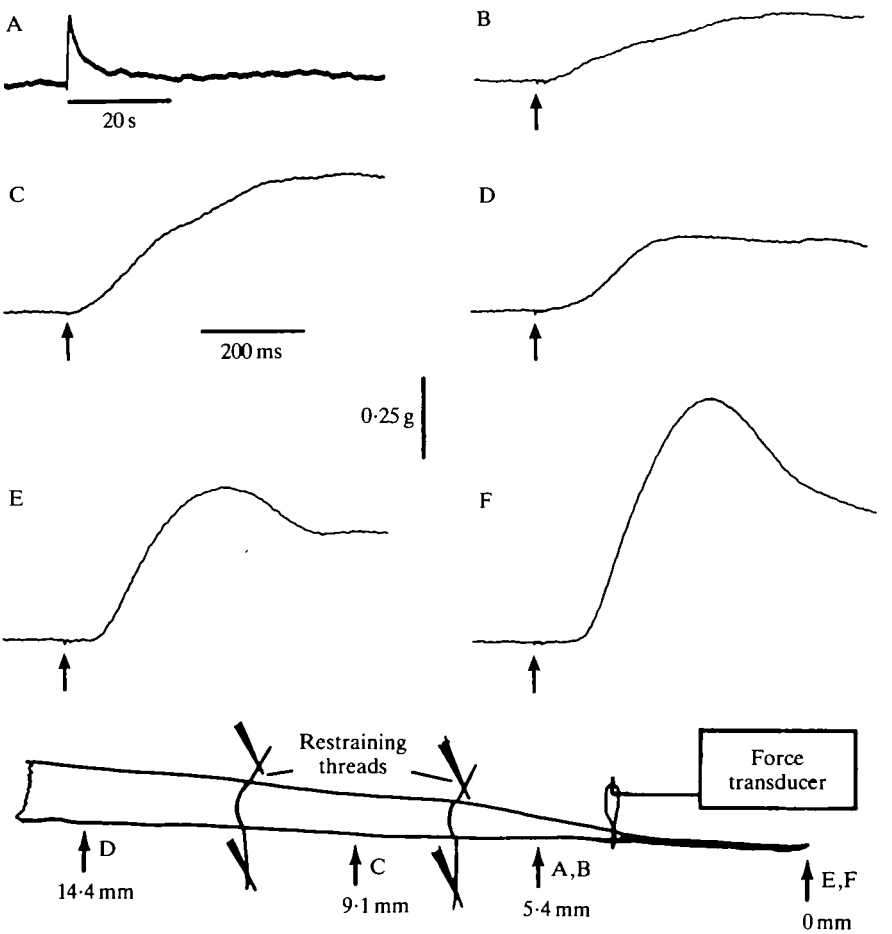

Fig. 5. Contractile responses of intervertebral muscles in an isolated (but otherwise intact) arm preparation. Inset shows the sites of stimulation (1-ms electrical shocks) corresponding to panels A–F and locations of the force transducer attachment and restraining threads. (A) Complete slow, twitch-like response recorded at a slow rate. (B–F) Initial portions of responses recorded at a 10-fold higher chart speed than in A. The delay with stimulation at the arm tip (E,F) is greater than with more centrally applied stimuli (B–D). Arrows show time when shock was applied.

also observed (Fig. 6B). As in the behavioural experiments described earlier, application of a bare needle alone (i.e. no tubefoot mounted on it) could also produce positive responses (Fig. 6C).

Fast electrical activity in the RNC following tubefoot stimulation

To extend electrical recordings to the fraction of a second following arm tip stimulation, the mechanically driven tubefoot was employed while recording from

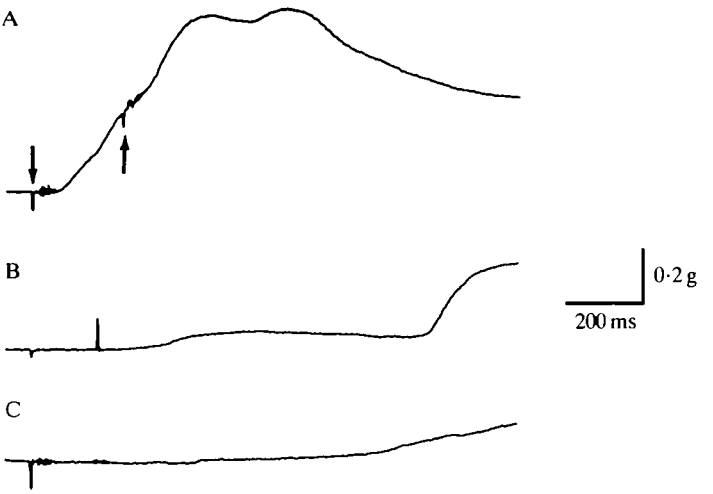

Fig. 6. Fast mechanical responses of an isolated arm to stimulation of the tip with a relay-driven tubefoot. Downward arrow (shown in A only) and the associated mechanical artifact indicate time of contact by the relay-driven probe. Upward arrow and artifact indicate when relay was deactivated to move the probe back to its original position. (A,B) Responses to stimulation by a *Pycnopodia* tubefoot. (C) Response to the needle alone which held the tubefoot in A and B.

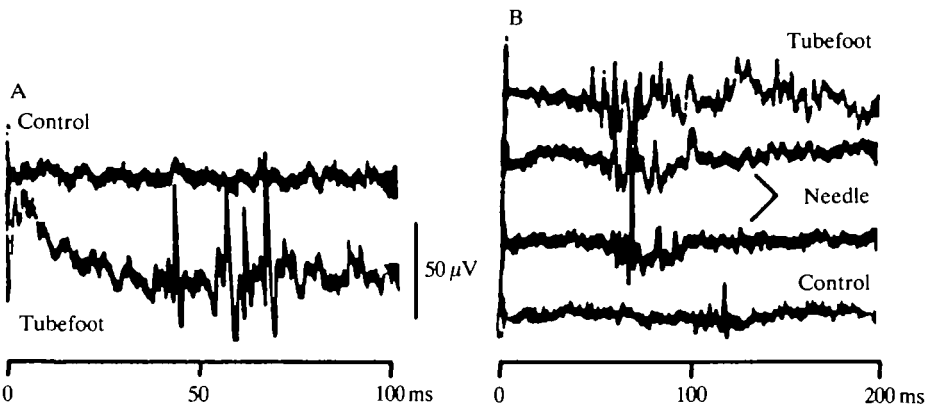

Fig. 7. Fast electrical activity in the radial nerve cord following relay-driven tubefoot stimulation of the arm tip. (A) Tubefoot trace with four large action potentials was obtained when tubefoot hit the arm tip. Control trace showing no electrical activity resulted from a near-miss. (B) Tubefoot and control traces obtained as in A. Needle traces show electrical activity triggered by contact of the arm tip by the relay-driven needle alone.

the exposed RNC. Fig. 7A shows typical results. The top trace shows a control response when the tubefoot was aimed just to miss the arm tip, and the bottom trace shows RNC activity recorded when the tubefoot hit the arm tip. Four fast spikes 50–100 μV in amplitude occur after a delay of 40–50 ms.

Activity like that in Fig. 7A could be reliably produced by tubefoot stimulation, but application of a needle only (i.e. no tubefoot mounted on it) could evoke similar activity. Fast activity appeared in the RNC with similar latency in both cases (e.g. Fig. 7B). Dividing the distance from the stimulus to the recording site by the latency (from onset of stimulus to peak of spike) defines conduction velocities of 41 cm s^{-1} for the tubefoot trace and 33 cm s^{-1} for the needle trace. Conduction velocity calculated in this way must be a lower limit, because some delay must exist in transducing the sensory input and setting up the initial action potential. Neuronal units responsible for generating the propagated impulse in Fig. 7B must therefore conduct at least 4–5 times as fast as the speed at which the wave of intervertebral muscle activation progresses centrally.

Identification of type 1 'giant' axon activity

Action potentials displayed in Fig. 7 are much faster and larger than those in Fig. 2, and these characteristics suggest that giant axons in the RNC are involved in these early electrical events. To test this idea more critically, and to identify the type of axons involved, fast electrical activity in the RNC in response to electrical stimulation alone was studied. Fig. 8 shows the RNC discharge accompanying shocks of different amplitudes applied 9·5 mm distal to the recording site. The smaller shocks (20 V; Fig. 8A) elicited a single component action potential (arrowhead 1) conducting at approximately 45 cm s^{-1}. Larger shocks (45 V; Fig. 8B) yielded spikes of increased amplitude at the same latency (arrowhead 1) and a smaller, slower event (arrowhead 2) about 30 ms later.

This pattern of electrical activity with increasing shock strength is similar to that described by Tuft & Gilly (1984), who showed that two distinct types of axons (1 and 2) are involved. Type 1 axons are the fastest and presumably largest axons in the RNC. Conduction velocity of the fastest spikes in the present study was $54 \pm 9{\cdot}6$ cm s^{-1} (mean ± S.D., $N = 18$) at 14°C. Tuft & Gilly (1984) gave a mean value of 139 cm s^{-1} at 20°C. In another species of ophiuroid, an average conduction velocity of 38 cm s^{-1} at 15°C was reported by Brehm (1977) for the most rapidly conducting units. It is thus reasonable to identify our fastest responses as activity of type 1 giant axons.

Activity in type 1 axons can also be identified through ion substitution experiments. Tuft & Gilly (1984) showed that removal of Ca^{2+} from the bathing medium did not affect the type 1 spike, but substitution of choline for Na^+ reversibly eliminated this activity. However, they showed that type 2 activity was impaired in a Ca^{2+}-free medium. Fig. 9 shows that the fastest electrical activity in our experiments also persists in a Ca^{2+}-free medium. Fig. 9A shows four sequential recordings made from an arm that had been dissected and soaked in Ca^{2+}-free sea water for approximately 60 min. Type 1 activity (conducting at 48 cm s^{-1}) in response to a

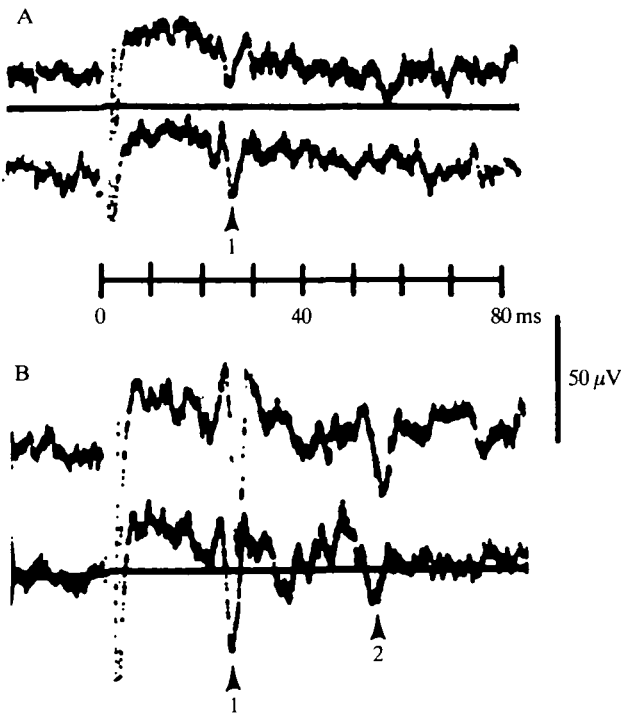

Fig. 8. Identification of low-threshold, fast electrical activity characteristic of type 1 giant axons in the radial nerve cord. (A) 20 V shocks yield short-latency spikes (arrowhead 1). (B) Stronger (45 V) shocks yield type 1 activity (arrowhead 1) as well as a slower spike (arrowhead 2).

strong shock was present, but no other activity was apparent. 12 min after re-admission of Ca^{2+} to the medium, the recordings in Fig. 9B were obtained in response to four shocks of increasing amplitude. Type 1 activity was still present (20 ms latency) and activity at later times now also appeared in response to strong shocks. Presumably, this reflects the recovery of function in Ca^{2+}-dependent type 2 axons.

All characteristics of the most rapid electrical events described above support the idea that they reflect activity in the giant type 1 axons of the RNC. Tuft & Gilly (1984) argued that these axons run uninterrupted by synapses for at least 1·5 cm and possibly the entire length of the arms. They also felt that conduction velocities measured did not strongly depend on positioning of the electrodes on the arm. Our results are consistent with these ideas. Fig. 10 is a plot of conduction velocity *vs* inter-electrode distance in 18 experiments. Linear regression analysis indicates the correlation (solid line) is very poor ($r^2 = 0{\cdot}075$). Thus, conduction velocity along type 1 axons may be fairly uniform over at least the distal-most 3 cm of arm. Variability below 1 cm probably reflects the limitations of the method used for determining velocity (see Materials and Methods).

Although the linear regression yields a poor fit to a single slope, this may not simply reflect scatter in the data. The apparent excess of high conduction velocities at

short inter-electrode distances suggests that velocity actually decreases over 0–1 cm and then becomes relatively constant for at least 2 cm. This changeover may be related to a transition from purely axonal conduction in individual type 1 fibres to trans-synaptic conduction between type 1 elements as proposed by Cobb (1985). Unfortunately, the variability in our short distance data precludes any detailed analysis of this important point.

Type 1 axons are activated by tubefoot stimulation

To make an association between type 1 axons and those units rapidly activated by tubefoot contact, a series of experiments was carried out in which RNC activity was recorded from the same preparation and a comparison of the responses to electrical and tubefoot stimulation was made. Electrical stimuli were first applied at an exposed section of RNC 8 mm from the arm tip (see inset to Fig. 11) to evoke activity reliably in type 1 axons. The RNC discharges due to electrical and tubefoot stimulation could then be more directly compared.

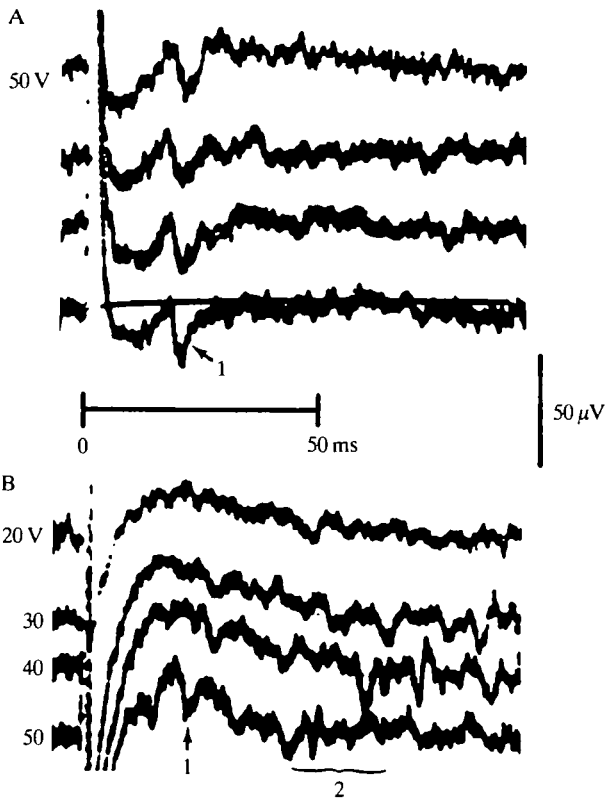

Fig. 9. Identification of type 1 axon activity in a Ca^{2+}-free medium. (A) 50 V shocks elicit only short-latency type 1 activity in a preparation that had been exposed to a Ca^{2+}-free medium for 60 min. (B) 12 min after returning the preparation in A to natural sea water shocks of increasing strength yield a two-type pattern of activity similar to that in Fig. 8.

Both modes of stimulation elicited action potentials similar (though not identical) in amplitude and latency (Fig. 11A,B). Conduction velocity of the fastest spike was about 45 cm s^{-1} in each case, a value characteristic of type 1 axons. Thus, axons of type 1 status (or a very similar one, based on conduction velocity) must be quickly

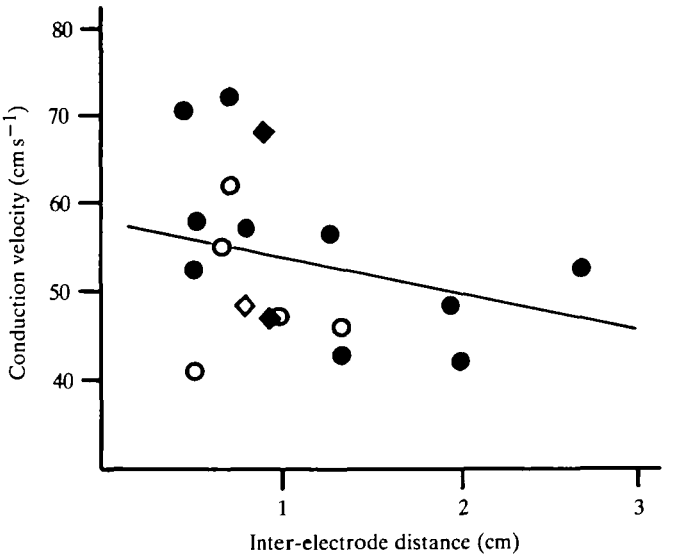

Fig. 10. Dependence of conduction velocity of type 1 axons on the distance between stimulating and recording electrodes. A very poor correlation exists, suggesting that type 1 axons propagate at a relatively uniform velocity of approximately 50 cm s^{-1} over at least 3 cm of arm length.

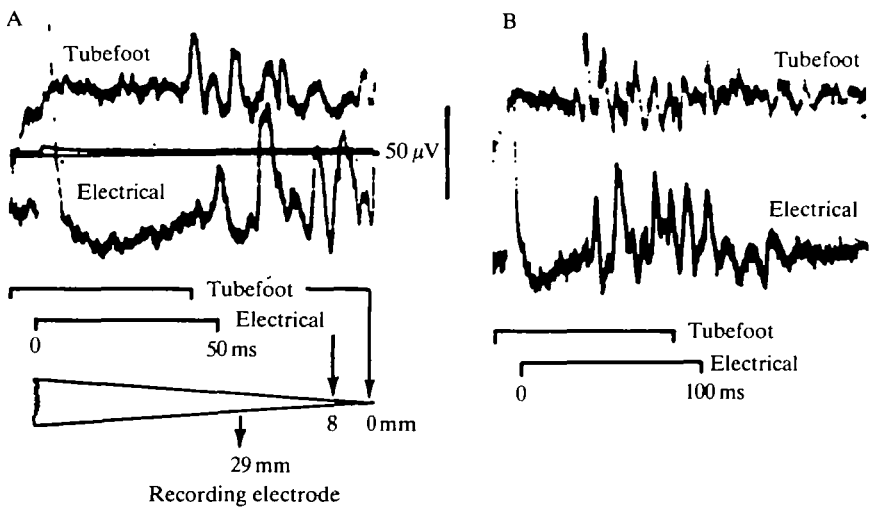

Fig. 11. Activation of type 1 axons by tubefoot and electrical stimulation in the same preparation. Inset shows sites of the two types of stimulation and the recording electrode. Note that 0 ms time points are slightly different for tubefoot and electrical stimulation, as indicated by scale bars. (A) Action potentials conducting at 45 cm s^{-1} are activated by both modes of stimulation. (B) Results of another trial, as in A but on a slower time base.

activated by tubefoot stimulation. Activity in these fibres propagated for at least 3 cm down the arm with the same velocity as in type 1 giant axons.

DISCUSSION

In this paper we have demonstrated that chemotactile stimulation of the sensitive terminal portion of an arm of *Ophiopteris* with a tubefoot from a predatory asteroid elicits a stereotyped escape response. The earliest behavioural phase of the escape response is a very rapid, snake-like recoiling of the stimulated arm away from the stimulus. This arm jerk is immediately followed by a coordinated rowing pattern of rapid locomotion. All of these movements are mediated by the segmental intervertebral musculature.

Stimulation of an arm tip in the above manner also rapidly activates a set of giant axons in the radial nerve cord (RNC) of the stimulated arm. This fast electrical activity propagates centrally much more rapidly than does the behavioural motor output constituting the arm jerk. Giant fibres involved in this fast electrical transmission appear to be of type 1 as described by Tuft & Gilly (1984). This identification is based on their low threshold, high conduction velocity and insensitivity to Ca^{2+}-free media relative to other RNC activity. Our results showing that type 1 transmission persists in Ca^{2+}-free media (see Fig. 9), while type 2 activity fails, suggest that type 1 axons can run for at least 8 mm without passing through a chemical synapse. This is in agreement with previous proposals (Brehm, 1977; Tuft & Gilly, 1984) that these units are 'through conducting' and cannot be interrupted at every segmental ganglion by a chemical synapse (Cobb, 1982; Cobb & Stubbs, 1981). So far, the longest ectoneural giant axons identified morphologically have been only 1 mm long (two segmental units in another species; Cobb, 1985).

The main thrust of this discussion concerns the functional role played by the giant type 1 axons, which must be among the largest in the RNC of *Ophiopteris*. We have no direct evidence for the function of these axons, but some functions can be discounted and others supported on logical grounds.

Several factors suggest that type 1 axons are *not* the final motor fibres directly responsible for intervertebral muscle activation.

First, the anatomy of the hyponeural motor neurones definitely does not support the view that large axons extend along the RNC for distances of up to 8 mm (Stubbs & Cobb, 1981; Cobb & Stubbs, 1981). This is a basic characteristic of type 1 fibres identified physiologically (see Tuft & Gilly, 1984).

Second, electrical activity recorded from the RNC 1 s or later after stimulation of the arm tip consists only of spikes of an amplitude much smaller than that expected for type 1 activity. Thus, at a time when vigorous locomotion would be occurring, activity of type 1 giant axons is not apparent. Conversely, selective stimulation of the lowest threshold type 1 units with weak electrical shocks applied directly to the RNC does not elicit any apparent motor activity involving the intervertebral muscles (unpublished observations).

Third, the timing of action potential propagation along type 1 axons relative to the wave of intervertebral muscle activation is not appropriate for a motor 'command' signal. From data plotted in Fig. 4, the propagation velocity for the most rapid phase of the wave of intervertebral muscle activation underlying the arm jerk was shown to be $7{\cdot}7\,cm\,s^{-1}$ (dashed line). Since latency for force development in response to direct stimulation in these muscles is quite brief at every point along the arm (20 ms or less, see Fig. 5), the command signal for muscle activation from motor neurones must also arrive at least 20–25 ms before movement commences at every segment along the arm. Any synaptic delay would increase this value but is not considered here. Propagation of the motor command signal would thus also occur at $7{\cdot}7\,cm\,s^{-1}$ with an initial delay of about 60 ms between the stimulus and the first command delivered (see Fig. 4). Such delays are consistently seen following electrical or chemotactile stimulation of the arm tip (see Figs 5, 6).

The conduction of action potentials along the giant fibres is far faster (approximately $50\,cm\,s^{-1}$) than the motor command and output. If impulses in *Ophiopteris* type 1 axons propagate at this velocity over most of the length of an arm, as suggested by Fig. 10 and as they appear to do in another species (Brehm, 1977), a serious mismatch becomes evident between the arrival of the electrical activity conveyed by type 1 axons and the command to activate the intervertebral muscles in a given segment. This mismatch leads to an unaccountable delay that grows progressively larger as the two signals propagate centrally at very different rates. It is difficult to see how they are causally related in any simple or direct way.

A much more likely role for type 1 giant fibres is one involving rapid communication of sensory input from a stimulated arm tip to the rest of the animal in order to mobilize a coordinated escape response. This is suggested by careful study of the escape response itself (see Fig. 1). If it is assumed that the arm tip was stimulated precisely when the first exposure occurred (time 0), then at the time of the next frame (400 ms) the fast arm jerk response is already complete. It is also evident that the two arms opposite to the stimulated one have moved by this time: they have already initiated the coordinated rowing phase of locomotion.

The total distance in the above case from the point of stimulation, down that arm, directly across the oral disk and out along the '2 o'clock' arm to where flexing of that arm has occurred is 13·6 cm. Information concerning the distant stimulus must therefore have propagated from arm to arm at an overall rate of at least $34\,cm\,s^{-1}$.

In reality, $34\,cm\,s^{-1}$ must be a lower limit. Since force production by the intervertebral muscles *closest* to the site of stimulation shows a latency of almost 100 ms under these conditions (see Figs 5, 6), a better estimate for the conduction time for the inter-arm information transfer would be 300 ms, corresponding to a propagation velocity of $45\,cm\,s^{-1}$. This value is close to our mean of $54\,cm\,s^{-1}$ for impulse conduction along type 1 axons. We suggest, therefore, that the rapid transfer of information from arm to arm demonstrated in Fig. 1 is directly mediated by these giant axons.

A simple arrangement of giant axons in the RNC like that depicted in Fig. 12 could account for these behavioural observations. We suggest that a type 1 giant fibre

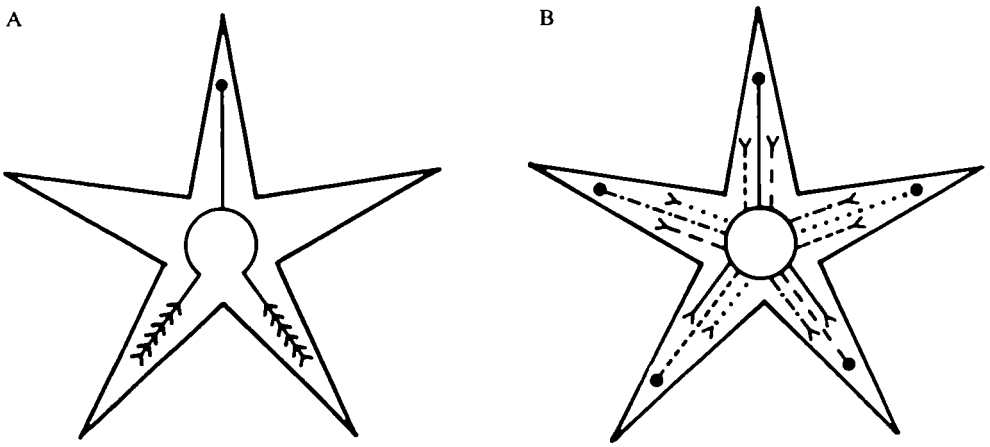

Fig. 12. Simple model of type 1 giant fibre pathway responsible for mobilizing the escape response of *Ophiopteris*. (A) A single type 1 axon is indicated with the site of sensory input (●) at the '12 o'clock' arm tip. The axon passes down the arm to the circumoral nerve ring where it bifurcates into the two most distant arms where excitatory influence on higher order hyponeural motor elements is exerted. (B) Repeating the element in A for each of the five arms yields a symmetrically sensitive and mobile animal with a minimum of three type 1 giant fibres per arm.

receives sensory input, presumably as a second-order (or higher) element, from several sensory modalities at one arm tip (filled circle in Fig. 12A), passes down the arm to the circumoral nerve ring where it bifurcates and sends a process out along each of the two most distant opposite arms. These branches exert indirect excitatory control over the segmental motor neurones (herringbone patterns), allowing for rapid initiation of the rowing phase of the escape response. Although it is likely that stimulation of the segment of giant axon in the arm with the sensory termination can also lead to motor neurone excitation (e.g. in triggering the arm jerk), this aspect is not included in the simple model of Fig. 12A.

It is not difficult to construct a symmetrically sensitive and mobile animal based on this scheme. Fig. 12B shows that only three giant axons per arm are required, and this is far fewer than exist morphologically. If each giant axon did not selectively bifurcate into the two most distant arms, but instead ramified into all four other arms, additional circuitry to inhibit rowing in the two arms closest to the stimulated one would also have to be postulated.

It is important to note that the model in Fig. 12 does not require the schematic type 1 axon in Fig. 12A, defined on purely functional grounds, to be composed of a single, individual neurone extending to the degree indicated. Although this is a reasonable possibility, another is that type 1 axons are serially linked by synapses, either chemical ones spaced relatively far apart (8 mm or more) or electrical junctions occurring more frequently. Electrical synapses might even occur on a segmental basis without seriously sacrificing the innate speed of the type 1 fibres. Such an arrangement would provide consonance with anatomical data suggesting that the ophiuroid

RNC is composed of segmental units (Cobb, 1982, 1985; Cobb & Stubbs, 1981; Stubbs & Cobb, 1981).

Although the precise location of type 1 axons in the RNC is not known, they are presumably ectoneural. Giant ectoneural axons have been described in several species of ophiuroids (Brehm, 1977; Cobb & Stubbs, 1981), and electron microscopic observations have confirmed their presence in *Ophiopteris* (A. Yee, unpublished results). Ectoneural cell bodies which give rise to some of the giant axons have also been identified (Cobb, 1982), and details of organization and spatial extent of some of the axons have now been established (Cobb, 1985). Functional classification of the morphologically identified axons cannot be made at this time, however.

Most importantly from the functional viewpoint, Cobb & Stubbs (1981) found longitudinal giant axons that run in bundles along the aboral surface of the ectoneural portion of the RNC, and some of these axons make chemical synapses with processes of hyponeural neurones in the segmental ganglia (Stubbs & Cobb, 1981). Cobb (1985) has recently reported intracellular recordings (in another species) from both ectoneural and hyponeural giant axons and cell bodies and determined the extent of processes from these elements by intracellular dye-filling techniques. Again, these ectoneural units may or may not represent type 1 fibres, but the morphological pathway for physiological transmission from ectoneural giant axons to the higher order hyponeural motor elements undoubtedly also exists in *Ophiopteris*.

In summary, the function of type 1 fibres in *Ophiopteris* is to mediate rapid transmission of sensory information over long distances, basically from arm tip to arm tip in several hundred milliseconds. One obviously valuable role would be the rapid mobilization of the initial power stroke in the coordinated rowing reaction in response to disturbance at a distant arm tip. There would seem to be no other way that the ophiuroid nervous system could accomplish this feat. Type 1 axons are probably also involved in coordinating the initial arm jerk, but the relatively short propagation distances involved make the picture less clear-cut. Details of transmission from the giant axons to higher order elements along the conduction path and the subsequent integration into the coordinated discharge of hyponeural motor neurones that ultimately produces the escape response in this animal remain totally unknown. The type 1 giant axon system and the arm jerk/rowing response provide a valuable model system for future study of neural integration in ophiuroids.

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