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AN ELECTROPHYSIOLOGICAL STUDY OF PARAPODIAL INNERVATION PATTERNS IN *APLYSIA FASCIATA*

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INTRODUCTION

Parapodial extensions of the foot are particularly well developed in *Aplysia fasciata*, in which species they function during swimming. As was indicated in earlier studies (Hughes & Tauc, 1962), many of the nerves which innervate the foot *sensu stricto* of other species supply the parapodia of *Aplysia fasciata*. In fact there is no detailed account of the branching of the nerves in *A. fasciata*; Mazarelli (1893) compared the different species, but not in this respect, and based his account of the nerves mainly on *A. depilans*. Hoffman (1939) has compared these results with those of Eales (1921) on *A. punctata* and those of McFarland (1909) on *A. dactylomela* and *A. tervinus*. Nevertheless, in our first studies with whole animal preparations (Hughes & Tauc, 1962), it was clear from experiments using electrical stimulation and recording that the account given by Eales (1921) based upon *A. punctata* was not strictly applicable, especially as the posterior pedal nerves in that species are clearly homologous with the posterior parapodial nerves which innervate the parapodia of *A. fasciata*.

Accordingly, a division of nerves leaving the pedal ganglia was made into pedal nerves proper innervating the foot, of which there are three, and the other main nerves which innervate the parapodia. These latter nerves were separated into anterior, middle and posterior, a subdivision based partly on the relative positions of origin, but especially on the region of the parapodium which they appear to innervate.

During the present study it became clear that a considerable overlap exists in the sensory area supplied by different parapodial nerves, and this contrasts with the more sharply defined dermatomes of mammals and the sensory innervation of the abdominal segments in the crayfish (Hughes & Wiersma, 1960) and of the earthworm (Prosser, 1935).

The main objective of this study was to distinguish different afferent units in the main nerves and to plot the distribution of the main regions of the parapodium which they innervate. During the course of the work further investigations were made on certain units which, in addition to responding to mechanical stimulation of the parapodial surface, were also responsive following electrical stimulation of distal branches of other parapodial nerves. Thus, following contraction of the parapodial muscles, such responses might provide some sort of feedback and have a proprioceptive function. This study is also of relevance to work being carried out in which recordings have been made from individual nerve cells in the pedal and pleural ganglia following peripheral stimulation (Hughes, Weevers & Hartley, 1969).

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MATERIALS AND METHODS

Medium-sized specimens (300–400 g) of *Aplysia fasciata* that had been freshly caught and kept in the aquarium were used throughout these experiments. Most were obtained at Arcachon but others were studied at Naples, where some comparison was also made with the innervation patterns of *A. depilans*. The size range used was

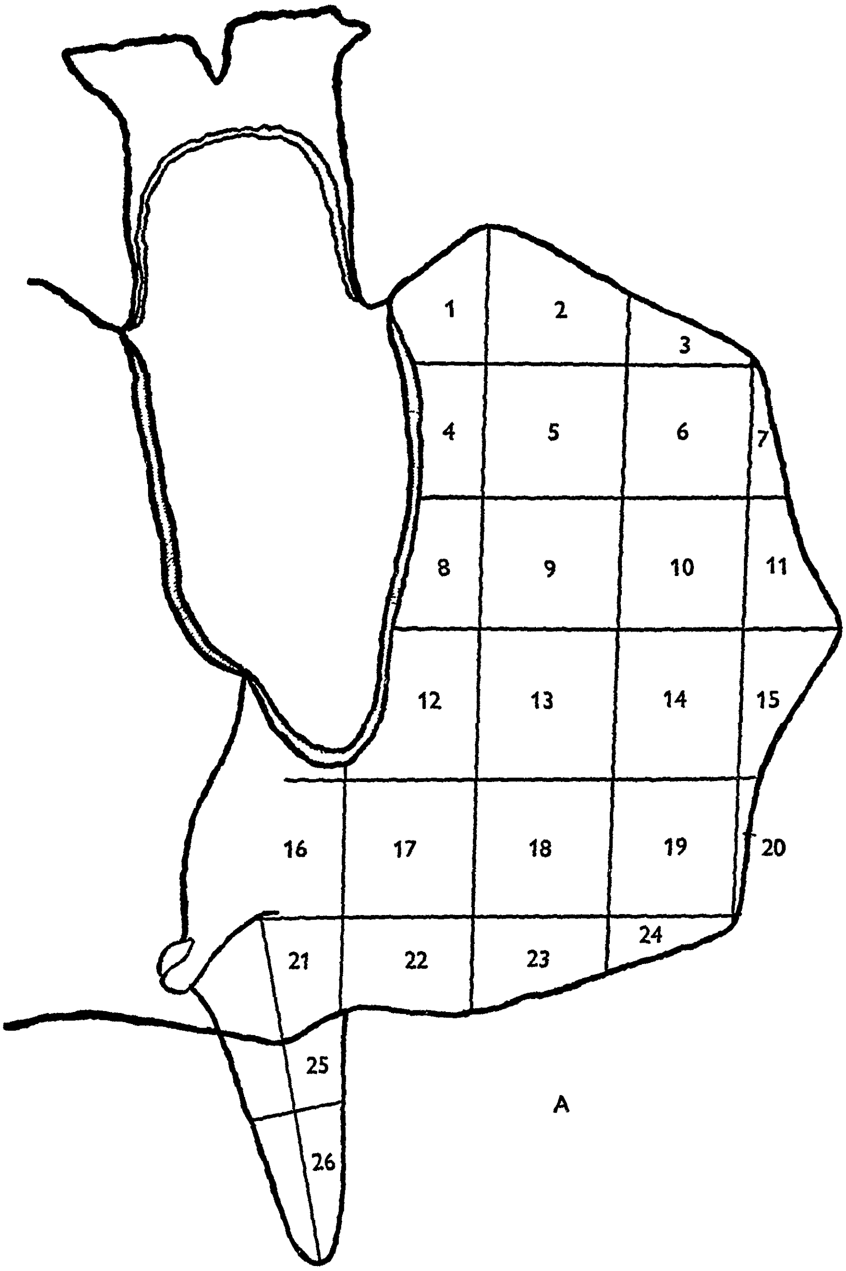
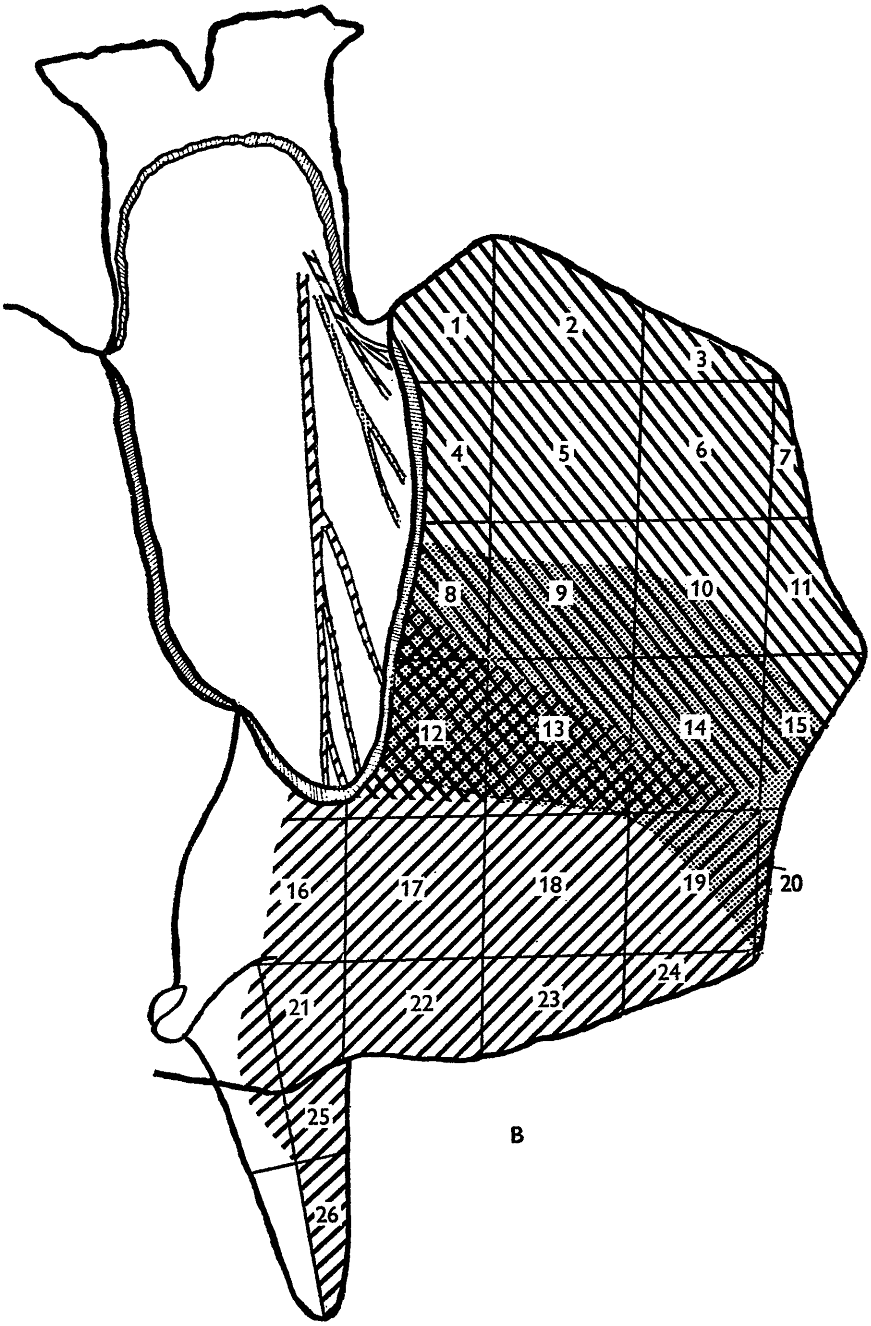


Fig. 1. A. For legend see facing page.

chosen partly because the innervation patterns are not too complex, and also because this size was being used in complementary studies on nervous control of swimming of *A. fasciata* (Hughes, Weevers & Hartley, 1969; Weevers & Hughes, 1971).

The animals were dissected from the dorsal side by means of an anterior median incision which followed posteriorly along the base of the right parapodium. The viscera were removed and the parapodia were pinned out; on the right side the pinning-out was done so as to divide the parapodium into 26 different areas (Text-



Text-fig. 1. A. Diagram showing the numbered areas into which the parapodia were divided during the pinning-out procedure. B. General pattern of innervation from the anterior, middle, and posterior parapodial nerves to the right parapodium. Different shading is used for the three nerves and shows the overlap between areas innervated by the anterior and posterior nerves as well as the way each overlap with the area of the middle parapodial nerve.

fig. 1 A) which could be stimulated separately. In most cases light mechanical stimulation was given with a brush to the dorsal surface of the parapodia; stronger stimulation was applied using a blunt seeker or a perspex cylinder attached to a camera cable-release. Extracellular platinum hook electrodes were placed under branches of the main nerve which were then cut proximal to the ganglion. For general mapping purposes the spikes were divided into small, medium and large units, depending on the spike height which ranged from 100 μV to 10 mV. Amplification was by means of Tektronix 122 pre-amplifiers and a 502A oscilloscope. Recordings were monitored throughout with a loudspeaker. Responses were often recorded on tape (Thermionic 3000) and photographed later. Electrical stimulation could be applied to the same nerve or to other nerves by means of a Grass S8 stimulator and an isolation transformer.

RESULTS

In the present work attention was mainly confined to a study of the innervation of the right parapodium. This was partly because it was easier to dissect and the pattern of innervation appeared to be more constant than on the left side. However, more detailed work showed that the innervation pattern on the right side also varies quite extensively. As far as can be judged from investigations so far carried out, an essentially similar arrangement applies to both parapodia but there are many differences in detail and also between individuals.

In most cases observed so far bilateral symmetry is surprisingly good (Text-fig. 4) but there are frequent exceptions.

Areas of the parapodium

The main difficulty of such studies on an animal like *Aplysia* is the lack of well-defined landmarks for use in comparison between different individuals. The following method was adopted, and although by no means precise it gave fairly comparable results from different specimens. In pinning-out the animal a single large pin was placed in the peripheral edge about half-way back, to divide the parapodium into more or less equal anterior and posterior halves. Another was placed posterior to this at the level at which the siphon and mantle cavity arise from the posterior region of the foot. A similar pin was placed at the same distance in front of the mid-point. Pins were also placed along the front and posterior edges so as to divide the parapodium into proximal and distal portions; other pins were then placed so as to define the grid shown in Text-fig. 1 A. Although the same procedure was carried out routinely, differences in the degree of contraction of the animal produced variations in the actual areas and some allowance was made for this when comparing different preparations.

The parapodial nerves

The present study is based on recordings from the parapodial nerves of over 50 individuals. A number of these were photographed and in all cases diagrams were made of the main branches leaving the pedal ganglion. In spite of much variation in detail the basic subdivision into anterior, middle and posterior parapodial nerves could be made out in nearly all individuals. The anterior nerve is relatively short and divides into many branches close to the ganglion. This and the posterior nerves are fairly stout but the middle parapodial nerve or nerves are quite thin. Often there are two middle parapodial nerves, one innervating rather more of the dorsal region and the other going more deeply. Both did not seem to be present in all preparations. The paired posterior parapodial nerves are the longest and each gives off branches, especially towards the periphery as it passes along the posterior regions of the parapodium. These nerves nearly always contain a red pigment. Text-fig. 1B is a diagram of the general pattern of innervation made out from these specimens, and data summarized in Table 1 illustrate common variations from the basic plan.

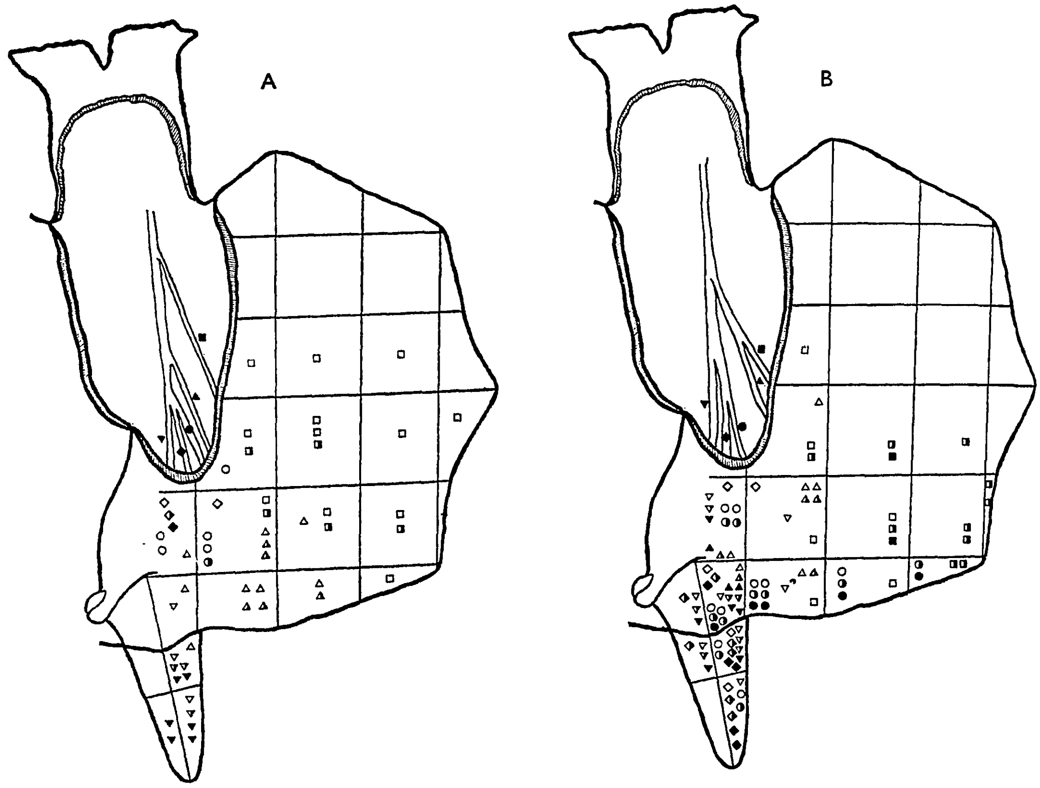
Table 1. Aplysia fasciata: *sensory innervation patterns of the right parapodium in ten individuals (A–J)*

(The areas are numbered according to Text-fig. 1A and the parapodial nerves (A, M, P), in which afferent responses were recorded following tactile stimulation are indicated.)

	A	B	C	D	E	F	G	H	I	J
1	—	—	A	—	A	A	A	A	A	A
2	—	—	A	—	A	A	A	A	A	A
3	—	—	A	—	A	A	A	A	A	A
4	—	—	A	—	AM	A	AM	AM	A	A
5	—	—	A	—	AM	AM	A	A	A	A
6	—	—	A	—	A	A	A	A	A	A
7	—	—	A	—	A	A	A	—	A	A
8	—	—	AM	M	AM	AM	AM	AM	AM	AM
9	—	—	AM	M	AM	AM	AM	AM	AM	AM
10	—	—	AM	M	AM	AM	AM	AM	AM	AM
11	—	—	M	—	A	AM	AM	M	—	A
12	—	P	MP	MP	P	P	MP	MP	MP	—
13	—	P	MP	MP	MP	MP	MP	MP	MP	M
14	—	P	MP	MP	MP	MP	M	M	MP	M
15	—	—	MP	M	P	M	M	M	MP	AM
16	P	P	P	P	P	P	P	P	P	—
17	P	P	P	P	P	P	P	P	P	M
18	P	P	P	P	P	MP	P	—	P	M
19	P	P	P	MP	MP	MP	P	—	P	M
20	P	P	P	MP	MP	MP	P	—	P	M
21	P	P	P	P	—	P	P	P	P	—
22	P	P	P	P	P	P	P	P	P	—
23	P	P	P	P	P	P	P	P	P	—
24	P	P	P	MP	P	P	P	P	P	M
25	P	P	P	—	P	P	P	P	P	—
26	P	P	P	—	P	P	P	P	P	—

The sensory fields

In general, the areas innervated by a given nerve were what one would expect from the position of the nerves when dissected close to the ganglia, and this became even clearer when branches of a given nerve were followed in the parapodial musculature. As the nerves radiate from the pedal ganglion, which is more or less level with the anterior parapodial border, so the sensory areas usually show a similar radiating pattern. The regions supplied by different fibres varied a great deal in area. Thus in some preparations a given major nerve branch might only contain a single unit which could be excited by stimulation of an area of about 2 mm^2. More usually such a nerve branch supplies at least three or four of areas 1–26 and individual units might be excitable by stimulation of areas as great as 30 mm^2. It also became clear that although these nerves were termed parapodial nerves, they also innervate part of the foot itself. The posterior parapodial nerve, for example, has branches which run to the posterior part of the foot, which has sometimes been referred to as the 'tail' because of its appearance during swimming in this species. But the posterior branch of the middle parapodial nerve, and also branches of the anterior parapodial nerve, enter



Text-fig. 2. Working diagrams for two preparations to show the pattern of distribution of responses in different branches of the right posterior parapodial nerves. Each branch of the nerve is indicated by a different shape of symbol and the intensity of the response by the number and type of symbol. Open symbols indicate impulse activity of small amplitude; half-black symbols indicate activity of moderate amplitude; black symbols indicate large spikes.

the foot musculature. Of course, the foot is mainly innervated by the three pedal nerves which have many branches, and the posterior one of these is very clear in these dissections, as described previously and used in recording and stimulation experiments (Hughes & Tauc, 1962).

Examples of the types of response obtained are shown in Plate 1. There are large differences in spike heights recorded extracellularly, ranging from 100 μV to 10 mV. The size of these spikes is not related to the size or thickness of the nerve. Responses of more than one type were recorded from most of the nerves. Many responses were slowly adapting, which was particularly true of the spikes of small and medium height. Some of the large spikes (10 mV) were markedly phasic; noticeable ones responded just posterior to the junction of the siphon with the foot and in the 'tail' itself (areas 21, 25). The ventral surface of the 'tail' is often even more responsive.

Most nerves appeared to contain at least some sensory fibres, although there were cases where these were very few or even absent, e.g. the posterior branch of the middle parapodial nerve (Text-fig. 3 B). In other cases where no sensory responses could be elicited a possible explanation is that the sensory endings were not accessible in preparations pinned out in this particular way. For example, sensory endings in the sole of the foot could not be directly stimulated.

As had been previously observed, there is definite evidence that sensory areas covering the whole parapodium are innervated by the main nerves that have been distinguished. Furthermore, individual parapodial nerves show a marked overlap in the areas innervated. This was also true at the level of single units within a given branch. Text-fig. 2 shows the results obtained from a typical preparation with areas innervated and some indication of the types of spike conducted by the nerves. The overall picture obtained from each preparation is that the whole surface is innervated in a fairly uniform way from different sensory fibres, but exactly which of the branches or main nerves contain fibres supplying a given part of the parapodium varies. The distribution seems to be related more to the local anatomy than to some developmental link between specific nerves and a particular part of the body surface as might be expected with more rigid neurogenesis.

From such preparations summary diagrams (Text-fig. 3) have been constructed and show the main areas innervated by the parapodial nerves. It might be emphasized that these diagrams should not be interpreted rigidly but only as an indication of the nerves which are likely to contain units excited by mechanical stimulation of particular regions of the parapodium.

Responses to electrical stimulation

It was found that when electrical stimuli are applied to the distal portion of some of the nerves, a contraction of the parapodial edge occurs. These contractions could be observed directly or recorded using a mechano-electrical transducer, and gave recordings similar to those obtained with sea anemones following repeated shocks at low frequency, i.e. they showed a staircase (Text-fig. 5). Detailed study of such nerve–muscle relationships is difficult because of the difficulty of obtaining the same preparation repeatedly, and was not attempted. Part of the complication is obviously due to the particular nature of the nerve/muscle network of the gastropod parapodium. However, recordings from some adjacent branches of the parapodial nerves showed evidence of sensory feedback following a single shock to motor fibres in the distal branch of the nerve. Responses were first found in individual branches of the same main parapodial nerve but later studies indicated their presence in branches of different nerves, and also in a given nerve branch following its stimulation (Plate 2). Some contraction could generally be observed when sensory recordings were obtained, but not in all cases. One cause of this is probably the condition of the preparation, contraction of the muscle network not necessarily leading to an overt response of the parapodial surface.

Usually, however, such responses were only obtained when some overlap in the sensory field of the two nerve branches had been discovered in the initial survey. Examples of the type of responses obtained are given in Plate 2 and Text-fig. 6. It was also observed that the same units responding in this way could be excited by

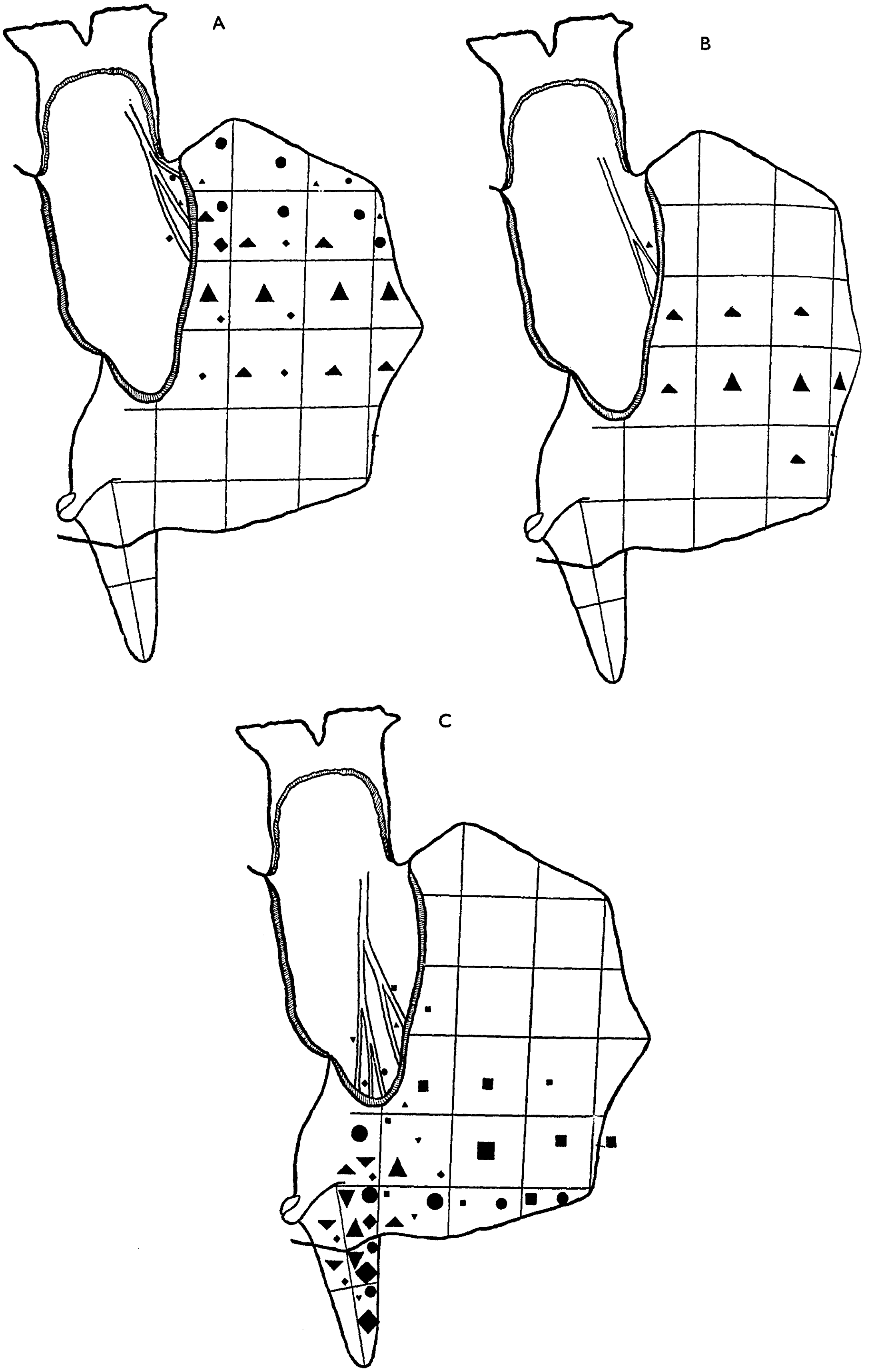
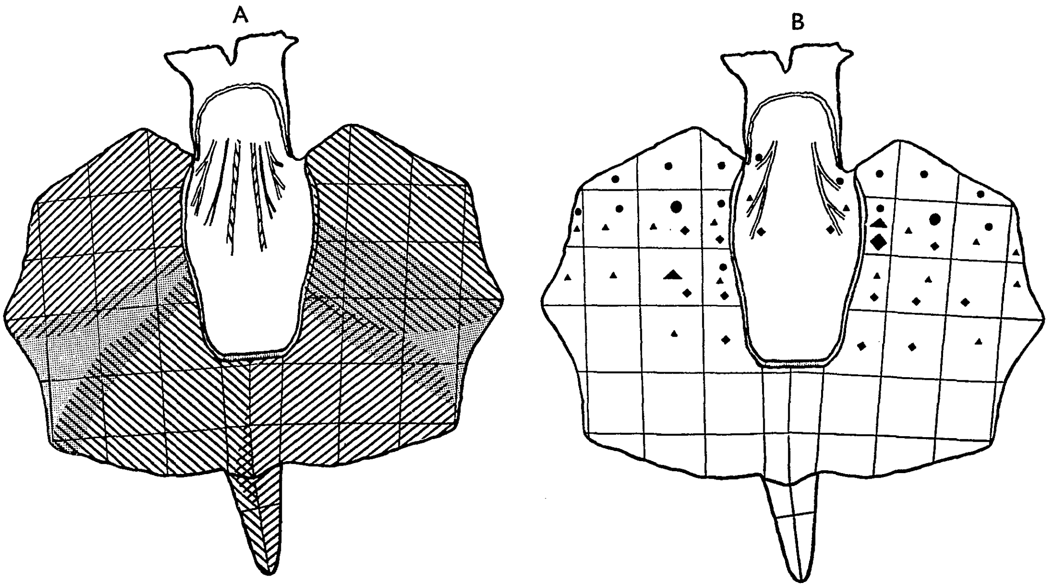
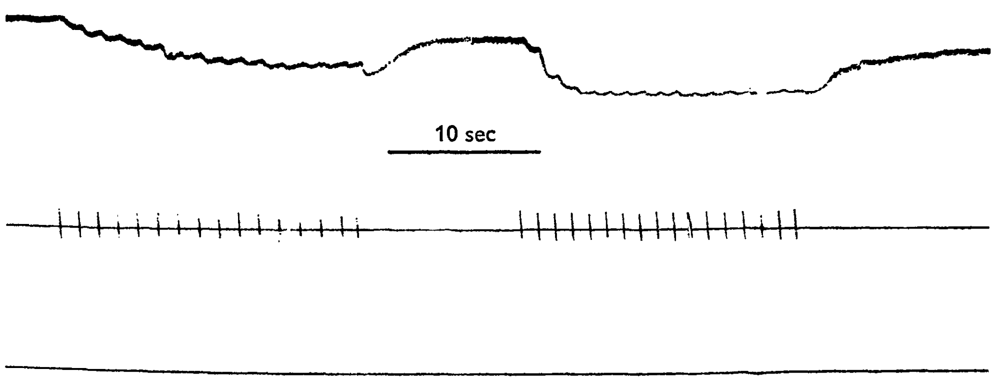


Fig. 3. For legend see facing page.



Text-fig. 4. Bilateral diagrams indicating differences in innervation on the two sides of the body. A, General pattern of innervation from the anterior, middle and posterior parapodial nerves. B, Areas innervated by 3 main branches of the right and left anterior parapodial nerves in a different preparation. Use of symbols is the same as in Text-fig. 3.

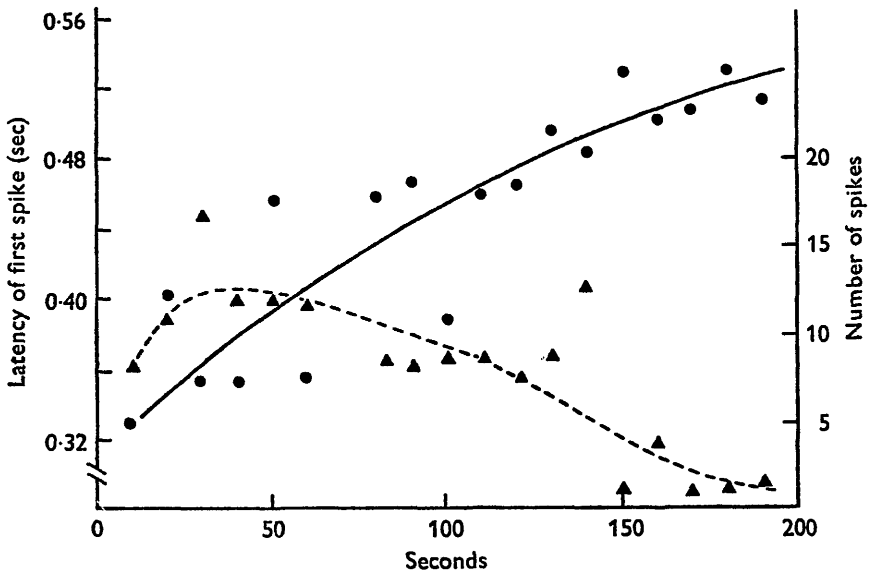


Text-fig. 5. Movement recordings from right parapodium of *Aplysia fasciata* following electrical stimulation of a branch of the posterior parapodial nerve. At low frequencies there is a contraction in response to each shock, which gives rise to a steepening staircase as the frequency is raised.

mechanical stimulation of the area where overlap occurred and this usually coincided with regions where a contraction was visible. In some cases reversal of the stimulating and recording electrodes did not always result in a reversal of the response.

Text-fig. 3. Diagrams derived from more detailed data such as that given in Text-fig. 2A and B. These three diagrams show the innervation pattern of (A) the anterior, (B) the middle, and (C) the posterior parapodial nerve to the right parapodium. Each nerve branch is indicated by a symbol of different shape, and the intensity of response elicited from different areas is indicated by size of the symbols. Smallest symbols indicate small spikes; medium-sized symbols indicate spikes of more than one size; large symbols indicate spikes of more than one size, some of which are very large.

Another characteristic feature of the response was a gradual decline in the response with its repetition. Preparations varied greatly in this respect. The behaviour of a common type has been plotted out in Text-fig. 6 and shows how there is a decline in the number of impulses in a burst and also an increase in the latency of the first impulse. In the present study no attempt has been made to identify the receptors involved in these and other responses, and there seem to be few observations on such receptors in the literature.



Text-fig. 6. Responses of a single unit in one of the more anterior branches of the right posterior parapodial nerve following stimulation of a posterior branch. Responses were recorded in a single unit which responded to touch of a small area at the junction of the regions responding to mechanical stimulation. The response declines progressively as shown by the increasing latency of the first spike and a decrease in number of spikes during each burst (dashed line).

DISCUSSION

This study has demonstrated electrophysiologically that the whole surface of the parapodia is served by receptors capable of detecting mechanical stimuli. Though the innervation pattern is related to the fanning-out of the nerves from the pedal ganglia, there is in no sense a segmental arrangement comparable to that found in other animals which have similar swimming organs, e.g. rays and skates. In these fish there are overlapping fields of sensory innervation which are greater than those of the segmental motor innervation (ten Cate, 1928). Comparable overlapping fields have also been found in the dogfish (Roberts, 1969). Among molluscs, ten Cate (1930) studied the innervation of the fins in *Sepia* using electrical stimulation and nerve-section techniques.

The pattern of innervation in *Aplysia* has confirmed the appropriateness of the naming of the parapodial nerves (i.e. anterior, middle, posterior) in relation to the regions of the parapodial innervation (Hughes & Tauc, 1962). In general, the innervation of the two parapodia is rather more symmetrical than had been expected, but many preparations have demonstrated differences in detail (Text-fig. 4). For example, a nerve on the left side appeared to be homologous with one of the right parapodial nerves, but electrophysiological recordings clearly showed that these two nerves innervated different regions of their respective parapodia. Throughout the work stimulation has mainly been restricted to the dorsal surface, but in some experiments receptors on the ventral surface may have been excited, especially with more intense stimulation. Responses from the tail region of the foot are of interest in relation to body symmetry because evidence is frequently found for innervation by fibres from the contralateral posterior parapodial nerve. There is no clear evidence of differences in sensitivity or concentration of sense organs in any special regions of the parapodia. Sensitivity of the receptor endings is not usually very great and it is doubtful whether they could detect water currents generated during swimming. Rather, they would be stimulated by contact with obstacles or the substratum.

Most parapodial nerves are mixed and usually contain motor fibres to the same region of the musculature as of the receptive field, but not in all cases. The general pattern of the efferent responses indicates a high degree of integration for a whole parapodium. Thus when records are taken from an intact parapodial nerve in response to mechanical stimulation of any part of the parapodium there results a change in the firing frequency of many units. However, in addition to this overall sensitivity there are local reflexes, particularly as demonstrated by the proprioceptive type of response recorded following electrical stimulation distal to a cut in a parapodial nerve, feedback from the periphery being mainly in associated branches of the same parapodial nerve. It seems possible that these proprioceptive reflexes may play a role in coordination of the swimming wave of the animal. The significance of the habituation of these reflex responses is difficult to interpret at present.

SUMMARY

1. Studies have been made of the patterns of parapodial innervation in *Aplysia fasciata* and *A. depilans* using electrophysiological methods.

2. Sensory fibres are chiefly found in the main parapodial nerves. The areas innervated by these nerves overlap somewhat and such overlapping extends to the branches of individual parapodial nerves.

3. Responses to mechanical stimulation of the parapodial surface gives rise to varying sizes of spike, the smaller spikes being usually more slowly adapting, the larger being highly phasic.

4. Although the general pattern of innervation is common to all specimens there are wide variations in detail.

5. Electrical stimulation of parapodial nerves produces mechanical contractions which are associated with responses in some of the sensory fibres. Usually the afferent units which are stimulated in this way have sensory fields within overlapping areas supplied by the two branches. Such proprioceptive feedback responses usually declined with repetition.

It is a pleasure to record my thanks to the Directors of the Marine Stations at Arcachon and Naples for the facilities that they so kindly placed at my disposal, and to their staffs for collecting the animals. Financial support for this work was provided through a grant from the Science Research Council.

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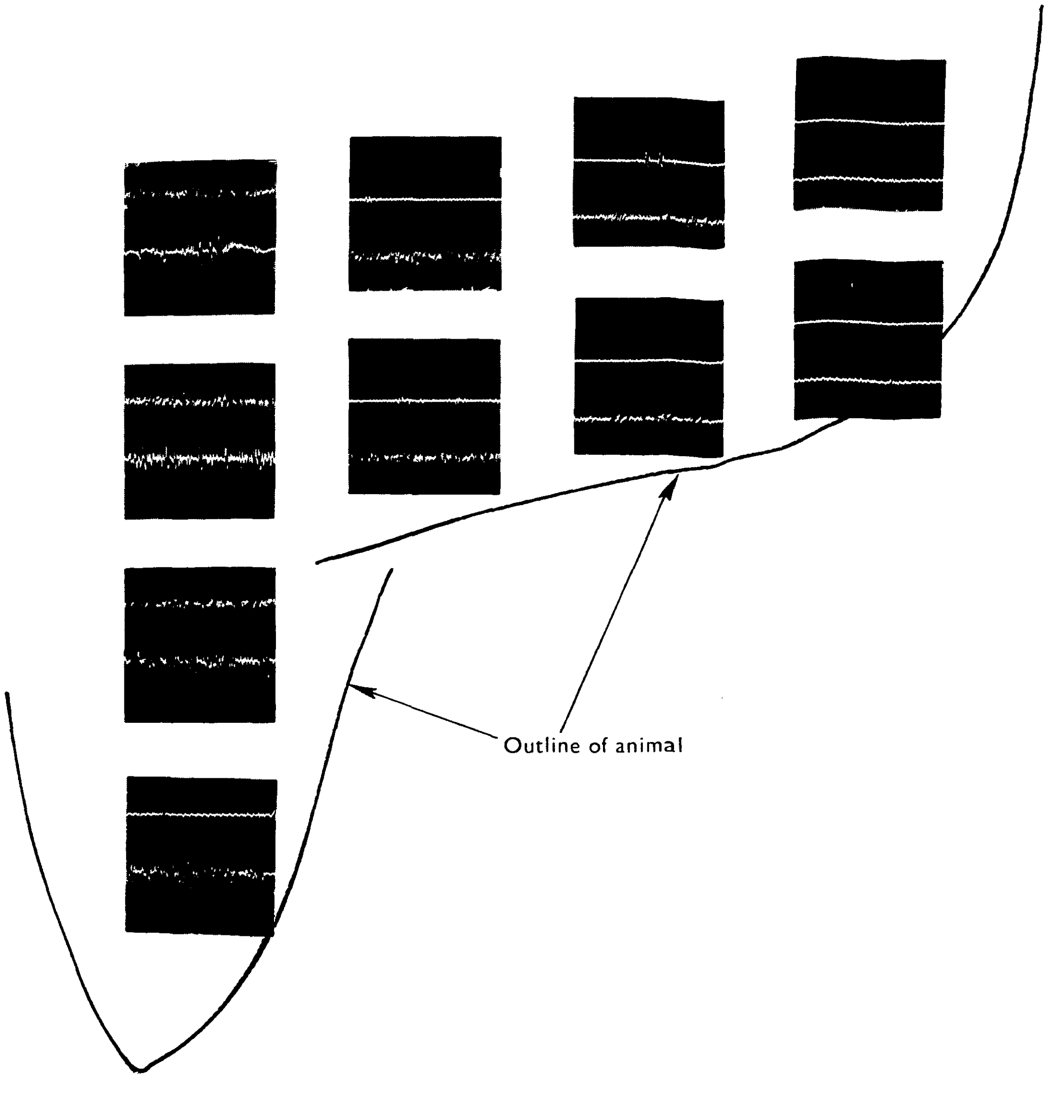
EXPLANATION OF PLATES

PLATE 1

Recordings from two branches of the right posterior parapodial nerve following stimulation of the areas as indicated by their position in the diagram.

PLATE 2

A and B: Stimulation of a branch of the posterior parapodial nerve at two different intensities. In A, a group of action potentials propagated peripherally from the stimulating electrodes. In B, at higher intensity of stimulation this response is followed, after a latency of about 0·5 sec, by a burst of afferent impulses. C and D are recordings from the right posterior parapodial nerve in a different preparation. C, Following stimulation of the same nerve proximally to the recording electrodes; following a centrifugal discharge there is a feedback response of units in the same nerve. D, Stimulation of a specific area of the right parapodium elicits impulses, some of which are clearly in the same fibres as were excited by the electrical stimulus: two pairs of electrodes recording from this same nerve.



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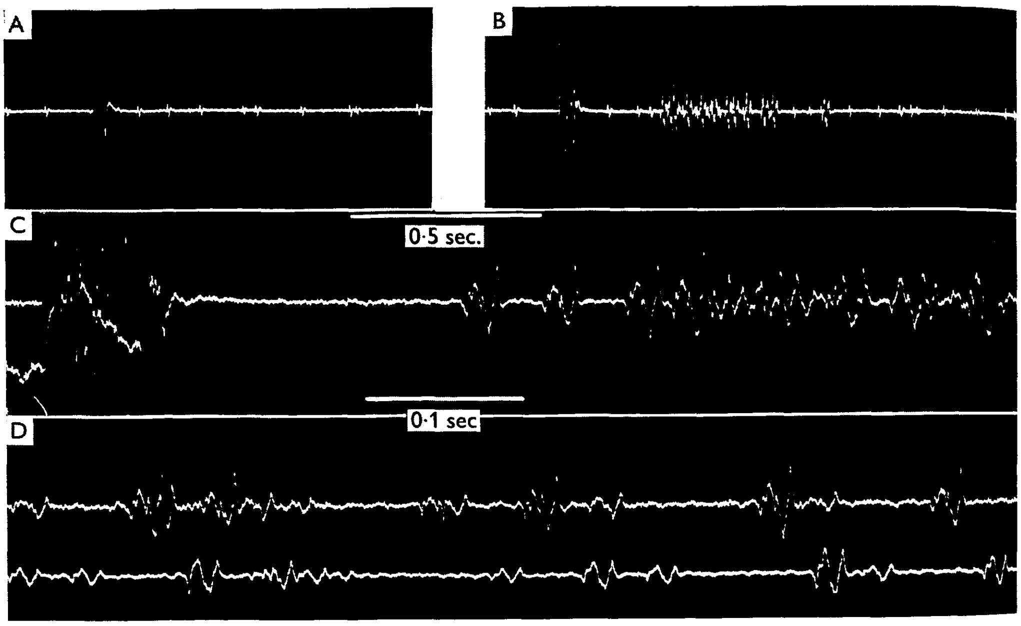

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