THE PEDAL NEURONS OF *APLYSIA PUNCTATA*

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**INTRODUCTION**

There are no detailed accounts of the connexions and branching of the axons of neurons in the pedal ganglia of opisthobranchs. Most of the experiments on these ganglia have been limited to cutting and stimulating nerve trunks, and using these techniques, Frohlich (1910) demonstrated the role of the pedal ganglia in inhibiting the tonic contraction of the isolated foot of *Aplysia limacina*. Ten Cate (1928) considered that the wave-like swimming movements of the parapod of this species were also maintained by inherent patterns of activity in the neurons of the pedal ganglia, and found that sectioning the pedal commissure eliminated the response of the parapodia to contralateral stimulation.

Herter (1931) and Turner & Nevius (1951) both studied pathways in the nervous system of pulmonate gastropods which involved the pedal ganglia, but the nature of their experiments could not reveal the precise configuration of the pathways they described. Nevertheless, they demonstrated several types of pathways through the pedal ganglia and found that they were influenced by activity of the cerebral ganglia descending in the cerebro-pedal connective. Unit activity of cells in the pedal ganglia of *Agriolimax*, and subsequent action potentials in the pedal nerves were recorded by Hughes & Kerkut (1956), who found that many cells exhibited a slow spontaneous activity that was sensitive to small changes in the concentration of the bathing medium.

The ability to distinguish antidromic from orthodromic action potentials whilst making intracellular recordings from neurons (Tauc, 1957), has been used in mapping the ramification of the branched axons of the giant cells in the abdominal and pleural ganglia of *Aplysia depilans* (Hughes & Tauc, 1962, 1963), and in the cerebral and pleural ganglia of *Tritonia hombergii* (Dorsett, 1967). These authors found neurons with many-branched axons which were distributed in a symmetrical fashion in nerves on both sides of the body.

The present paper is a study made on the distribution of the axons and synaptic pathways to 175 neurons sampled from the pedal ganglia of 15 specimens of *Aplysia punctata*.

**MATERIAL AND METHODS**

*Aplysia punctata* can occasionally be found in some numbers on the west coast of Anglesey, both on the shore and in the sublittoral zone. Animals were collected and kept under circulation in the laboratory where they survived well for several weeks. For the experiments, animals of about 12 cm. in length were pinned in an extended position and the entire central nervous system was quickly removed. This was then
transferred to a smaller dish and pinned, ventral side uppermost, so that the pedal ganglia overlaid the pleurals and the cerebals. After cleaning off the connective tissue surrounding the nerves and ganglia, the three pedal nerves were visible as shown in Fig. 1. Suction electrodes were then placed on the pedal nerves of one side, close to the ganglion, and a further electrode was placed on the commissure. The connective tissue covering the ganglion was then raised clear of the underlying neurons and cut with small scissors. The sides of the cut normally retract to expose the neurons. These are normally between 100 and 200 μ in diameter and uniformly pigmented, so there is no easy way of recognising individual cells from preparation to preparation except by their position relative to the main nerve trunks. The surface of the ganglion was therefore sampled systematically with a micro-electrode, records being kept of the position and responses of each cell.

The recording techniques were those previously used by Hughes & Tauc (1963) and Dorsett (1967). Nerves containing an axon of a neuron were identified by triggering the upper beam of the oscilloscope on the rising edge of the intracellular spike whilst displaying an external recording from the nerve on the lower beam. The associated action potential is identified by its constant position with respect to the intracellular spike. As many of the action potentials in the nerves are very small and only separated from amplifier noise with difficulty, the nerves were alternatively stimulated through the suction electrodes and the subsequent action potential was recorded from the neuron in the pedal ganglion. This method has the advantage of supplying information on both antidromic and orthodromic pathways to the cell.

Fig. 1. A tracing made from a photograph of the ventral aspect of a dissected pedal ganglion of *Aplysia*. Only some of the neurons are represented. CPe, Cerebro-pedal connective; Pe, pedal commissure; PPe, pleuro-pedal connective; int, interneurons; 2nd, 3rd nuc, areas of neurons with axons in the respective pedal nerves; 1–3, pedal nerves.
RESULTS

Neurons associated with the anterior pedal nerve

Only a small proportion of the total number of neurons sampled in these experiments sent axons into the first pedal nerve. As this nerve arises from a slightly more dorsal position on the ganglion than the other two pedal nerves the majority of anterior pedal motor neurons may lie on the side that was not exposed by the dissection.

Fig. 2. Types of anterior pedal motor neurons. The axon from the neuron has a thicker line and afferent pathways a thinner line. Axons in the pedal commissure are drawn towards the top of the figure and those in the other pedal nerves are denoted by the subscript.

The neurons identified as having an axon in the anterior pedal nerve may be broadly classified into two types (Fig. 2). There are a number of unipolar cells which, in addition to the axon in N.1, may also have synaptic contacts with fibres entering the ganglion in the first and third nerves. This is also probably true of the second nerve, although none was found in the present experiments. The second type of cell is a bipolar cell which sends one branch of its neuron into the first nerve, whilst the other enters the pedal commissure and crosses to the opposite ganglion. The bipolar neurons also receive synaptic inputs from fibres in the ipsilateral pedal nerves and from fibres entering the ganglion from the pedal commissure.

Experiments using the recording technique reveal that many of the axons in the first nerve show very small action potentials (Fig. 3 A) which are more clearly seen by photographing a number of superimposed sweeps. There are also a number of presumably larger axons which show action potentials well above the noise level (Fig. 3 B). The first pedal nerve divides into two major branches shortly after arising from the ganglion and a number of experiments were made to decide whether axons passing into N.1 divided into both branches. No such axons were found although an individual anterior pedal motor neuron often received excitation from orthodromic pathways in both nerves. Stimulation of a nerve containing both an axon and afferent pathways to the cell frequently elicits an AS spike followed by one or two orthodromic ones (Fig. 3 C). A similar response was noted by Hughes (1967) in the LGC of Aplysia fasciata after stimulating the pedal and parapodial nerves. It is probably due to the slower conduction velocity and long duration of the compound e.p.s.p.'s of the fibres in the afferent pathway.
Fig. 3. Responses of anterior pedal motor neurons. A, B, Intracellular spikes from two neurons with axons in the anterior nerve. The externally recorded action potential (arrowed) in A is more clearly seen by superimposing ten successive sweeps, whereas the fibre in B is larger and more easily seen; C, an antidromic spike followed by two orthodromic ones following stimulation of the anterior branch of N. 1; D, an orthodromic spike in the same neuron after stimulation of the posterior branch.

Neurons associated with the median pedal nerve

A larger number of neurons were found associated with the second pedal nerve and in some cases these were grouped together, which made possible a tentative location of a second pedal nucleus of motor neurons (Fig. 1). With increased numbers of neurons a greater variety of connexions have been established for this group than for the anterior pedal nerve (Fig. 4). Again the neurons can be categorized into unipolar and bipolar types, the former being more commonly found than the latter. Fibres synapsing with the unipolar neurons were found in all three pedal nerves and in the commissure in a variety of combinations (Fig. 5 A). In the majority of cases these afferents are excitatory, but in one instance the fibre in the commissure was inhibitory (Fig. 5 D). The efferent spikes of group II motor neurons, as recorded in the second nerve, are often small (Fig. 5 B) and difficult to detect above the noise level of the amplifier, and frequently more useful information could be obtained using the stimulating technique.

In the bipolar neurons of this group the second branch of the axon is again found in the commissure, no instance being recorded of it entering another pedal nerve. Afferent fibres in the other pedal nerves and the commissure also synapse with the bipolar
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neurons (Fig. 4), these pathways being mostly excitatory with the exception of the posterior pedal nerve which occasionally produced inhibition.

Fibres in the pleuro-visceral connective also synapse with neurons of this group, but this pathway was not examined in any detail.

![Diagram of median pedal motor neurons](image)

**Fig. 4. Median pedal motor neurons.** Unipolar and bipolar types are shown, together with their afferent connexions. The same conventions are used as in Fig. 3, but inhibitory pathways are indicated by broken lines. *PVC*, Pathway in pleuro-visceral connective.

**Neurons associated with the posterior pedal nerve**

The motor neurons associated with the third pedal nerve conform to the pattern established for the other pedal nerves. Both unipolar and bipolar neurons are found (Fig. 6), the latter having one branch in the commissure. The action potentials from the axons of these neurons recorded externally in the third nerve are relatively large, and show conduction velocities ranging from 50 to 100 cm./sec. Stimulation of N. 3 produces antidromic spikes in neurons of this group (Fig. 7 C) which may be followed by an orthodromic response when afferent pathways to the cell are also present (Fig. 7 A). The presence of an inhibitory pathway in the third nerve was also confirmed in the group III motor neurons, the antidromic spike being followed after a delay of 50 msec. by an i.p.s.p. (Fig. 7 D), which corresponds to the delay of the more usual orthodromic excitation (Fig. 7 A).

**Interneurons**

Some 25% of the neurons sampled in the present investigation did not send axons into the pedal nerves or commissure. The inference to be drawn from this is that the
axons from these neurons either pass into the cerebro-pedal or pleuro-pedal connectives, or remain in the pedal ganglion where they synapse with the primary motor neurons. Until further information on these cells becomes available they have therefore been classified as interneurons.

Stimulation of any of the ipsilateral pedal nerves may produce an e.p.s.p. and orthodromic spike in this type of cell (Fig. 8A–C) which commonly receive inputs

Fig. 5. Responses of median pedal motor neurons. A, Superimposed results of activity in a single neuron to stimulation of the pedal nerves and commissure. Stimulation of N.2 resulted in an AS (2 A) and orthodromic spike (20). Orthodromic responses follow stimulation of the commissure (C) and nerve 3; B, a neuron with a small action potential recorded in N.2; C–E, a neuron with an axon in N.2 responds to stimulation with an AS spike (C), while the commissure (D) and N. 3 (E) give inhibitory and excitatory orthodromic responses; F, orthodromic excitation of a neuron in this group after stimulation of the commissure.
Fig. 6. Posterior pedal motor neurons. Unipolar and bipolar types are shown together with their afferent connexions. Note the inhibitory pathway in the third nerve.

Fig. 7. Responses of posterior pedal motor neurons. A, antidromic and orthodromic spikes after stimulation of N. 3; B, the intracellular spike and action potential recorded externally in N. 3; C, the AS spike in a group III motor neuron with no afferent pathway in N. 3; D, AS spike and inhibitory potential after stimulation of the third nerve.
from more than one nerve, including the commissure. The variety of the connexions identified for the interneurons is summarized in Fig. 9. A greater proportion of the afferent pathways in the commissure produce inhibition in the interneurons (Fig. 8E) than in the motor neurons described in the previous sections. The inhibitory potentials have an amplitude of about 5 mV. and a duration of 100–150 msec. and it is interesting to find evidence of the inhibitory pathway in N. 3 converging on the interneurons, although in this case (Fig. 8D) inhibition was followed by a post-inhibitory rebound.

Fig. 8. Interneurons. A–C, Orthodromic responses of an interneuron to stimulation of N. 1, the pedal commissure, and N. 3 respectively; D, stimulation of N. 3 producing an inhibitory potential followed by post-inhibitory excitation; E, an inhibitory potential produced by stimulation of the commissure; F, an orthodromic spike after a long delay following stimulation of N. 2.
Occasionally one finds units which respond with orthodromic spikes to stimulation of peripheral nerves, but after delays of 300–400 msec. (Fig. 8F). These may involve multi-synaptic pathways through the cerebral ganglia such as those described by Herter (1931).

Fig. 9. Interneurons. These cells were apparently without axons in the pedal nerves or commissure.

Stimulation of the contralateral pedal nerves

All the observations made in the preceding sections have been in response to stimulation of the ipsilateral pedal nerves. These have established the presence of a group of bipolar neurons associated with each nerve which send one branch of their axon into the pedal commissure. These cells, in common with the unipolar and interneurons, may also receive synaptic inputs from fibres crossing the commissure from the opposite side. As neither the unipolar or interneurons have axons in the commissure, it seems probable that some of the fibres which synapse with them are from the bipolar cells in the opposite ganglion or from peripherally located sensory cells whose axons enter the ganglion through the contralateral pedal nerves and the commissure.

A series of experiments were made in which recordings were taken from neurons in one pedal ganglion whilst stimulating the pedal nerves of the opposite side. In no case were antidromic spikes recorded in neurons in response to this type of stimulation, which means that the axons of the bipolar cells do not leave the opposite pedal
ganglion, at least, not in the pedal nerves. Examples of some of the orthodromic pathways established by this type of experiment are illustrated in Fig. 10. In the majority of cases stimulation of only one of the three pedal nerves resulted in compound e.p.s.p.'s large enough to reach spike threshold (Fig. 11 A, C), but some cells (Fig. 11 B) show a series of e.p.s.p.'s from all three nerves, none of which was sufficient to fire the cell. It may be that cells of this type require summation of excitation from more than one synaptic field in order to produce the action potential. When cells produce spikes in response to stimulation of more than one nerve (Fig. 11 D) there is often a difference in the rate of development of the compound e.p.s.p. and therefore in spike latency.

**Simultaneous intracellular recording from both pedal ganglia**

If the axons of the bipolar neurons do not pass into the opposite pedal nerves, the possibility exists that they synapse with cells in the ganglion. Intracellular recordings made simultaneously from cells in opposite pedal ganglia were found to produce one of two types of interaction. Certain pairs of cells respond to stimulation of a pedal nerve with orthodromic spikes of short latency (Fig. 12 A). The e.p.s.p. in the ipsilateral cell occurs approximately 25 msec after stimulation whilst that in the contralateral cell is delayed a further 15 msec which corresponds to the delay in crossing the commissure. Reducing the intensity of the stimulus leads to a variable increase in the interval between the onset of the e.p.s.p. and the spike in both cells, but the ipsilateral cell always fires before the cell of the other side.

A second type of interaction observed between pairs of cells is illustrated in Fig. 12 B, C. Here, stimulation of the median nerve resulted in the double antidromic and orthodromic spike in the ipsilateral cell. The contralateral cell followed with an orthodromic spike, the e.p.s.p. following the AS spike of the ipsilateral cell after a delay of about 12 msec, but this could be delayed some 80 msec by an intervening inhibitory potential.

Occasionally, much longer delays were observed between the AS spike in the ipsilateral cell and the occurrence of e.p.s.p.'s in its partner in the opposite ganglion. The
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contralateral cell in Fig. 12D shows two e.p.s.p.'s 30 and 100 msec after the spike in the ipsilateral cell. Both these delays seem too long for a monosynaptic pathway in the commissure, and may involve interneurons or pathways in the cerebral loop.

Fig. 11. Responses of pedal neurons to stimulation of contralateral nerves. A, A neuron showing several e.p.s.p.'s and an orthodromic spike after stimulation of N. 2; B, a neuron responding to stimulation of the pedal nerves with e.p.s.p.'s, none of which reached spike threshold; C, a neuron responding to stimulation of the third nerve; D, this cell was excited by stimulation of both nerve 1 and 2. Note the difference in latency.

DISCUSSION

The multiple branching and symmetrical pattern of axon distribution typical of the giant cells in the abdominal and pleural ganglia of Aplysia (Hughes & Tauc, 1963) does not seem to be characteristic of the neurons in the pedal ganglia. The majority of these are unipolar or bipolar cells which send one axon into one of the pedal nerves and, in the case of the bipolars, the other into the pedal commissure. It is probable that they are primary motorneurons, initiating and maintaining the locomotory waves in the musculature of the foot (Frohlich, 1910). Experimental evidence suggests that neurons in certain areas of the cell cortex are associated with particular nerves, each motor centre having its complement of unipolar and bipolar cells.

Not all of the neurons have axons in the pedal nerves or commissure, and if these are interneurons, as is suggested here, it is probable that they have integrative functions within the ganglion or provide the close linkage which has been suggested be-
tween the pedal and cerebral ganglia (Turner & Nevius, 1951). Orthodromic pathways to these cells exist in all the pedal nerves and in the commissure, which would seem to be one prerequisite for this purpose.

If the right and left pedal ganglia contain the motor centres responsible for the coordination of the locomotory movements, the primary function of the commissure would be to relay information integrating the activity of motor units on both sides. As neither the unipolar or interneurons have axons in the commissure, it seems that the responsibility for this exchange must lie with the bipolar cells. A very simple model suggesting how this might be achieved is shown in Fig. 13A. The bipolar cell of one side synapses with a unipolar neuron on the other, by means of its axon which enters the commissure. There is no reason why these connexions should not be made with bipolar cells or interneurons, although it is thought unlikely that there would be reciprocal innervation between pairs of bipolar cells as this might lead to continuous re-excitation.

In its essential features this model is similar to the relationship found between the LGC and RGC in the unique specimen of *Aplysia fasciata* described by Hughes.
Pedal neurons of Aplysia (1967), in which the giant cells had synaptic contacts with each other. But for the rather unusual nature of the synaptic potential at this junction these neurons might well have formed a reciprocating system.

Support for the existence of an arrangement suggested in the model is obtained from the results shown in Fig. 12B, C. The AS spike in the ipsilateral cell is followed by an orthodromic spike in its partner after a delay consistent with the time taken to cross the commissure. In this case the system is complicated by the presence of an inhibitory potential generated in another pathway activated by the stimulus, but this only delays the orthodromic spike and is not sufficient to block it.

A second function of the commissure is to carry sensory or proprioceptive information to the pedal ganglia from receptors located on the contralateral side (Fig. 13B). It seems clear, from results such as those presented in Fig. 12A, that there is strong evidence for pathways such as these.

Fig. 13. A, A simple integrative relationship between neurons in the left and right pedal ganglia, based on the evidence presented here; B, a second possible relationship in which neurons in each ganglia share a common orthodromic supply. It has not been established whether this is by a single branched axon or by two, as shown here.

SUMMARY

1. Three classes of neurons have been identified in the pedal ganglia of Aplysia punctata.

2. The motor neurons, which may be unipolar or bipolar, have the axon passing into one of the pedal nerves and in the case of the bipolars, the other branch entering the pedal commissure. This synapses with neurons on the opposite side, thus providing an integrative link between the ganglia.

3. The interneurons are without axons in the pedal nerves or commissure, although afferent pathways to these cells and the motor neurons occur in the pedal nerves in a variety of combinations.

4. The pathways in the ipsilateral nerves are for the most part excitatory, but inhibitory fibres occur in the posterior pedal nerve.

5. Inhibitory potentials were often obtained in the interneurons by stimulation of the pedal commissure.

6. A second type of coordinative pathway is provided by fibres which enter the CNS in one of the pedal nerves and terminate on neurons in both pedal ganglia.
REFERENCES


