

ASPECTS OF THE NEUROPHYSIOLOGY OF *BUCCINUM UNDATUM* L. (GASTROPODA)

II. CENTRAL ORGANISATION*

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INTRODUCTION

Neurophysiological studies on molluscan nerve cells have provided a large proportion of the knowledge concerning neuronal physiology (Tauc, 1956; Tauc & Gerschenfeld, 1962; Tauc & Hughes, 1963 *a, b*; Arvanitaki & Chalazonitis, 1955; Hodgkin & Huxley, 1952) and nervous integration (Bullock, 1948; Kerkut & Cottrell, 1963; Kerkut & Thomas, 1963; Kerkut & Walker, 1962). This work has been largely confined to species with axons and cells of large size and has thus left many gaps in the knowledge of more general aspects of molluscan neurophysiology. Papers concerning the latter are relatively few in number and rather scattered both in the date of their publication and in their interests.

Much of the early physiological study was concentrated on the nature of the foot muscle tone, its mechanism and nervous regulation (Jordan, 1901; Postma, 1941). One exception was the work of ten Cate (1928) upon the protective movements of the 'wings' of *Aplysia*. In the course of this he demonstrated that stimulation of the wing nerves of one side of *Aplysia* brought about responses in regions innervated by the other ipsilateral nerves and also the contralateral ones. Similar responses could also be elicited by stimulation of the pleuro-visceral connectives. By removing the other ganglia it was established that these effects were mediated by the pedal ganglia with contralateral transmission through the pedal commissure.

More recent work includes that by Turner & Nevius (1951) on *Ariolimax*, Hughes & Tauc (1961, 1962) on *Aplysia* and Kerkut & Walker (1962) on *Helix*. Turner & Nevius demonstrated the convergence of many efferent pathways on to motor neurones in the pedal ganglia, viz. 'Impulses may be recorded in the pedal nerves following stimulation of almost any nerve leading to the central nervous system.'

The work of Hughes & Tauc plotted the paths of the giant axons of *Aplysia*, dealt with the effects of mechanical stimulation of peripheral areas, the patterns of axonal branching and synaptic connexions, and neuronal pathways. In the course of these experiments stimulation 'of the sensory pathways via the normal receptor mechanisms' was employed to a considerable extent. Similar means were employed in the present experiments on *Buccinum* for the same reason, namely 'to obtain information of greater physiological significance in respect of the normal functions and behaviour of the animal'.

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The study of *Helix* by Kerkut & Walker (1962) is of interest in relation to the central organization of the gastropod CNS since one section of the results is devoted to mapping the cell connexions in various ganglia. They demonstrated a number of ortho- and antidromic pathways related to the activity of single cells in the supra- and suboesophageal ganglia.

The work upon the molluscan CNS is thus rather fragmentary, though indicative of a considerable degree of complexity. Bearing the results of the above works in mind experiments upon *Buccinum* CNS were carried out with four objects in mind. The central reactions to mechanical stimulation of the mantle had already aroused interest (Laverack & Bailey, 1963) and these were investigated in more detail. The reflexes involved in the distinctive retraction response formed a second obvious subject for investigation. Contralateral co-ordination involving the stimulation of peripheral receptors was also studied. Finally the convergence of various mechanoreceptive paths in divers central ganglia was determined.

Further, smaller investigations upon centrally determined patterned efferent activity by single preganglionic shocks and axonal branching were also carried out.

MATERIALS AND METHODS

In the course of the experiments to be described in this paper three methods of recording nervous activity were used. The basic details of the method of exposing the nervous system are given in Laverack & Bailey (1963). In all experiments cold filtered sea water was used as a physiological saline.

Method 1

In this case the C.N.S. was left *in situ*, all the surrounding connective tissue being carefully removed. Recordings were made from selected nerves by means of two independently movable platinum-wire electrodes. The indifferent electrodes were placed in the body musculature. Nervous activity was displayed via two similar preamplifiers in a conventional manner. Simultaneous activity in nerves from widely separated regions could thus be monitored. One of the selected nerves was left intact and carried afferent activity into the central complex from the stimulated area. The second nerve was cut peripheral to the recording electrode so that efferent activity alone was recorded. The remainder of the nerves in the body cavity were then cut to isolate the ganglionic complex from any other afferent activity. Mechanical, and in some cases also chemical, stimuli were then applied to the region with intact afferent connexions both before and after section of the various commissures and connectives that link the ganglia. Diminution or loss of the reflex efferent activity was taken to indicate the interruption of part of the functional connexions between the selected nerves. Preganglionic electrical stimuli were also used to stimulate afferent fibres in some experiments.

Method 2

The convergence of afferent pathways in the CNS was investigated by means of localized recording via indium micro-electrodes (see Bailey & Laverack, 1965). This technique involved some extension of the dissection in order to reduce the movements of the preparation and facilitate the insertion of the recording electrode.

In order to eliminate movement the connective tissue was removed from both the CNS and nerves after which the ventral body wall was cut longitudinally in the median line so that the preparation was almost bisected. A small, shaped, Perspex spacer was then inserted beneath the CNS, into the gap created in the body wall, and pinned to the wax floor of the dissection dish. The two sides of the ventral body wall were then pressed firmly against the Perspex and pinned down. Co-ordinated muscular activity was virtually abolished by this means and the CNS was left resting upon a hard surface which facilitated electrode penetration. To reduce movements of the CNS, caused by the muscles of the nerve sheath and the gut, the oesophagus anterior and posterior to the CNS was pinned down through holes drilled at intervals along the Perspex spacer.

It was found necessary to remove the sheath from the dorsal side of the ganglia immediately prior to the insertion of the electrode in order to assist the penetration. Using this technique it proved possible to observe the effects of various stimuli applied to different regions of the animal upon the same small group of central neurones.

Method 3

Intracellular glass saline-filled microelectrodes were used to study axonal branching and post-synaptic potentials of single neurones of the supra-intestinal ganglion. For this purpose the preparations were set up as described in Bailey & Laverack, 1965.

Stimulation

The stimuli used in the course of these experiments were mechanical and electrical. The mechanical stimulus most frequently used was a simple tactile one applied by using a paint-brush. In some experiments dealing with the reflex activity due to activity of mantle movement receptors movement stimuli were applied by the methods described previously (Laverack & Bailey, 1963). The electrical stimuli were shocks produced by discharging a diode. These were applied through a 1:1 transformer. Both amplitude and frequency could be varied at will.

RESULTS

Reflex pathways related to mechanical stimulation of the left mantle region

It had previously been reported that reflex efferent activity could be recorded in the pallial nerves of *Buccinum* in response to peripheral movement stimuli (Laverack & Bailey, 1963). By means of dual-channel recording it was possible to monitor concurrent activity in any two separate pallial nerves. Fig. 1 is a diagrammatic representation of the eight major ganglia of the anterior central nervous complex. The cerebral commissure and the buccal ganglia are omitted for reasons of clarity. Superimposed on this outline are some of the overall pathways which have been demonstrated in the reflex activity originating in the afferent fibres in one pallial nerve and emerging from the CNS to be recorded in the efferent fibres of a second pallial nerve. These pathways are drawn as continuous lines since no valid indication of the position, or number, of synapses involved could be seen using the experimental methods herein described. It proved impossible to relate individual afferent and efferent units in any of the experiments because of the small size and multiplicity of potentials in the bursts of activity which were recorded.

The pathways numbered 1-3 in Fig. 1 relate to reflex efferent activity passing back down the pallial nerves. Pathway 1 demonstrates that some reflex activity may be a function of the isolated pleural ganglion without reference to any other ganglionic interaction. Pathway 2 shows a longer loop which passes through the left pleural ganglion to the ipsilateral pedal ganglion. The afferent and efferent paths both traverse the left pleuro-pedal connective. Pathway 3 involves the pedal ganglion of both sides though the activity originates in the left pallial nerves and the efferent burst passes down an adjacent nerve. The pathway passes through the pleural and left pedal ganglion to the right pedal ganglion by means of the pedal commissure.

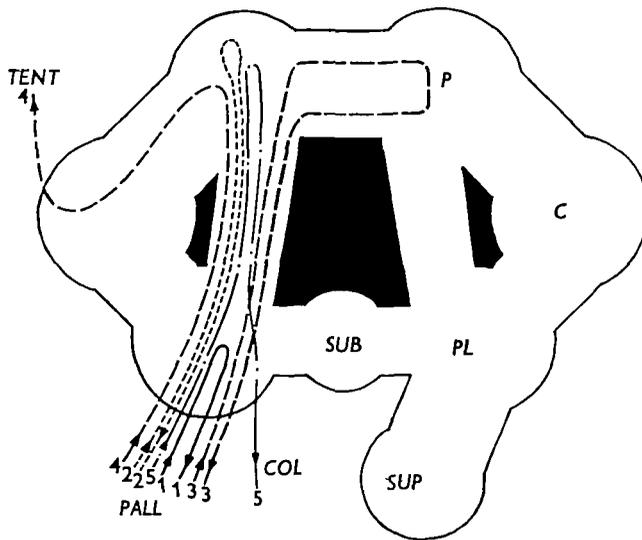


Fig. 1. Reflex pathways relating to mechanical stimulation of the mantle region. C, Cerebral ganglion; COL, columella nerve; P, pedal ganglion; PALL, pallial nerves; PL, pleural ganglion; SUB, sub-intestinal ganglion; SUP, supra-intestinal ganglion; TENT, tentacular nerve; 1-5, pathways.

Reflex activity can be initiated in nerves other than the pallial nerves of the left side, as shown by pathways 4 and 5. Pathway 4 shows that activity invoked in the left mantle region passes through the left cerebral ganglion. Efferent activity can thus be observed in the left tentacular nerves as a result of afferent activity from the left pleural ganglion. Pathway 5 demonstrates that activity arising in the mantle also passes to the columellar muscle. This route passes through the left pleural and left pedal ganglion before emerging from the former.

Reflex pathways associated with the retraction response

The protective retraction response is a general feature of the gastropods. Strong mechanical stimulation of the head region of *Buccinum* will produce such a response, and the efferent activity occasioned by such stimuli was investigated.

The tentacular nerves of the left side were left intact whilst the remainder of the nerves were cut to isolate the CNS. The nervous activity in the tentacular nerves and various others was then monitored concurrently.

Fig. 2 shows only the complete pathways which were established by these means. As before, reflexes involving 1, 2, 3 or in one case (pathway 5) 5 ganglia could be found. In the last case the left pedal ganglion was involved twice. Mechanical stimulation of the head region thus has an even more widespread effect in the CNS than similar stimulation of the mantle. There is no indication of the number of neurones implicated in the chain.

Some of the pathways shown to be involved in the retraction response are more direct than most of those involved in the mantle movement reflexes, e.g. pathways 1-3 in Fig. 2 (cf. Fig. 1), and it is possible that these are related to the initial rapid

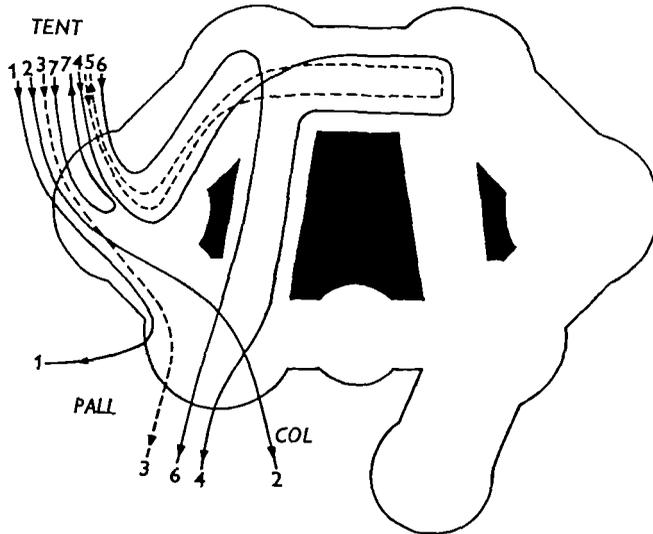


Fig. 2. Reflex pathways relating to the retraction response. Legend as for Fig. 1. (See text.)

movement which precedes the full retraction. The initial response consists of the contraction of the tentacles, siphon and the head region only. This contraction can be elicited in the whole animal by relatively light mechanical stimuli which, if un-repeated, will not cause the full retraction by contraction of the columella retractor. Repeated light stimulation, or a single strong stimulus, produce the initial fast response which then develops into the full retraction response whereby the shell is lifted over the whole head region leaving only the foot extended. Further stimulation will then cause the retraction of the foot. The reflex pathways passing through the pedal ganglia, nos. 4-6 in Fig. 2, are therefore most likely to be concerned in the full retraction response.

Pathway 7 indicates that, as in the pleural ganglion, some of the reflex arcs are completed within the ganglion to which the afferent pathways run. This type of reflex may be concerned with the retraction of the tentacle.

Central nervous pathways relating to contralateral co-ordination

Efferent nervous activity monitored from the left pallial nerves showed that bursts of action potentials could be elicited by mechanical stimuli applied to the tentacular and mantle regions of the right side of the body.

Fig. 3 shows only the complete pathways which were demonstrated. Pathways 1 and 2 were activated by stimulating the right tentacle and they crossed the central complex from right to left via the cerebral commissure, though by analogy with the pathways shown in Fig. 1 there may also be connexions with the pedal and pleural ganglia of the right side. The pathways arising in the right pallial nerves (3-5) show that each of the three commissures linking the paired ganglia of the anterior complex carry connexions between receptors in the right mantle and motor axons to the left mantle.

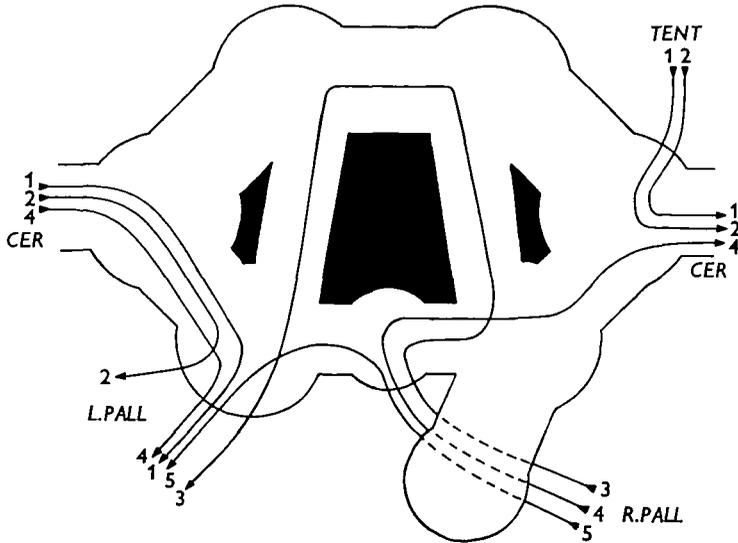


Fig. 3. Reflex pathways relating to contralateral co-ordination. *CER*, Cerebral commissure, through which pathways 1, 2 and 4 pass from the right to the left cerebral ganglion; *L.PALL*, left pallial nerves; *R.PALL*, right pallial nerves. Remainder as in Fig. 1.

Central effects of afferent activity

The insertion of indium microelectrodes into selected ganglia confirmed the pathways which have already been described, since the recorded activity in any ganglion could be influenced in some way by the stimulation of afferent pathways in the nerves which had been used to elucidate reflex arcs passing through that ganglion in the earlier experiments.

The use of these microelectrodes made it possible to detect the excitatory, and in a few cases the inhibitory, effects of afferent impulses on the neurones of the central nervous complex. Excitatory effects causing an increase in on-going activity or eliciting action potentials from previously silent neurones were recorded (see Fig. 4A and B). With this technique inhibitory effects were noted by the reduction of impulse frequencies of active neurones as in Fig. 4C.

Convergence of afferent pathways in the central nervous complex

Using the indium electrodes it was also possible to observe the effects of stimulation of various regions of the body on the same group of central neurones. Fig. 5 illustrates four cases of convergence of afferent pathways on to neurones in various ganglia. In

each case the ganglionic neurones are affected by at least two pathways which converge from opposite sides of the body. Mechanical stimulation of the various body regions produced similar effects on the central neurones, which in most cases was excitatory. The pathways deduced from these experimental results are assumed to take the

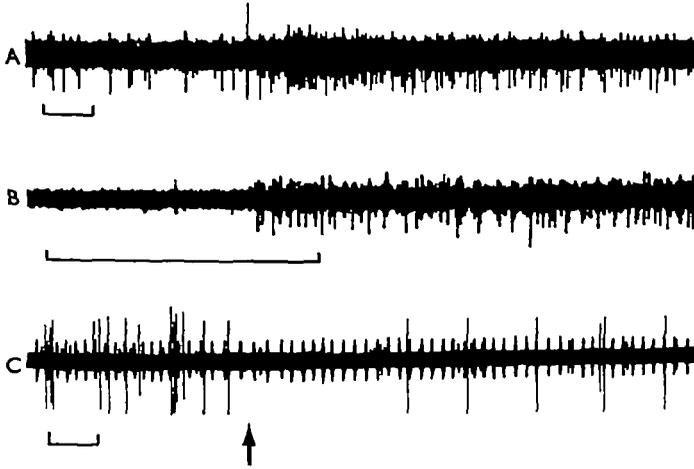


Fig. 4. Responses of central neurones. A, Increase in activity in pedal ganglion in response to touching the head. B, Responses of 'silent' neurones in the supra-intestinal ganglion elicited by acid stimulus applied to the osphradium. C, Active neurone in the pedal ganglion inhibited by touching the tentacle of the same side. Time-marks represent 1 sec. in each case. Arrow represents onset of stimulus.

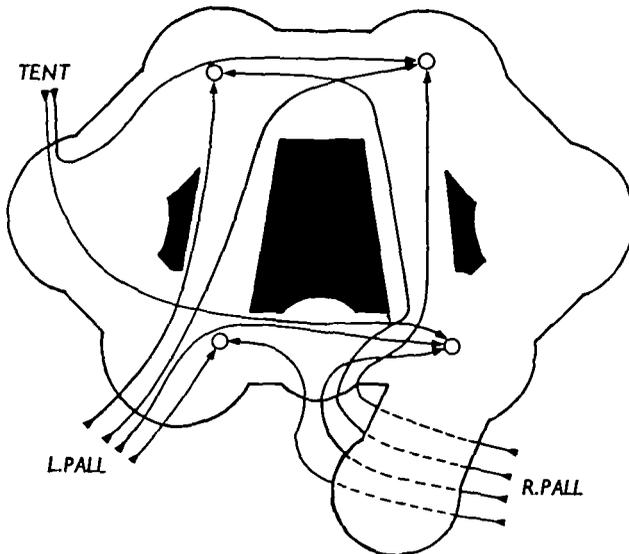


Fig. 5. Convergence of afferent pathways upon central neurones. Circles represent site of recording electrode. Remainder of legend as in Fig. 3 (also see text).

shortest route between the afferent nerve and the central recording site because it proved impossible to cut commissures and connectives with the recording electrode *in situ* and retain the desired recording site. The exact route taken by any nervous

activity therefore remained unknown in these cases, and the pathways drawn in Fig. 5 merely indicate the known convergences. The points of convergence of afferent impulses from the same regions of the body are dispersed throughout the ganglia of the central complex. In order to reach such different localities afferent impulses must take widely divergent routes through the ganglia.

The converging pathways demonstrated by these results are all of similar afferent origin; that is, all arise in response to some form of mechanical stimulation. In a later section of the results it will be seen that afferent pathways from different types of receptor also converge on to central neurones and can have similar or opposite effects upon their activity.

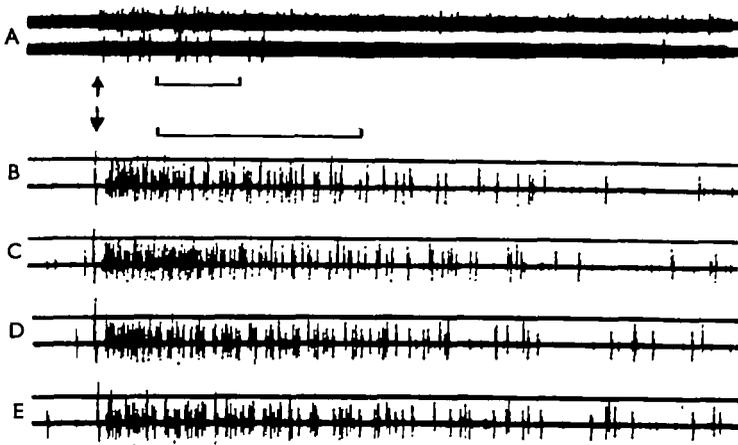


Fig. 6. Patterned efferent activity evoked in a pallial nerve (lower trace) in response to pre-ganglionic electrical stimuli applied to a tentacular nerve of the same side. A, Response in both nerves elicited by touching the head. B, C, D, E, Responses in the pallial nerve to successive shocks applied to the tentacular nerve at intervals of 4.5 sec. Arrows indicate onset of stimulus. Time-marks represent 1 sec., upper for A, and lower for B-E.

Efferent responses to preganglionic electrical stimulation

Patterned trains of efferent impulses in several axons caused by single preganglionic shocks have been recorded using the isolated cerebral ganglion of *Mya* (Horridge, 1958, 1961). In view of the divergent reflex areas demonstrated in the CNS of *Buccinum*, which are activated by stimulation of a relatively small region of the body, a few similar experiments were carried out on the latter animal. In this case, however, the C.N.S. and the majority of the nerves were left intact in order to determine whether similar patterned responses could be evoked from the complete CNS. The maximal electrical shocks were applied to a selected nerve and the evoked activity was monitored postganglionically from a second nerve. Fig. 6 shows the result of one such experiment. From this it may be seen that similar patterns of impulses from a number of axons were evoked by each of the four successive stimuli. The intensity of the response and the residual train of activity both increased slightly and there is a decrease in the central delay, but the overall pattern of the efferent response remains much the same.

spikes can also arise in one cell in the unstimulated preparation. Both spikes are affected in a similar manner by the same stimulus which caused the excitation of both pathways. Fig. 7C shows the 'double spike' phenomenon. The spike illustrated is one of a train of impulses of identical form arising in a single cell of the unstimulated preparation. The two synchronously active sites, which must represent two separate axonal branches since two such active sites in one branch would lead to a cancelling out of the spikes, continued to fire at the same interval and with the same small delay for several minutes.

Post-synaptic potentials

These were seen in only a few of the penetrated central neurones of *Buccinum*. Fig. 8 shows one example of each type of post-synaptic potential. The IPSPs in A were elicited by a mechanical stimulus applied to the osphradium whilst the EPSPs in B occurred in the unstimulated preparation and were mainly subthreshold.

The infrequent occurrence of records of this type suggests either that the actual position of the tip of the recording electrode in the soma of the neurone is critical for the observation of post-synaptic potentials or that in relatively few cases are the cell bodies invaded by such potentials—that is, when they arise at synapses close to, or on, the soma.

DISCUSSION

Reflex pathways in the CNS

The results from the experiments upon the 'mantle reflexes' (see Fig. 1) yield several points of interest. The reflex paths contained in the isolated pleural ganglion demonstrate that afferent neurones may directly or indirectly activate adjacent motor neurones. This is similar to the case in *Octopus* (Gray, 1960), where mantle mechanoreceptor axons synapse with motor units in the stellgate ganglion. The significance of the *Octopus* reflexes is not understood but those in *Buccinum* may well be concerned in the retraction response, especially considering that the siphonal afferent pathways pass into the same ganglion and the mechanoreceptors of the siphonal musculature can be expected to be stimulated by the retraction of the siphon. This hypothesis is the more likely when it is known that sudden mantle movements cause efferent activity in the tentacular and columella nerves, that is, to other regions intimately concerned in the retraction response.

Mechanical stimulation of the left side of the mantle causes reflex activity at various levels in the CNS since one, two or three ganglia may be involved in the reflex arcs. Hughes & Tauc (1962) have shown that both synaptic and through-conducting pathways can exist in the pedal ganglia of *Aplysia*. Since simple bipolar interneurones appear to be lacking in *Buccinum* ganglia (Bailey & Laverack, 1965), reflex paths synapsing on to the unipolar cells will also affect the activity at a number of synapses common to the particular interneurone affected. Fairly widespread effects might therefore be expected, as is borne out by the records of small groups of central neurones responding to a given peripheral stimulus.

The significance of the longer pathways, whether they are synaptic or direct, is less easy to understand. Turner & Nevius (1951) have demonstrated similar 'neurone loops' and branching axons in the CNS of *Ariolimax*. The co-existence of these two features in any reflex path would make possible the dispersion of nervous activity into

other neurone networks adjacent to the reflex arc, besides returning motor impulses to the mantle region. The neurone loops illustrated in Fig. 1 all involve at least one of the pedal ganglia and thus such stimuli might be expected to cause considerable activity in the pedal ganglia. The foot, however, is the last region affected by the retraction response, and considerable stimulation is necessary to cause this effect in the whole animal. In this context another aspect of the work of Turner & Nevius (1951) is of significance since they found that the largest axons of the pedal nerve required the summation of impulses from several pre-synaptic regions to initiate post-synaptic responses. This need for considerable pre-synaptic activity may therefore be satisfied by the many reflex pathways involving the pedal ganglia.

The results summarized in Fig. 2, with the retraction response specifically in mind, show many points of similarity to those concerning the mantle reflexes. Reflex activity arises in the cerebral ganglia in a similar manner to that in the pleural (cf. pathway 1 in Fig. 1 with pathway 7 in Fig. 2). It is possible that this type of activity is involved in the initial quick responses of the tentacles. However, it should be noted that the initial rapid contraction is retained when the tentacular nerves are severed. The change in behaviour resulting from such a lesion is not in the contraction itself but appears to lie in its prolongation. Light tactile stimulation of a tentacle with its innervation intact leads to a contraction which lasts several seconds before the tentacle is again extended. When the tentacle is isolated the contraction is relaxed almost immediately. Cutting the nerves also results in a maintained partial contraction of the tentacles. It would appear therefore that the complete innervation is unnecessary for the initial rapid contraction but is essential for both maintained contraction and full extension of the tentacles.

Besides the local reflex discussed above, tentacular afferent activity also results in efferent bursts in the pallial and columellar nerves. Some of the pathways take the shortest possible route through the ganglia whilst others pass through one or both pedal ganglia. It would seem, therefore, that the closely integrated tentacular, mantle and columella regions are closely related to pedal activity by the nervous involvement of the pedal ganglia. The protective retraction response of *Buccinum* would thus appear to be controlled in a similar manner to the protective response of *Aplysia* (ten Cate, 1928).

The pathways resulting in contralateral efferent activity, summarized in Fig. 3, show that both commissures and the sub-intestinal-pleural connective of *Buccinum* carry contralateral reflex pathways from the right to left mantle regions (see pathways 3-5). Contralateral pathways from the right tentacular region to the left mantle also pass through the cerebral commissure and do not involve the pedal ganglia in at least some cases. Portions of pathways which are probably similar to those in *Buccinum* have been found in *Aplysia* (Hughes & Tauc, 1962) and *Helix* (Kerkut & Walker, 1962) but the full extent of these reflex paths has not been worked out in these other species, so a close comparison is not possible. The dispersion of information throughout the various ganglia and the presence of both ipsi- and contralateral afferent pathways passing along all the available anatomical connexions all tend to make unlikely the existence of specific areas of peripheral representation in the CNS.

The convergence of afferent pathways upon interneurons or motor neurons in various molluscan ganglia has been demonstrated by Turner & Nevius (1951) and

Kerkut & Walker (1962). The former authors showed convergence of afferent pathways from both sides of the body on to efferent neurones in the pedal ganglia. They also found that post-synaptic potentials in pedal motor axons of *Ariolimax* had the same form when any of three different afferent paths were electrically stimulated. Kerkut & Walker demonstrated by similar means the widely dispersed orthodromic pathways of neurones in the supra- and suboesophageal ganglia of *Helix*.

The convergent pathways shown in *Buccinum* (see Fig. 5) indicate that central neurones will respond to widely dispersed peripheral stimuli of both ipsi- and contra-lateral origin. The maximum number of convergent paths was found to be three, but a wider range of stimuli might have shown a greater number since it has already been demonstrated that single central neurones will respond to peripheral stimuli of different modalities (Bailey & Laverack, 1965).

The patterned efferent activity induced by single preganglionic shocks has been described in *Mya* (Horridge, 1961), whence it arose from a single, isolated ganglion. Similar experiments upon *Buccinum* revealed that patterned responses could be evoked from the entire CNS when a single nerve was maximally stimulated. Since such patterns can result from an isolated ganglion or the whole CNS, it would appear that the diffuse nature of the central pathways has a lesser effect upon the final motor output to any region than might be expected. The demonstrated dispersion of reflex pathways through the CNS may be compensated by their ultimate convergence on to a relatively small number of motor neurones for any given region. Thus for a given response—for example, retraction—the first impulse to arrive at the motor neurone synapses may be able to initiate the motor impulse pattern for the response. Afferent impulses arriving later via longer neurone loops may extend or modify the response but are not responsible for its initiation. Such a hypothesis would account for the rapid initial retraction response and the synaptic summation which appears necessary for the retraction of the foot.

Naturally occurring, centrally determined patterns of motor impulses are known to occur in a number of invertebrates (see Horridge, 1961, 1964). Two hypotheses concerning the mechanism of such activity have been proposed. Maynard (1955) suggested that the final motor output is the result of a series of interneurones each in turn exciting the next in the chain. Horridge (1961, 1964) on the other hand suggests that the concept of a 'circuit diagram' be replaced by a chemical specificity in the relationship between one group of neurones and another. The anatomy of the synaptic connexions thus becomes of lesser importance so long as the chemical sensitivity of the neurones and the transmitter substances they produce are genetically fixed.

These theories are difficult to separate experimentally and recent evidence from vertebrate neurophysiological studies suggest that features of both may be combined in certain cases. Fisher & Coury (1964) have shown in the rat brain that neural pathways of considerable extent can be selectively stimulated by the injection of cholinergic drugs into a variety of brain structures. Thus tracts of fibres with at least a partially ordered histological pattern, a specific chemical sensitivity and a predictable behavioural effect are known to exist in vertebrates. Similar systems are therefore anticipated in the invertebrates.

Central neurone anatomy

The results indicating the branching nature of the dendrites of central cells may be compared with those of Tauc & Hughes (1963*b*), showing that this feature is common to *Aplysia* and *Buccinum*, and probably throughout the Gastropoda.

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