J. exp. Biol. **113**, 337–349 (1984)
Printed in Great Britain © *The Company of Biologists Limited 1984*

IONIC BASIS OF ACTION POTENTIAL PROPAGATION ALONG TWO CLASSES OF 'GIANT' AXONS IN THE OPHIUROID, *OPHIOPTERIS PAPILLOSA*

BY PAMELA J. TUFT AND WM. F. GILLY

Hopkins Marine Station of Stanford University, Department of Biological Sciences, Pacific Grove, CA 93950, U.S.A.

Accepted 9 April 1984

SUMMARY

1. Electrical activity was recorded from the radial nerve cord with extracellular suction electrodes. Spontaneous unitary spikes are 20–50 μV in amplitude with a duration of about 10 ms.

2. Compound action potentials evoked by brief electrical shocks consist of two distinct fast spikes followed by a much slower wave of activity. Fast spikes are graded in amplitude to a maximal level, with little change in time course, over a considerable range of stimulus intensities.

3. The larger of the two fast spikes (spike 1) has a lower threshold and higher conduction velocity: $139 \pm 14{\cdot}3\,\mathrm{cm\,s^{-1}}$ (mean ± S.D., $N = 6$) *vs* $55 \pm 7{\cdot}4\,\mathrm{cm\,s^{-1}}$ for spike 2.

4. Spike 1 is reversibly eliminated by replacement of Na with choline in the bathing medium; spike 2 is unaffected.

5. Spike 2 is reduced in amplitude by removal of Ca from the bathing medium; spike 1 is unaffected.

6. Cadmium (2–10 $\mathrm{mmol\,l^{-1}}$) reversibly blocks spikes 1 and 2. Tetrodotoxin (TTX, 1–2 $\mu\mathrm{mol\,l^{-1}}$) does not affect either spike.

7. These results support the existence of at least two classes of relatively large axons. Type 1 axons, generating spike 1, rely on a TTX-insensitive Na action potential, whereas type 2 axons may utilize a Ca action potential.

8. Each spike represents summed activity of a relatively small number of axons probably arranged in bundles. The nature of morphological pathways involved in ophiuroid neural conduction is discussed.

INTRODUCTION

Electrical activity in the echinoderm nervous system has not been extensively studied. Much of our understanding of this important phylum comes from work on ophiuroids, commonly known as brittle stars (Cobb, 1982; Pentreath & Cobb, 1982). Many of these animals are highly mobile, show complex behaviour and have relatively large, accessible neural elements. They thus provide distinct advantages for neurobiological experimentation. Propagation of electrical activity along the ophiuroid

Key words: Ophiuroid, nerve, ionic.

radial nerve cord (RNC) was first described by Brehm (1977). He observed brief unitary spikes, apparently generated by axons, that propagated without decrement and postulated these to be involved in control of bioluminescence. A much slower wave of activity that spread decrementally from the site of stimulation was also described. Based on these characteristics and its demonstrated Ca sensitivity, Brehm interpreted the slow wave to be largely synaptic in nature and to reflect integrative activity in the RNC. Electrical activity underlying photosensitivity has recently been studied in a different species (Stubbs, 1982).

Morphological investigations of the RNC (Brehm, 1977; Cobb & Stubbs, 1981) have revealed 'giant' ectoneural axons up to 10–20 μm in diameter. These axons are probably of mixed motor and sensory types and run longitudinally, both individually and in bundles. The hyponeural portion of the nerve also contains large motor axons (Pentreath & Cottrell, 1971). Axons of such size are at least an order of magnitude larger than those typically found in echinoderms (Hyman, 1955) and undoubtedly were those recorded from in the electrophysiological studies.

This paper describes electrical activity propagated along the RNC of *Ophiopteris papillosa*. Both spontaneous and stimulated activity can be recorded. Identification and analysis of a characteristic compound action potential is presented, and the effects of ionic substitutions on such activity are described. Evidence suggests that individual components of the compound action potential represent recordings from separate axonal classes within the RNC. The most rapidly conducting, and presumably largest, units appear to have activity mediated by TTX-insensitive Na channels.

METHODS

Specimens of *Ophiopteris papillosa* were collected subtidally in Monterey Bay near Hopkins Marine Station, Pacific Grove, CA. They were maintained in running natural sea water at 14 °C.

Animals were anaesthetized for approximately 10 min in a solution of 1 part isotonic $MgCl_2$ to 5 parts natural sea water (NSW). Following this, the segmental arm plates on the oral surface were manually removed from one arm to reveal the ectoneural surface of the radial nerve cord (RNC). NSW was then flushed over the specimen until spontaneous motor activity resumed. If activity did not return within 30 min, the preparation was discarded. For recording spontaneous electrical activity, the central disk (oral side up) was pinned to a Sylgard (Dow Corning)-floored dish, thereby allowing free arm movement. In all other cases the entire arm under study was immobilized by pinning down with U-shaped pins.

A bipolar stimulating electrode (70 μm diameter Pt wires spaced 250 μm apart and insulated except for the tips) was symmetrically straddled over the RNC. Rectangular pulses of 1 ms duration (except for the strength-duration experiment in Fig. 5) were generated by a Grass S-6 stimulator.

Suction recording electrodes were made from 1 ml disposable syringes drawn under a flame to tip diameters of about 200 μm and filled with NSW. Recordings were obtained by measuring differentially between Ag: AgCl wires, one inside the syringe and one wrapped around the outside of the tip. Signals were amplified by a Tektronix Type 122 preamplifier with the high-pass filter set at 0·8 Hz (coupling time constant

Table 1. *Solutions employed*

Solution	Constituent concentrations (mmol l^{-1})					
	NaCl	$CaCl_2$	KCl	$MgCl_2$	Tris	Choline-Cl
0-Ca SW*	440	0	10	40	10	0
0-Na SW	0	10	10	20	10	420
60-Ca SW	440	60	10	10	10	0
2- and 10-Cd SW	425	60	10	10	10	0

Cd was used at 2 and 10 mmol l^{-1}.
* Buffered with 2 mmol l^{-1} EGTA.

0·2 s). Final recordings were photographed from a Tektronix 5111 storage oscilloscope.

Electrical activity could be recorded from almost any point on the nerve cord, as well as from the intervertebral muscles. The most reliable and reproducible results, however, were obtained from the slightly swollen 'ganglionic' regions in each segment of the RNC where nerves branch out to the tube feet. These regions contain both cell bodies and neuropile as well as many axonal processes (see Fig. 6 in Cobb & Stubbs, 1981). All recordings reported here were made at these locations with the suction electrode making a seal on the ectoneural surface. In all cases the entire RNC was intact and uncut. It made no difference to the form of the potentials when the positions of recording and stimulating electrodes were switched. Interelectrode distance was 1·5 cm.

Some experiments were conducted with the RNC totally submerged. In order to avoid changes in solution level during the ionic substitution experiments, all recordings were made in a drained dish with 30 ml of solution being slowly dripped onto the RNC prior to recording. Recording and stimulation sites in these cases were just above the solution surface.

All experiments were conducted at 19 °C with solutions prepared as indicated in Table 1 and maintained at pH 7·8.

RESULTS

Spontaneous electrical activity during arm movements

Although spontaneous electrical activity in ophiuroid RNC has not been previously reported, the reasons for this are unclear. In the present experiments spontaneous activity was always observed once a good seal was obtained with the recording electrode on the RNC in an unrestrained arm. Such activity is illustrated in Fig. 1 and consists of bursts of individual spikes with amplitudes of 20–50 μV and durations of 10–20 ms (measured at half-peak amplitude). Fig. 1A shows a single spike recorded on a fast time base. As argued below (see also Brehm, 1977) these spikes probably represent activity of single axons. Fig. 1B–D shows bursts of spikes that appear similar to the one in Fig. 1A. Activity like this was regularly seen both during flexion of the arm in the horizontal plane (Fig. 1B,C) and during movements of the oral disk and genital bursae only (i.e. arm itself did not move; Fig. 1D). In each case, more

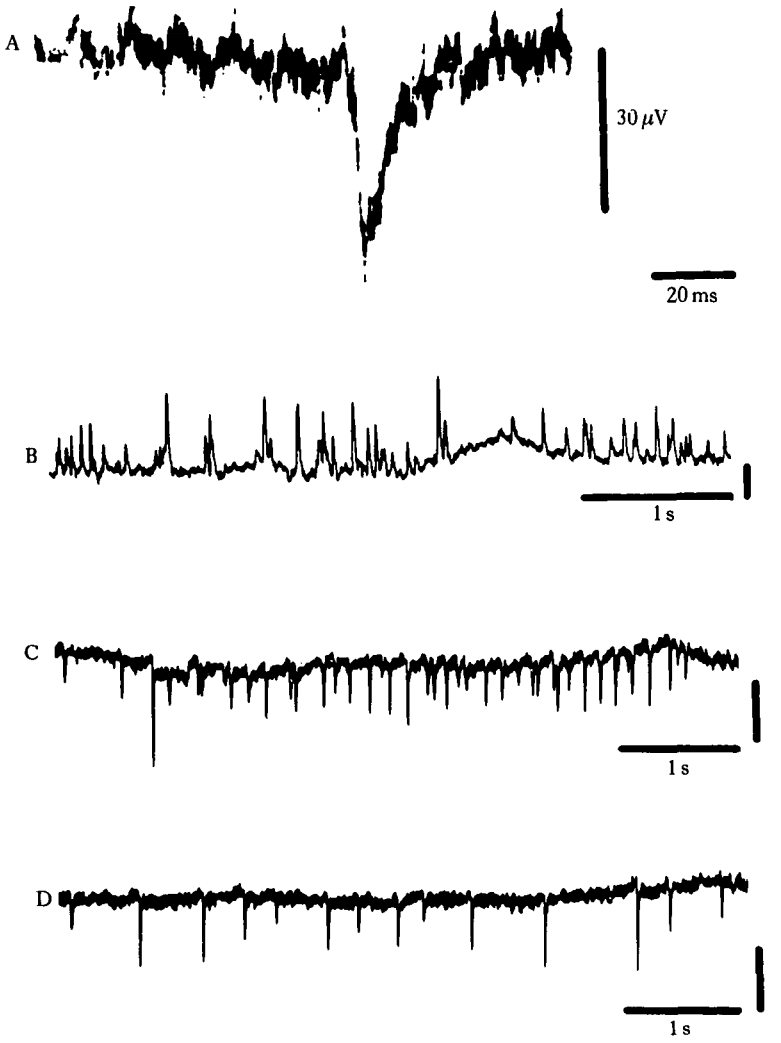

Fig. 1. Spontaneous electrical activity of RNC in natural sea water. (A) Single spike at high sweep speed. (B) Chart recording showing a train of discrete spikes associated with spontaneous arm movements (polarity inverted). (C) Train of single spikes associated with arm movements as in B in a different animal. (D) Larger spikes accompanying movements of central disk and genital bursae. All vertical scale bars correspond to 30 μV.

than one spike amplitude is visible, implying that several units in the RNC were active. No electrical activity was noted in association with spontaneous tube feet movement. Detailed correlations between behaviour and electrical activity were not carried out in the present study.

Similar electrical activity occurs upon RNC stimulation

Activity could be recorded in response to both mechanical and electrical stimulation. Mechanical stimulation consisted of manually agitating the oral arm plates or lateral spines with a plastic probe. A single brief shock constituted electrical stimulation. Although both methods gave similar responses, electrical stimulation was more

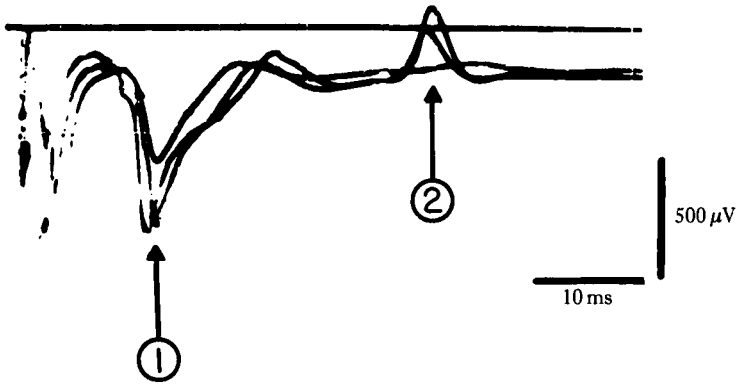

Fig. 2. Details of the compound action potential evoked by electrical stimulation of the RNC. Two distinct spikes are present (arrows 1 and 2); both are clearly separated from the shock artifact. Four traces correspond to stimuli of 0, 16, 32 and 48 V, all of 1 ms duration and spaced 1 min apart. Amplitudes of both spike components are graded with stimulus strength.

controllable, and the remainder of the results to be described were thus obtained.

The basic pattern of electrical activity consistently observed is indicated in Fig. 2. Four traces are illustrated for shocks of 0, 16, 32 and 48 V amplitude (1 ms duration).

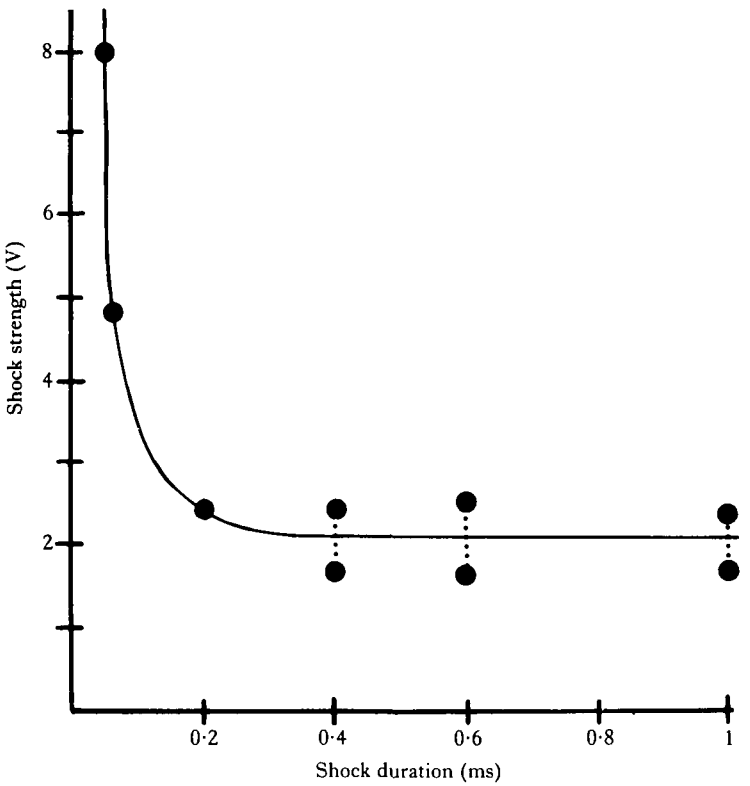

Fig. 3. Strength-duration curve for first detectable electrical activity. Smooth curve was drawn by eye. A decrease in stimulus duration requires an increase in shock strength to elicit a just detectable response. Vertical dotted lines represent range of strengths found for the indicated durations in repeated trials.

Such responses were only observed for stimuli above a definite threshold and consisted of a multi-component compound action potential with two distinct early spikes (arrows 1 and 2) followed by a much slower wave of activity (not illustrated).

This three-part pattern is like that described by Brehm (1977), for another ophiuroid. The slow wave was shown by Brehm to be Ca-dependent and probably reflects synaptically mediated activity. This component was not studied in the present work. The rest of this paper concerns the two fast spikes.

Characteristics of the fast spikes

Fast electrical activity (spikes 1 and 2 as in Fig. 2) with certain characteristics could be recorded in every successful experiment. Typically, a graded response was observed for the first spike over a significant range of stimulus intensities. Gradation was also observed for the second spike, but was less marked. The gradation range was defined by a threshold voltage at which activity became first detectable and a maximal level where activity was essentially constant in amplitude for any further increase in shock strength. As can be seen in Fig. 2, responses changed primarily in amplitude over the gradation range, while the time course remained fairly constant.

Although spikes 1 and 2 are graded in amplitude over a range of stimulus intensities, a definite threshold exists at which activity becomes just detectable. Fig. 3 is a strength-duration curve analysing first detectable activity. The range of long pulse values

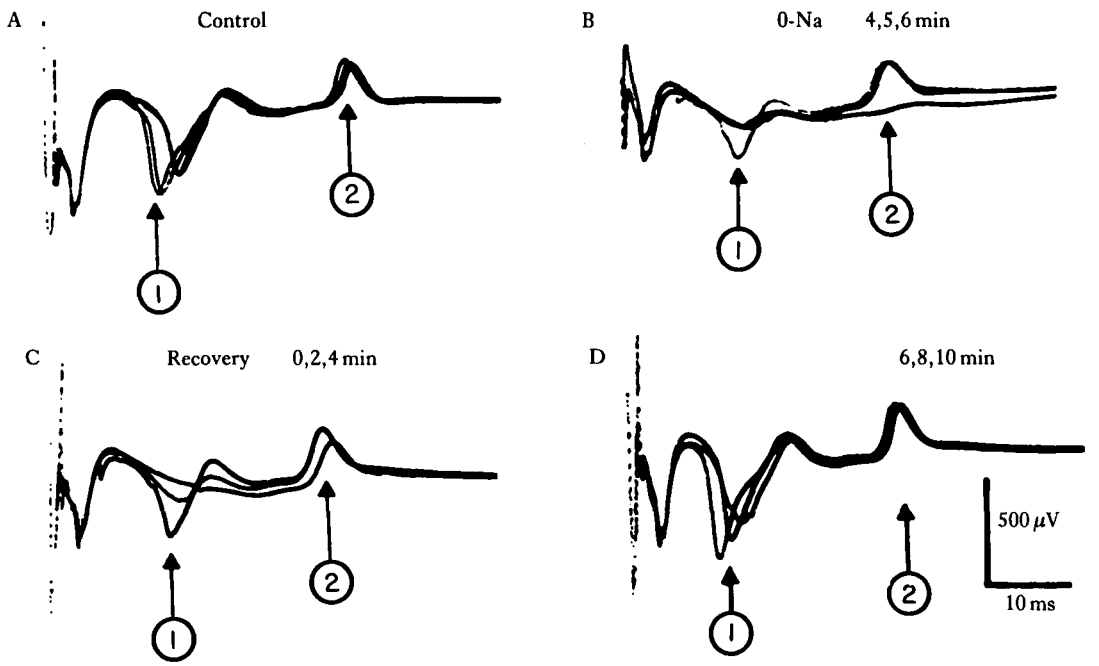

Fig. 4. Blocking of spike 1 by removal of Na from the bathing medium. (A) Control records obtained in natural sea water taken at 2-min intervals. (B) Reduction of spike 1 at 4 min following admission of 0-Na SW (choline substituted) and complete blocking of the spike at 5 and 6 min. (C) Progressive recovery of electrical activity following readmission of natural sea water. Records taken at 0, 2 and 4 min. (D) Continued recovery as in C, but at 6, 8 and 10 min total elapsed time in natural sea water. Stimulus for every record was 32 V, 1 ms duration.

(0·4–1 ms) defines an asymptote of about 2 V. Below 0·2 ms duration, threshold increases steeply, reaching 8 V at 0·08 ms. Just detectable responses were similar to the unitary spikes pictured in Fig. 1, both in amplitude and time course. Furthermore, their time of occurrence following the shock was very similar to spike 1 responses obtained with larger stimuli. The lowest threshold activity seen with very small shocks probably reflects excitation of only one or a very few units, and the large, full-sized spikes (e.g. Fig. 2) might thus indicate nearly simultaneous activation of many small units with similar properties. This idea will be elaborated upon in the Discussion.

Spike components 1 and 2 of the compound action potential have characteristic conduction velocities (measured as inter-electrode distance divided by time-to-peak) which are distinctly different. Experiments with six different nerve cords in NSW yielded a mean conduction velocity for the first spike of 139 cm s^{-1} (±14·3 S.D.) with a range of 115–151 cm s^{-1}. Spike duration was approximately 10 ms. The second spike propagated at a speed of 55 cm s^{-1} (±7·4 S.D.) with a range of 47–68 cm s^{-1}. Duration of the second spike ranged from 7–16 ms.

Characteristic ionic sensitivities of the fast spikes

Complete removal of Na from the bathing medium consistently inhibited the faster spike 1. Fig. 4A shows three records obtained in the control NSW, and Fig. 4B shows

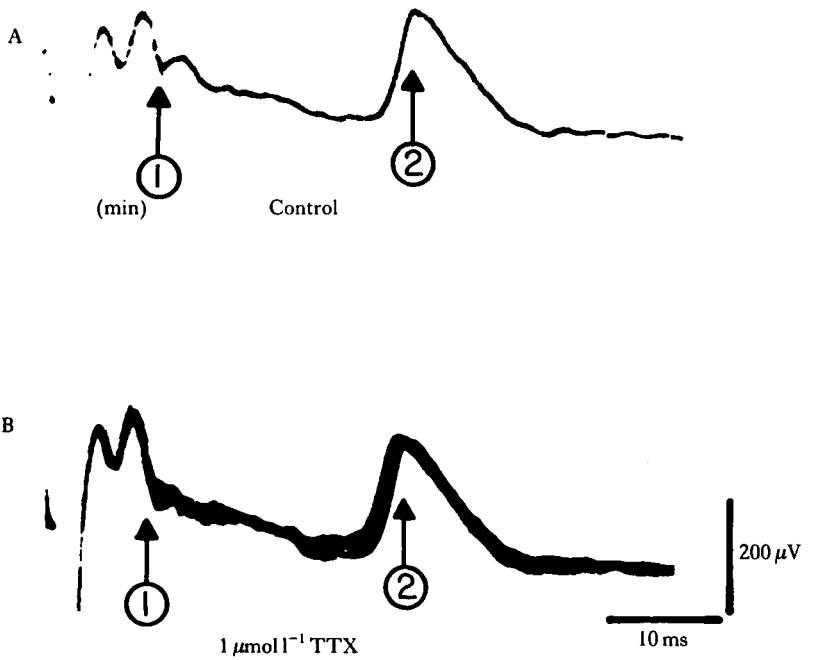

Fig. 5. Lack of effect of tetrodotoxin (TTX) on RNC electrical activity. (A) Control trace in natural sea water. (B) Superimposed traces taken every 2 min following application of 1 μmol l^{-1} TTX. Activity was monitored for 15 min, but no effect was seen (not illustrated). Other experiments with TTX at concentrations up to 2 μmol l^{-1} confirmed this.

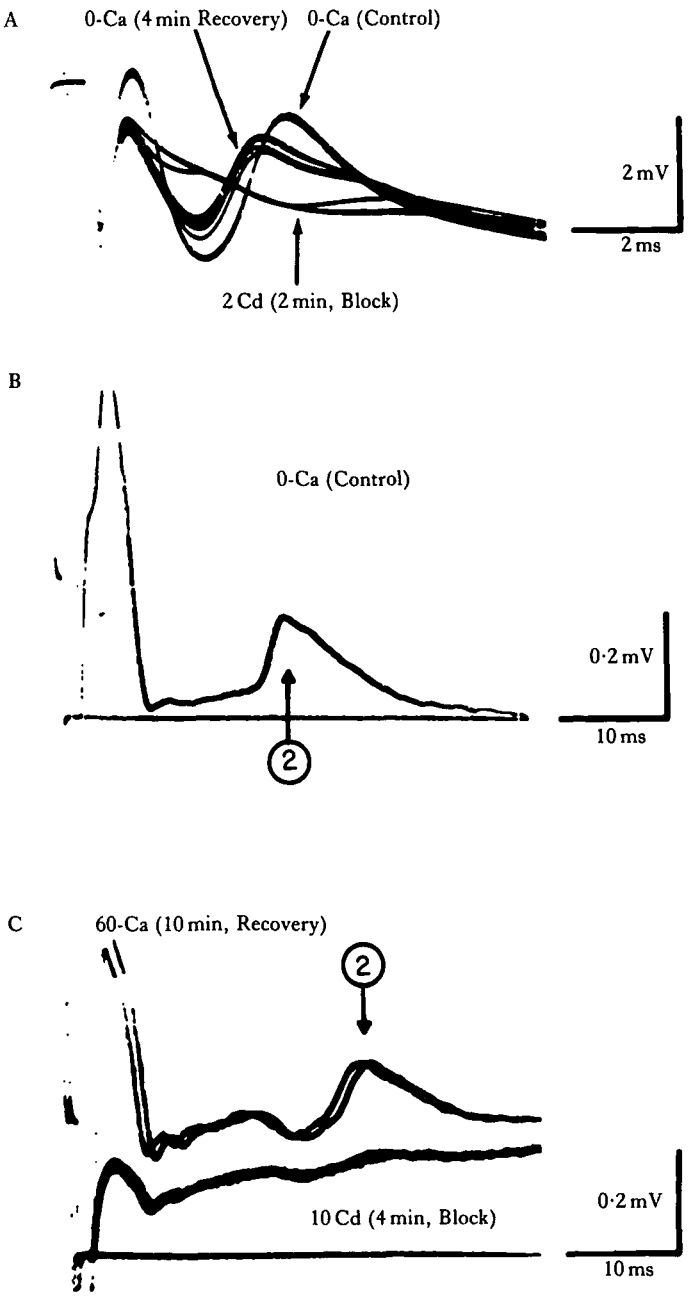

Fig. 6. Blocking of spikes 1 and 2 by cadmium ions. (A) Effect of 2 mmol l^{-1} Cd on spike 1. Control record was taken in 0-Ca SW. 2 Cd trace obtained after applying Cd-containing artificial sea water (see Table 1). Superimposed recovery traces taken at 4 min following Cd washout with natural sea water and every 2 min thereafter. (B),(C) Blocking of spike 2 in a different RNC. Control trace (in 0-Ca SW) is shown in (B). Block in 10 mmol l^{-1} Cd-containing artificial sea water (see Table 1) is shown in (C). Recovery in 60 mmol l^{-1} Ca artificial sea water (see Table 1) is also shown.

responses obtained 4, 5 and 6 min following replacement of Na with choline (0-Na SW, see Table 1). Spike 1 failed completely, and full recovery occurred upon readmission of Na (Fig. 4C,D). The second spike (2) was essentially unaffected by Na removal (Fig. 4A,B). We have no explanation for the apparent failure of spike 2 in the 5-min record of Fig. 4B. The response was completely normal at 6 min. This was the only case of such an apparently random failure of spike 2 in Na-free medium.

Results similar to those in Fig. 4 were obtained in two other Na-removal experiments with the same preparation and in one other RNC. Several experiments with Tris as a Na substitute, rather than choline, also gave similar results, but reversibility of the spike 1 block was very poor.

Several experiments with Ca-free sea water (0-Ca SW, see Table 1) consistently indicated no change in spike 1 and a decrease in spike 2 amplitude by a factor of 0·7–0·5 (not illustrated). Reversal of this effect was sometimes (but not always) seen when NSW was reintroduced. High-Ca sea water (60-Ca SW, see Table 1) raised threshold for both spikes slightly, with a more pronounced effect occurring for the second spike.

Effects of Na- and Ca-channel blockers

Tetrodotoxin (TTX), a very specific Na channel blocker, in concentrations of 1–2 μmol l^{-1} had no effect on the compound action potential (Fig. 5). Since spike 1 depends on Na, it appears that this activity involves TTX-insensitive Na-channels. Such channels are not uncommon (Narahashi, 1974).

Cadmium ions, potent blockers of many Ca-channels (Hagiwara & Byerly, 1981; but see also Discussion), blocked all electrical activity of the RNC at 2–10 mmol l^{-1}. Fig. 6A shows results from an experiment in 0-Ca SW on spike 1. Addition of 2 mmol l^{-1} Cd to the medium abolished the spike within 2 min, and removal of Cd led to significant recovery after 4 min. In another experiment (control records in 0-Ca SW, Fig. 6B) addition of 10 mmol l^{-1} Cd completely blocked spike 2 within 4 min. Recovery was essentially completed in 10 min after washing out Cd with 60-Ca SW (Fig. 6C).

DISCUSSION

Electrical activity described here is similar to that reported for other ophiuroids (Brehm, 1977; Stubbs, 1982) and in other echinoderms as well: echinoids (Takahashi, 1964; Sandeman, 1965; Millott & Okumura, 1968) and asteroids (Binyon & Hasler, 1970). In each case activity evoked by electrical stimulation consists of fast action potential-like spikes followed by a much slower wave. Subdivision of the faster response into two distinct kinetic components was first proposed for an echinoid (Sandeman, 1965). Duration of the compound action potential in echinoids is much longer than that in ophiuroids, however, making any exact comparison difficult. Brehm (1977) observed 'through conducting' spikes in an ophiuroid which propagated in an 'all-or-none' manner at velocities up to 78 cm s^{-1}. His conduction velocities measured at 15 °C are similar to those found in the present study at 19 °C, in which the faster spike 1 showed a mean velocity of 139 cm s^{-1}, and spike 2 propagated at 55 cm s^{-1}. Thus, it is very likely that the two fast spikes consistently

observed in these experiments reflect activity in units like those described by Brehm and presumably are axons of the 'giant' type discussed in the Introduction.

The present study is the first to demonstrate the ionic dependence of these rapidly conducting axons. Brehm (1977) studied the effects of ionic substitutions on electrical activity of the ophiuroid RNC and found a definite Ca requirement for the slow wave (component 3 in this report) but no dependence on Na. However, his data (Fig. 7) did not resolve activity of the faster, 'all-or-none' spikes (i.e. spikes 1 and 2 in this report) in the ionic substitution experiments.

Existence of these two distinct fast spikes, the more rapidly conducting of which is Na-dependent, leads to the hypothesis that these recordings reflect activity in two functionally distinct classes of axons. The consistent difference in conduction velocities of spikes 1 and 2 by a factor of about 2·5 provides one basis for this assertion. The faster conduction velocity and apparently lower threshold of spike 1 responses (cf. results in conjunction with Fig. 3) suggest that the underlying 'Type 1' axons may be the larger of the two.

Characteristic ionic sensitivities of the two spikes also support the idea of separate axon types. Spike 1 exhibits a pronounced Na requirement for propagation. The second spike is more or less unaffected by Na removal, but the possibility of incomplete solution changes cannot be completely dismissed. Removal of Ca, on the other hand, does affect spike 2. Amplitude of this component was reduced by up to 50% in 0-Ca SW. Thus, spike 2 may be mainly Ca-dependent, but Na may also be involved. Persistence of spike 2 in Ca-free media can probably be explained by the presence of sufficient residual Ca in the tissue to support a reduced level of excitability.

Pharmacology reported here is also consistent with the proposed Na-dependent spike 1 and Ca-dependent spike 2. Both spikes were blocked by Cd and were not affected by high concentrations of TTX. Cadmium would be expected to block a Ca-mediated action potential, consistent with results observed for spike 2. Cadmium also inhibits Na- (and K-) channel activation in squid giant axon quite effectively by greatly slowing their opening kinetics (Gilly & Armstrong, 1982*a*,*b* and unpublished data). This inhibition would probably be manifest as a dramatic elevation of action potential threshold, perhaps beyond the experimentally accessible range, in experiments using stimulating/recording techniques like those employed here. No electrical activity in the present experiments was ever seen in the presence of Cd with the largest shocks available. Thus, blocking of spike 1 by Cd seems consistent with the known effects of Cd on Na channels. Results with TTX do not present any serious threat to our hypothesis, for many examples of TTX-insensitive Na channels are known (Narahashi, 1974).

Based on these arguments we propose that type 1 axons, having an action potential generated by Na-channels, and type 2 axons, relying primarily on Ca-channels, are responsible for propagation of spikes 1 and 2, respectively, in the radial nerve cord of *Ophiopteris papillosa*.

What is the nature of the morphological pathways involved in the conduction of these spikes along distinct axons as proposed here? Considering the extracellular recording methods and the large amplitudes and graded characteristics of the observed spikes, it is unlikely that either spike 1 or 2 represent excitation of single axons. However, the individual large spikes may represent activity in a number of axons with

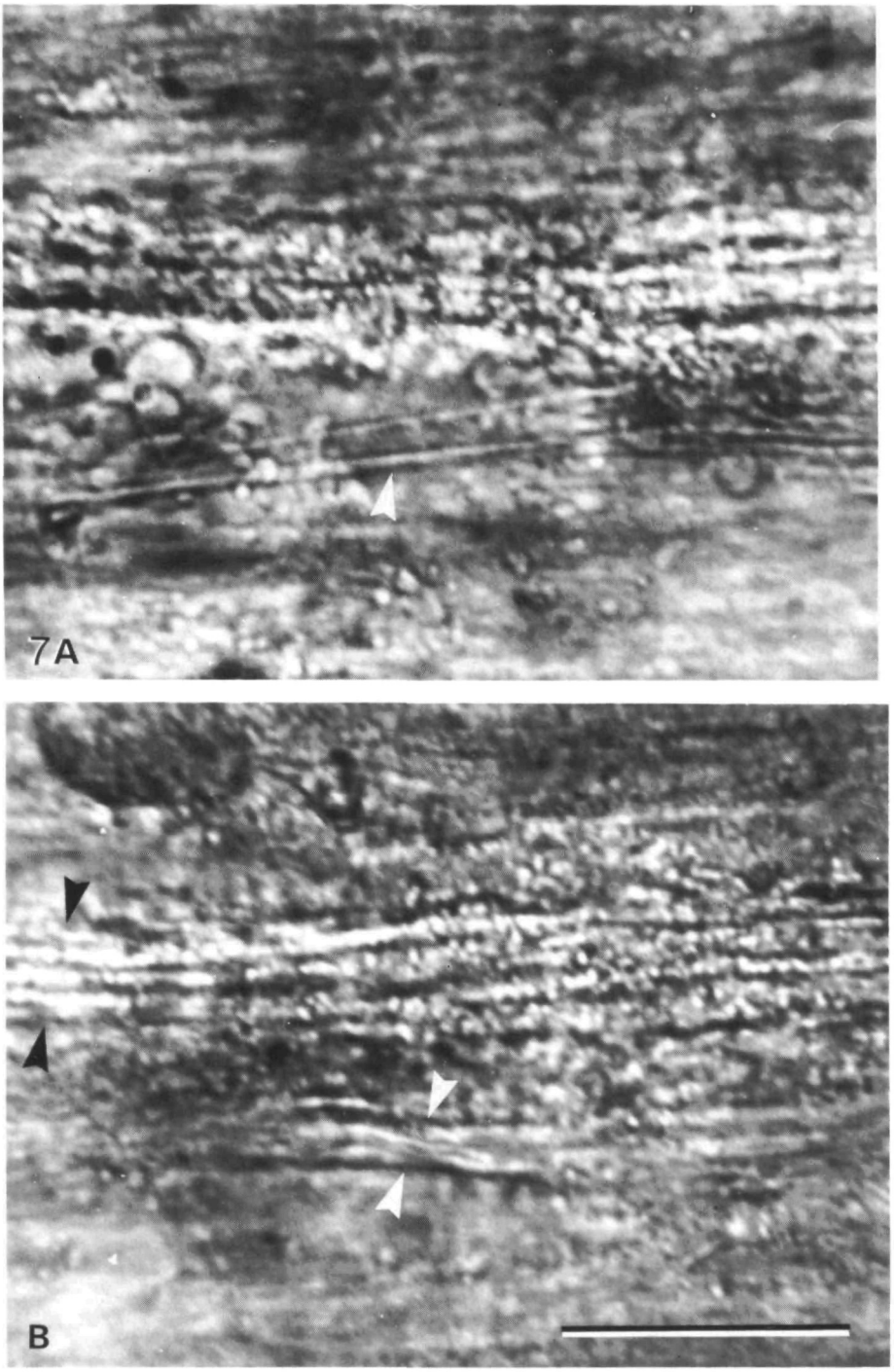

Fig. 7. Light micrographs of living radial nerve cord. (A) Single longitudinally-orientated axon is indicated by arrow. (B) Bundles of axons are indicated by arrows. See text for additional details. Scale bar, 50 μm.

similar characteristics. Thus, graded responses over a definite range of stimulus strengths can be explained as gradual recruitment of more axons, perhaps within a small bundle, up to a final, set number. This idea is consistent with the fairly constant time course of the spike over the range of graded amplitudes found for both spikes (Fig. 2). If each axon synchronously generated a unitary spike of about 35 μV amplitude (Fig. 1A), then maximal amplitudes of spikes 1 and 2 suggest that the number of axons in a bundle may be fairly small, perhaps 20 or so.

The presence of 'giant' axons in ophiuroid RNC is well established: up to 10 μm diameter in *Ophiothrix fragilis* (Pentreath & Cottrell, 1971), up to 8 μm in *Ophiopsila californica* (Brehm, 1977), up to 20 μm in *Ophiura texturata* (Cobb & Stubbs, 1981). Moreover, that those axons run closely opposed to one another in bundles is apparent from Brehm's (1977) photomicrographs and constitutes a major feature of the proposed layout of ophiuroid neural pathways as indicated by Cobb & Stubbs (1981).

Similar bundles of 'giant' axons exist in *Ophiopteris papillosa*. Fig. 7 shows light micrographs of optical sections through a living *Ophiopteris* radial nerve using Nomarski optics. Longitudinally-orientated axons (single arrow) of about 4–5 μm diameter are visible in Fig. 7A. Cell bodies of 10–15 μm diameter are clustered on the left-hand edge of the plate. Bundles of axons are indicated in Fig. 7B. A large bundle (20–25 μm diameter) is outlined by black arrows, and its constituent axons are readily visible. In the smaller bundle (about 10 μm diameter, white arrows) axons form a rope-like arrangement in which they apparently spiral over one another. These axonal bundles are located in an interganglionic region of radial nerve, with the larger bundle running closer to the RNC's midline. While we cannot say with certainty that our recordings were made from these precise elements, the possibility of recording from two different axonal classes obviously exists in the species employed here. Exact identification and morphological characterization of the axons and axonal bundles involved warrant further investigation.

Involvement of synapses in conduction along ophiuroid axons has been a subject of some dispute, and our results also concern this issue. Brehm (1977) argued that his 'all-or-none' spikes did not pass through synapses, but Cobb & Stubbs (1981) have challenged this interpretation based on morphological studies. Their results, involving degeneration experiments, may not be conclusive either, however, as the entire axon may not have died following sectioning of the RNC. It is also possible that the two groups looked at different classes of axons altogether.

Involvement of synapses in propagation along our type 1 axons seems very unlikely. If these axons extend only the length of single interganglionic regions, about 1 mm (Cobb & Stubbs, 1981; Cobb, 1982), then over the recording distance of 1·5 cm, 14 or 15 synapses would occur. Assuming a minimum delay of 0·7 ms per synapse (obligatory delay for frog neuromuscular junction at 18 °C; Katz, 1966), total delay time for the distance travelled would be about 10 ms, yielding a delay time per unit length of 0·0067 s cm^{-1}. Mean apparent conduction time per unit length reported here for spike 1 is 1 s per 139 cm (0·0072 s cm^{-1}). The difference between this value and the hypothetical delay time above would thus define 'true' axonal conduction time per unit length to be 0·00053 s cm^{-1}. This corresponds to a conduction velocity of 1894 cm s^{-1}, which is almost exactly that reported for a 476-μm diameter squid axon at 18·5 °C (Hodgkin & Huxley, 1952) and unreasonably large for any axons in

ophiuroids. Scaling down the squid axon to 4 μm diameter (cf. Fig. 7), by assuming a direct proportionality between conduction velocity and the square root of fibre diameter, leads to a predicted velocity of 174 cm s^{-1}, a value very close to our observed spike 1 mean of 139 cm s^{-1}.

These simple calculations indicate that type 1 axons cannot be interrupted at each ganglion by a conventional, chemically-transmitting synapse. Involvement of electrical synapses of course cannot be ruled out. As discussed by Cobb & Stubbs (1981), there appears to be no good morphological evidence for either type of synapse in the giant fibre pathway, primarily because of the difficulties involved with unambiguous identification of synapses in invertebrates (Cobb & Pentreath, 1977, 1978).

The same conclusion cannot be made for type 2 axons. A similar calculation for the second spike (mean velocity of 55 cm s^{-1}), yields a predicted true axonal conduction velocity of 87 cm s^{-1}. This is reasonable for axons of the size in ophiuroid nerve. It is thus possible that spike 2 reflects propagation along large axons (i.e. about the size of type 1 fibres) through one synapse per ganglion. Of course, it also remains possible that a class of considerably smaller axons may propagate without any involvement of synapses, as in the type 1 fibres. Our data cannot distinguish between these possibilities, and the apparent Ca-sensitivity of spike 2 is consistent with either idea.

In conclusion, the compound action potential represents recordings from two predominant classes of presumably ectoneural axons in the ophiuroid RNC. Indications of separate axonal classes are evidenced by distinct ionic sensitivities and conduction velocities. The most rapidly conducting axons (type 1) require Na for propagation, while the slower ones (type 2) seem to be primarily Ca-dependent. Axons of similar properties are probably arranged in bundles or grouped in close proximity. Exact morphological and functional identification of these axons remains to be established.

We thank Dr Stuart Thompson, Brad Jones and Peter Ruben for aid in improving extracellular recording methods. We are extremely grateful to Freya Sommer for collecting animals.

REFERENCES

BINYON, J. & HASLER, B. (1970). Electrophysiology of the starfish radial nerve cord. *Comp. Biochem. Physiol.* **32**, 747–753.

BREHM, P. (1977). Electrophysiology and luminescence of an ophiuroid radial nerve. *J. exp. Biol.* **71**, 213–227.

COBB, J. L. S. (1982). The anatomical basis of integratory mechanisms in echinoderms. *International Echinoderms Conference, Tampa Bay*, (ed. J. M. Lawrence), pp. 409–412. Rotterdam: A. A. Balkema.

COBB, J. L. S. & PENTREATH, V. W. (1977). Anatomical studies of simple invertebrate synapses utilizing stage rotation electron microscopy and densitometry. *Tissue Cell* **9**, 125–135.

COBB, J. L. S. & PENTREATH, V. W. (1978). Comparison of the morphology of synapses in invertebrate and vertebrate nervous systems: analysis of the significance of the anatomical differences and interpretation of the morphological specializations. *Prog. Neurobiol.* **10**, 231–252.

COBB, J. L. S. & STUBBS, T. R. (1981). The giant neurone system in ophiuroids. 1. The general morphology of the radial nerve cords and circumoral nerve ring. *Cell Tissue Res.* **219**, 197–207.

GILLY, W. F. & ARMSTRONG, C. M. (1982*a*). Slowing of sodium channel opening kinetics in squid axon by extracellular zinc. *J. gen. Physiol.* **79**, 935–964.

GILLY, W. F. & ARMSTRONG, C. M. (1982*b*). Divalent cations and the activation kinetics of potassium channels in squid giant axons. *J. gen. Physiol.* **79**, 965–996.

HAGIWARA, S. & BYERLY, L. (1981). Calcium channel. *A. Rev. Neurosci.* **4**, 69–125.

HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol., Lond.* **117**, 500–544.

HYMAN, L. M. (1955). *The Invertebrates. Echinodermata,* Vol. IV. New York: McGraw-Hill. 763 pp.

KATZ, B. (1966). *Nerve, Muscle and Synapse*. New York: McGraw-Hill. 193 pp.

MILLOTT, N. & OKUMURA, H. (1968). The electrical activity of the radial nerve in *Diadema antillarum* Phillipi and certain other echinoids. *J. exp. Biol.* **48**, 279–289.

NARAHASHI, T. (1974). Chemicals as tools in the study of excitable membranes. *Physiol. Rev.* **54**, 813–889.

PENTREATH, V. W. & COBB, J. L. S. (1982). Echinodermata. In *Electrical Conduction and Behavior in 'Simple' Invertebrates*, (ed. G. A. B. Shelton), pp. 440–472. Oxford: Clarendon Press.

PENTREATH, V. W. & COTTRELL, G. A. (1971). 'Giant' neurons and neurosecretion in the hyponeural tissue of *Ophiothrix fragilis* Ablidgaard. *J. exp. mar. Biol. Ecol.* **6**, 249–264.

SANDEMAN, D. C. (1965). Electrical activity in the radial nerve cord and ampullae of sea urchins. *J. exp. Biol.* **43**, 247–256.

STUBBS, T. R. (1982). The neurophysiology of photosensitivy in ophiuroids. In *International Echinoderms Conference, Tampa Bay*, (ed. J. M. Lawrence), pp. 403–408. Rotterdam: A. A. Balkema.

TAKAHASHI, K. (1964). Electrical responses to light stimuli in the isolated radial nerve of the sea urchin *Diadema setosum* (Leske). *Nature, Lond.* **201**, 1343–1344.