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# NERVE PATHWAYS AND REFLEX SIPHON WITHDRAWAL IN THE SURF CLAM

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## INTRODUCTION

Ganglion cells in the central nervous system of gastropod molluscs have proved especially useful in investigations of functional neuron architecture and the physiology of excitable membranes (e.g. Arvanitaki & Chalazonitis, 1961; Tauc & Hughes, 1963; Tauc, 1962*a*, *b*). Large size, favourable electrical properties and accessibility—all have contributed to the successful role these structures have played in developing a comprehension of nervous processes at the cellular level. Gastropod central neurons are particularly useful because certain ones can be consistently identified in different preparations; their structure and functioning can therefore be thoroughly investigated and catalogued with regard to electrical properties, modes of excitation, and pharmacological sensitivity (Tauc & Gerschenfeld, 1961, 1962; Kerkut & Walker, 1962). Information about sufficient numbers of such cells would provide a valuable functional index for concomitant studies of behaviour and its neurophysiological basis.

The bivalve molluscs appear to offer more attractive behavioural subjects than either the opistobranch or pulmonate gastropods. The variety of behaviour in these more sedentary forms is understandably less complex, and the responses tend to be reflex and reliable. These animals thus provide stable responding systems for investigation of the cellular mechanisms which underlie simple behaviour. As a case in point Hecht (1920) was able to establish the photochemical nature of the primary sensory process mediating siphon withdrawal in *Mya*, merely by performing measurements of the latency of that photically-evoked reflex under various experimental conditions. The sensory physiology of this reflex has since been thoroughly investigated in *Spisula* (Kennedy, 1960).

Using the razor clam, *Ensis*, Drew (1908) was the first to show that electrical stimulation of nerve roots on one side of a ganglion results in symmetrical excitation of structures on both sides. For example, contraction of muscles in the siphon and mantle on both sides of the animal was obtained by electrical stimulation of the posterior pallial nerve on one side alone. These observations have been confirmed in *Mya* by Horridge (1958), who used extracellular recording techniques, as well as behavioural observations, to demonstrate that asymmetrical stimulation of afferent pathways results in bilateral postsynaptic activity in nerves and connectives. Horridge's findings were thus able to account for the symmetrical contraction of the siphonal and pallial muscles on both sides of the clam, in response to a highly localized threshold stimulus; moreover, they provided a logical mechanism to explain another of Drew's observations,

namely, that excitation in response to strong tactile stimulation can spread via any anatomically available nervous pathway to involve muscles in distant parts of the animal. Subdissection of nerve trunks for purposes of single-unit extracellular recording has not met with success in bivalves, due primarily to the small size and delicate nature of the individual fibres. Probably for this reason a detailed analysis of synaptic connexions in *Mya* was not attempted by Horridge, and the precise nature of the pathways mediating symmetrical responses could not, therefore, be determined. Evidence in fact indicates that both bilaterally branched and unilateral efferent axons occur in the pallial nerves of *Mya* and *Spisula*, and it was of interest to find out which pathway is primarily responsible for reflex behaviour.

The results presented in this report are based on an electrophysiological investigation of the nerve pathways concerned with reflex siphon withdrawal in the surf clam. Intracellular recordings from cell bodies in the visceral ganglion have shown that somata of efferent neurons having large fibres to the siphons occur in specific identifiable regions, the pallial integrating centres. Axons of individual cells in these regions are distributed to one or (rarely) two of the three main branches of the posterior pallial nerves on the side of origin; they do not cross to the opposite side of the ganglion. Highly convergent input to these neurons occurs via sensory fibres from every branch on the ipsilateral and contralateral side as well, and this arrangement apparently forms the physiological basis for symmetrical siphon withdrawal. Pathways other than those which occur within the pallial integrating centres may be involved in the prolonged closure response of *Spisula*; the most likely assumption is that this aspect of behaviour is controlled by small fibres from efferent cells in some other region of the ganglion.

## MATERIALS AND METHODS

Male and female individuals of the surf clam, *Spisula solidissima*, were used in the course of this investigation. This animal is the largest bivalve on the Atlantic Coast and possesses correspondingly large nervous structures. Many of the experiments were performed at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the summer of 1964. However, clams withstood shipment to Charlottesville exceedingly well, and they remained in good condition for many weeks when maintained in circulating artificial sea water at 10° C. Results obtained in the laboratory at Virginia were similar to those obtained at Woods Hole.

Dissections were performed by transecting the adductor muscles of an animal and removing the left valve. The visceral ganglion and its associated nerves could then be removed from their situation, just ventral to the posterior adductor muscle, and placed in a suitable chamber for further dissection and recording. Although the ganglion and nerve trunks are embedded in extremely tough and elastic connective tissues, these structures can be dissected free without difficulty because of their marked pigmentation. The compounds implicated in their coloration have been discussed by Kennedy (1960).

Recordings of electrical activity were performed in natural or artificial sea water, the latter having the following composition: NaCl, 423 mM.; KCl, 9·00 mM.; $CaCl_2$, 9·27 mM; $MgCl_2$, 22·94 mM.; $MgSO_4$, 25·50 mM.; $NaHCO_3$, 2·15 mM. For intracellular recording the visceral ganglion was pinned to the wax floor of the recording

chamber by a few adhering strands of connective tissue, and the pallial nerve branches were then picked up on pairs of silver-silver chloride electrodes for stimulation. Micropipette electrodes filled with 3M. KCl and having resistances of 20–50 MΩ were employed. Signals were detected by a high impedance, neutralized capacitance pre-amplifier and displayed on an oscillograph. Extracellular recordings of sensory discharges were obtained from the cut central end of pallial and siphonal nerve branches.

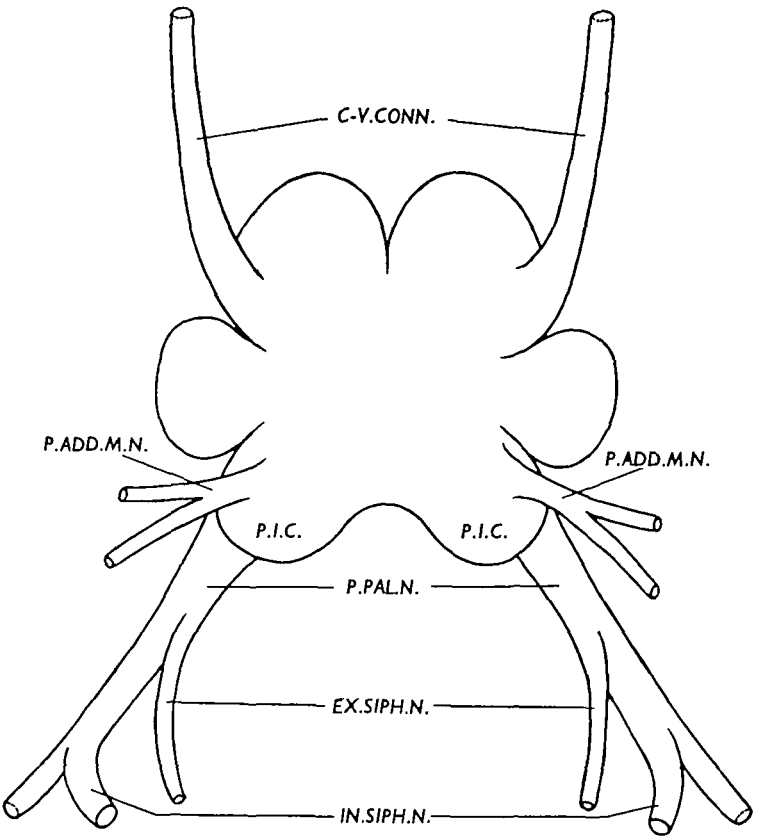


Fig. 1. Diagram of the visceral ganglion of *Spisula* as viewed from the dorsal surface. The paired branchial nerves, which emerge from the ventrolateral aspects of the ganglion, have not been included. *C-V. CONN.*, Cerebrovisceral connectives; *P. ADD. M. N.*, nerves to the posterior adductor muscle; *P. PAL. N.*, posterior pallial nerves; *P.I.C.*, pallial integrating centres; *EX. SIPH. N.*, excurrent siphon nerve; *IN. SIPH. M.*, incurrent siphon nerve.

The siphonal structure was separated from the rest of the mantle, cut through the mid-line on the ventral side, and then pinned to the floor of a wax block. Nerves were lifted above the surface of the bathing solution and placed upon a fine platinum wire electrode; a grounded chlorided silver wire placed in the solution served as the indifferent electrode. Action potentials were picked up by a high-gain a.c. pre-amplifier with a band pass of 35–10,000 cyc./sec. Mechanical stimuli were applied to the siphonal tentacles by means of a small glass stylus mounted in a crystal-driven phonograph cartridge which was excited by electrical pulses of varying duration and intensity from a conventional laboratory stimulator. For electrical stimulation 1·0 msec pulses

were delivered to the nerve trunks through isolating transformers. Recordings of compound action potentials, for measuring conduction velocities, were performed at 15° C. in a constant temperature room. All other observations were made at room temperatures, which varied between 18 and 21° C.

## RESULTS

### (1) *Anatomical*

A diagram of the visceral ganglion of *Spisula*, together with its major nerve supply, is shown in Fig. 1. In large animals, 5 or 6 in. long, this structure may exceed 2 mm. in width, and, routinely, lengths of pallial nerve 15 mm. long can be dissected before branching occurs into the muscle of the siphons and the posterior mantle. Several large brown-coloured cell bodies are usually visible in the conspicuous paired lobes which comprise the anterior of the ganglion. Such cells are probably concerned with transmission in the cerebrovisceral connectives, to judge from their anatomical relationship with those nerves; but this supposition has not been examined electrophysiologically. The posterior pallial nerves emerge from the postero-ventral aspects of the ganglion on either side; two separate major branches are soon given to the excurrent and incurrent siphons, respectively. The more distal branch, to the incurrent siphon, is a good deal larger than the branch to the excurrent siphon, and reflects the larger size of the structure it innervates.

### (2) *Responses to tactile and electrical stimuli*

In the intact animal weak tactile stimulation of the tentacles which ring the openings of both siphons results in strictly localized responses, usually amounting to withdrawal of the stimulated tentacle(s) alone. Stronger stimulation may cause a discernable increase in the perimeter of the responding region; usually however, the result is a much stronger rapid symmetrical withdrawal of both siphons, followed immediately by a transient twitch-like contraction of the adductor muscles. This response sequence which causes water to be forced out through the openings of the siphons, is a simple reflex, and the structures involved return to their former position within a minute. Siphon withdrawal itself is always symmetrical, involving both halves of both siphons, no matter how restricted the stimulus which produced it. The response is unaltered by cutting completely through the mid-line region of fusion of both siphons, while leaving the visceral ganglion and its nerve supply intact. This indicates that contralateral activation of the siphonal retractor muscles must be controlled by pathways within the ganglion itself, since peripheral branching of either afferent or efferent nervous connexions to the contralateral regions of the siphons must be effectively eliminated by the operation. To confirm these findings the entire siphonal structures were removed intact from several clams, and the posterior pallial nerves were cut near their region of entry into the visceral ganglion. In such preparations harsh stimulation of a tentacle on one side caused a response in the siphon muscles on that side only. When the severed central ends of the pallial nerves were stimulated electrically with trains of shocks at 100/sec., contraction of the siphonal retractor muscles on either side occurred only when the ipsilateral nerve was stimulated. This again indicates that contralateral branching at the periphery by efferent fibres of the

pallial nerve does not occur. Preliminary extracellular recordings of impulse traffic in the combined siphonal-pallial nerve on one side of the intact animal in response to stimulation of the opposite nerve did show that a few fast through-conducting fibres were present, as well as large numbers of synaptically driven units. Therefore, it seemed at first that symmetrical siphon withdrawal could be simply accounted for by symmetrical distribution of bilaterally branching efferent fibres. The results of intracellular recordings presented below indicate that such an arrangement is not necessary to account for the observed behaviour.

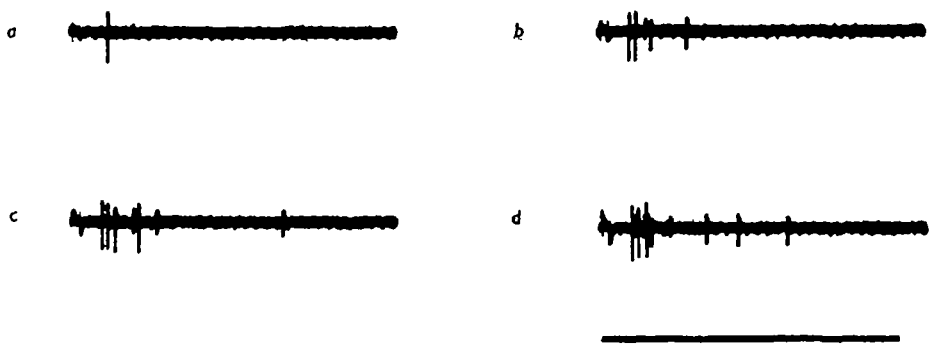


Fig. 2. Electrical responses recorded extracellularly from the axons of touch-sensitive cells in the tentacles of the incurrent siphon. The intensity of the electrical pulse to the mechanical stimulator was gradually increased in *a–d*. Calibration: 100 msec.

Prolonged or repetitive mechanical stimulation delivered to any region of the siphons or the mantle will result in sustained closure of the valves and retraction of the siphons, mantle, and foot. This response may be maintained for many minutes or hours, and it therefore long outlasts the stimulus producing it; it is of interest because the neural mechanisms involved in its initiation and maintainance may bear on the question of prolonged behaviour patterns in general. Extracellular recordings from *Mya* have implicated a population of neurons with small efferent fibres in the maintenance of prolonged closure (Pumphrey, 1938). Although the nervous activity is distributed via the central nervous system it seemed necessary to investigate the possibility of peripheral influences. Accordingly, electrical responses from touch-sensitive cells located in the tentacles, on the siphons and on the mantle were briefly examined in several different preparations. In fact, as can be seen from the records of Figs. 2 and 3, the responses of stimulated cells are quite conventional. An increase in the intensity of the mechanical stimulus delivered to the tentacles recruits sensory cells as well as increasing the response of those cells already active. Repeated stimulation of a receptor population makes them refractory (Fig. 3*b–e*). Disadaptation occurs rapidly, however, and a 60 sec. period during which no stimulus is applied may result in complete recovery of original sensitivity (Fig. 3*f*). In the records of Fig. 3*h* and *g* a receptor cell which responded to a weak mechanical stimulus with a single impulse failed after several repetitive stimuli recurring at 10 per sec. These results thus do not support the possibility that the closure response of *Spisula* is driven by prolonged

input from peripheral tactile receptors; however, the role of proprioceptors in such activity has not specifically been investigated and therefore cannot be ruled out.

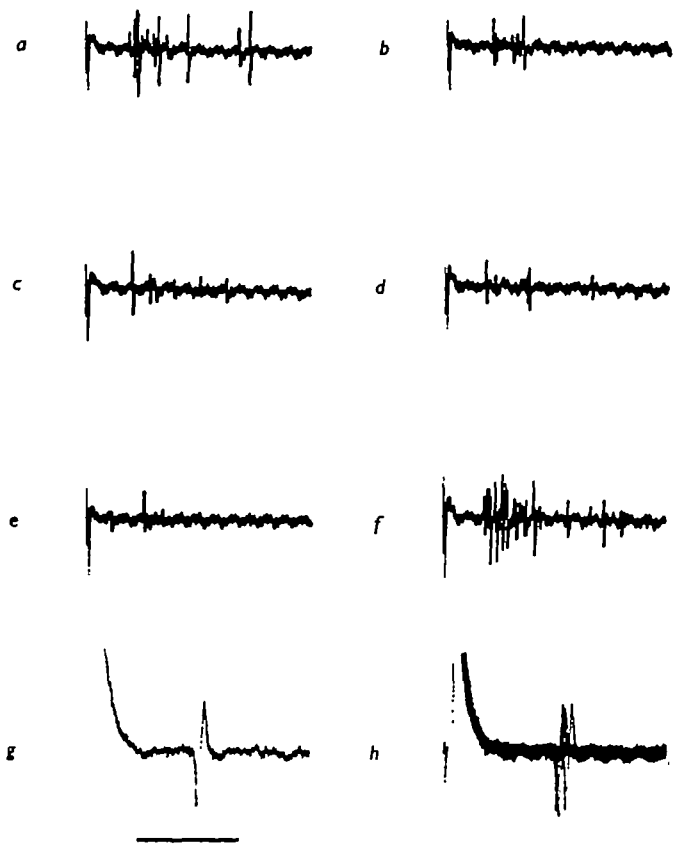


Fig. 3. Extracellular records from tactile cells on the incurrent siphon in response to consecutive stimuli recurring at 1 per second. Records in *a–e* show the response to maximal stimuli at $t=0$, 3, 5, 7, and 13 sec. respectively. Stimulation was then stopped for a period of 60 sec., at the end of which time the response in *f* was obtained to the same stimulus. In *g*, a sensory cell in the mantle near the siphons responded to a weak mechanical stimulus with a single action potential. When the stimuli recurred at a frequency of 10 per sec. adaptation occurred after three or four responses. Calibration: *a–f*, 100 msec.; *g–h*, 25 msec.

### (3) *Measurements of conduction velocity*

Measurements of conduction velocities were made on eleven different lengths of combined pallial-incurrent siphonal nerve, ranging from 1·0 to 1·5 mm. in length. In order to standardize conditions, the measurements were performed at 15° C. in a constant temperature room. The major length of the nerve to be tested was submerged in the grounded sae-water bath; an external recording lead was used to lift the cut distal end of the siphonal nerve just above the surface of the water, while the central end rested, also above the surface, on a pair of chlorided silver wire stimulating electrodes. Records shown in Fig. 4 illustrate the typical configuration of compound responses to a stimulus series of increasing intensity. With the greatest intensity used four distinct negative-going peaks are evident, which represent fibre populations with conduction

velocities ranging from 0·1 to 1·0 m./sec. when peak latencies are measured. The peak with the largest amplitude is also the fastest; since its size can be graded by adjusting stimulus strength it must be composed of the summed activity of a number of separate fibres which, to judge from their low threshold and high speed, are among

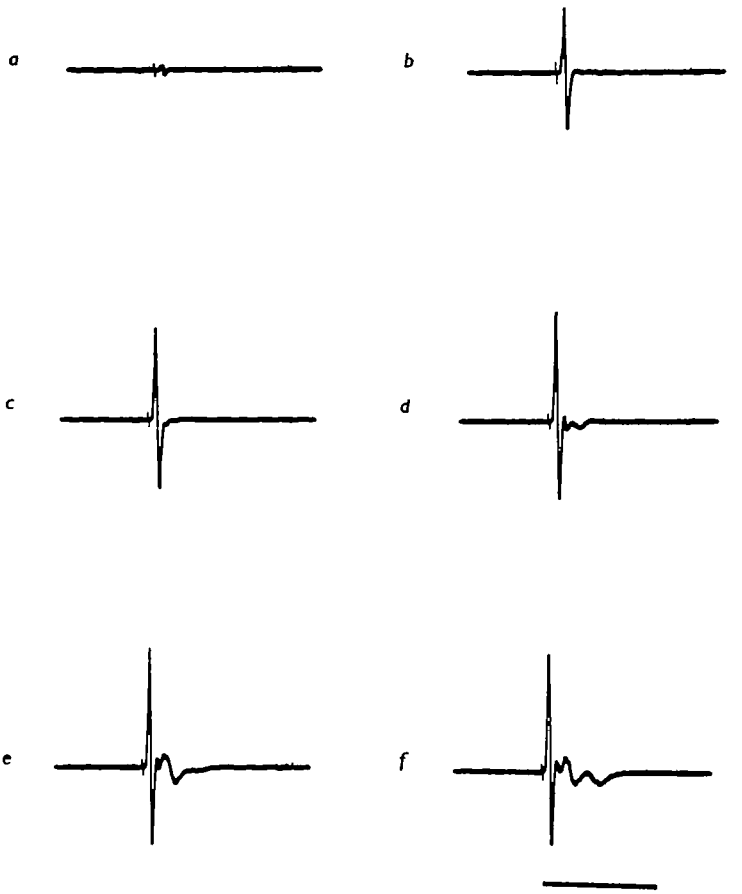


Fig. 4. Compound action potentials evoked in a length of incurrent siphon nerve by electrical stimuli of increasing strength (*a–f*). Fast, low-threshold responses, such as those evoked in *a*, appear to inflect the positive phase of the larger response recorded in *b*. Increasing negativity of the active recording lead is indicated by a downward deflection of the trace. Calibration: 100 msec.

the largest found in the nerve. However, some still faster fibres, with conduction velocities of up to 1·5 m./sec. were observed by adjusting the stimulus intensity in the region of threshold for the first response of any size to occur. Fig. 4*a* shows a small fast action potential of this type, and others appear to inflect the positive phase of the largest response in Fig. 4*b*. Fig. 5 is a histogram showing the distribution of conduction velocities in eleven different lengths of incurrent siphonal nerve. These data may be compared with the values given in Table 1, for 16 efferent cells, whose conduction velocities were measured directly by timing the latency of the intracellularly recorded antidromic impulse. Since the intracellular measurements were made at temperatures of up to 4° C. higher than the whole nerve recordings, somewhat faster conduction

speeds are to be expected; if a $Q_{10}$ of 2 is assumed for these axons, then a 20–40% increase in conduction velocity over the measurements made at 15° C. should be observed, and the efferent neurons should therefore be assigned to that population of fibres represented by the second negative peak in Fig. 4, i.e. the group with a distribution maximum at 0·5 m./sec. in Fig. 5.

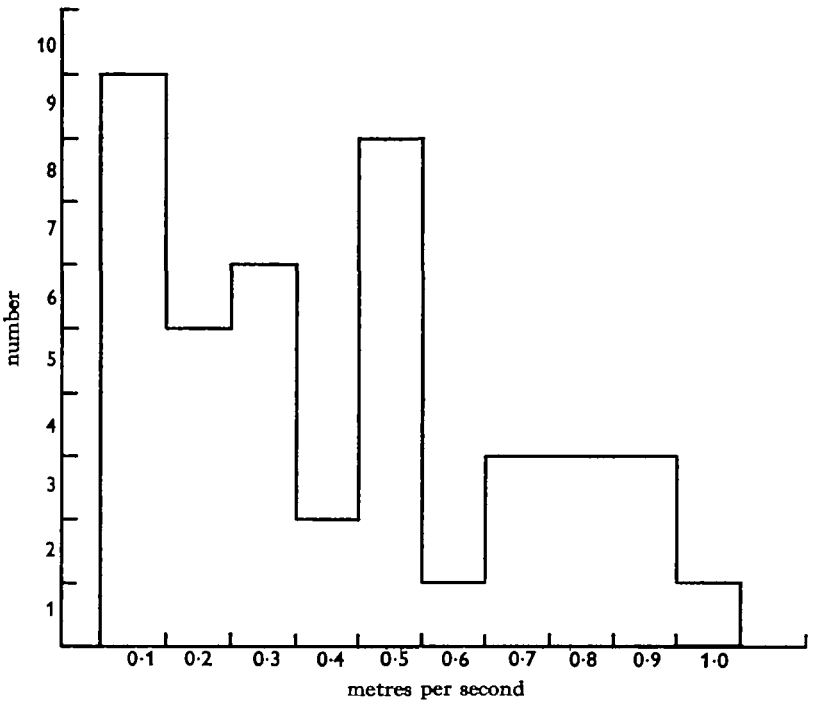


Fig. 5. Histogram indicating the distribution of fibres in the incurrent siphon nerve with respect to conduction velocity. The data were taken from measurements on eleven different lengths of nerve at 15 °C. The vertical scale indicates number of negative peaks measured. The horizontal scale is conduction velocity.

Table. 1. *Conduction velocities of efferent neurons as measured by latency of the antidromic response*

| Cell no. | Conduction velocity (m/sec.) | Temp. (°C.) | Cell no. | Conduction velocity (m./sec) | Temp. (°C.) |
|---|---|---|---|---|---|
| 1 | 1·30 | 19·0 | 9 | 0·50 | 19·0 |
| 2 | 0·75 | 18·0 | 10 | 1·20 | 19·0 |
| 3 | 0·71 | 17·5 | 11 | 0·75 | 19·0 |
| 4 | 0·67 | 18·0 | 12 | 0·67 | 19·5 |
| 5 | 0·85 | 18·0 | 13 | 0·59 | 18·0 |
| 6 | 0·67 | 18·0 | 14 | 0·62 | 18·0 |
| 7 | 0·70 | 18·5 | 15 | 0·55 | 18·0 |
| 8 | 0·70 | 19·0 | 16 | 0·62 | 18·5 |

### (4) *Postsynaptic responses*

Intracellular recordings were obtained after the transparent connective tissue envelope which surrounds the visceral ganglion had been torn or partially removed. At first, various regions of the ganglion were probed with micropipettes, while electrical stimuli were being delivered to the siphonal nerve branches on either side. However, it soon became evident that excitation of impaled units by those specific

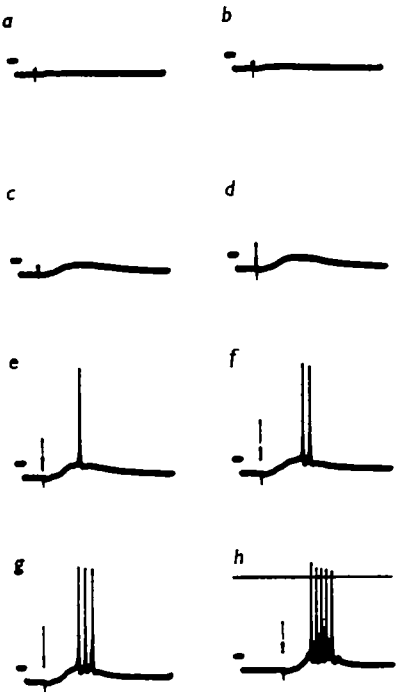


Fig. 6. Intracellularly recorded activity from a ganglion cell in response to electrical stimuli of increasing intensity delivered to the contralateral combined pallial-incurrent siphon nerve. The horizontal line in *h* indicates zero potential level; the resting membrane potential of the cell was about −65 mV. Calibration signal: 10 mV. and 10 msec.

pathways was consistently obtained only in the paired regions designated '*P.I.C.*' in Fig. 1. Responses from cells in these areas were found to be similar, when the discharge characteristics were compared in different units; it can be concluded that they represent a rather homogeneous population of neurons which respond in a similar fashion to input from many parallel channels on both sides of the animal. Fig. 6 illustrates records from a cell soma in response to a series of presynaptic volleys of increasing magnitude in pathways on the contralateral side of the ganglion. The classical features of synaptic excitation can be observed, including graded depolarizations, shift in the latency of the first spike, and increase in spike frequency and number as the input is intensified. These records are not typical in at least one respect, however; overshooting spikes are usually *not* observed for longer than a few milliseconds following penetration of a cell by the electrode. The somata of most cells in the pallial regions of the ganglion are small, averaging 15–20 $\mu$ in diameter, and penetration of

the cell membrane by an electrode is often followed by a decline in the original resting membrane potential. Changes in excitability must occur, and, as a result, invasion of the soma by conducted action potentials is rarely maintained. It may be argued that spikes normally do not invade the soma membrane but only do so transiently when the potential drop occasioned by penetration of the membrane briefly enhances the safety factor for invasion. No particular evidence in the present investigation bears on

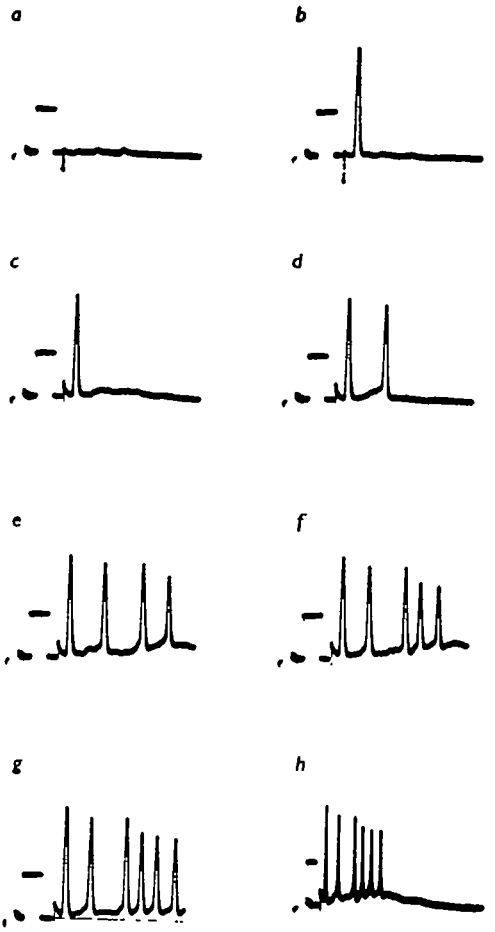


Fig. 7. Intracellular records from an efferent neuron in response to an intensity series in the ipsilateral incurrent siphon nerve. An antidromic response, evoked at low stimulus intensities, has a peak latency equal to that of the first visible postsynaptic response in *a*. Records in *g* and *h* were photographed at different sweep speeds in response to stimuli of identical strength. Calibration: 10 mV. and 10 msec.

this question one way or the other; however, Mendelson (personal communication) claims that polarization of impaled cells in the *Spisula* visceral ganglion by passing inward current across the membrane will often reinstate spike overshoot and thus, presumably, soma invasion. Such a finding suggests that excitation of the soma membrane by activity in other regions of the neuron occurs normally, but fails in penetrated units as a result of injury-induced depolarization. In fact, non-invasion of the soma

is probably not a reliable index of the physiological state of the rest of the cell (Tauc & Hughes, 1963); synaptic connexions between most molluscan nerve cells apparently occur only at axonal loci and the soma membrane may be invaded only secondarily by an active response (Young, 1939; Tauc, 1962*a*).

Detailed observations of postsynaptic responses were usually made using as the input pathways afferent fibres from the incurrent siphonal nerve alone. This branch is the largest of the three, and it usually produces the strongest synaptic excitation. In some preparations, however, stimulating electrodes were placed as well on the pallial nerve distal to the siphon. Afferent fibres in both of these pathways make synaptic connexions with the same cells which receive input from the incurrent nerve, and spatial summation of subthreshold EPSP's from any combination of the three afferent pathways will cause impulses to be triggered when the same stimuli delivered individually to the separate nerves will not. Convergence of afferent fibres upon postsynaptic cells in the pallial integrating centres can thus be conclusively demonstrated. On the other hand, axons of the efferent cells themselves seem to be restricted to a single nerve branch, as judged by the usual presence of antidromic activity in only one of the three branches following stimulation. Only in a small percentage of cases were antidromically conducted spikes simultaneously evoked by stimuli in both the incurrent and excurrent siphonal nerves. As indicated below, contralateral branching of efferent neurons from this region of the ganglion is never observed.

The records of Fig. 7 are typical of the response patterns observed following ipsilateral excitation. At the lowest stimulus intensity small, probably unitary, subthreshold EPSP's are evoked after various latencies. Stronger stimuli then usually excite the axons of the impaled cell at the stimulation site, and an antidromically conducted impulse is recorded by the electrode in the soma. The antidromic nature of this response was often confirmed by testing its ability to follow repetitive stimuli well above 10/sec without any shift in latency. With increasing stimulus intensities the recruitment of additional afferent fibres leads to enhanced EPSP amplitude, and orthodromic impulses are generated. In the present investigation postsynaptic potentials did not have sufficient amplitude in every unit penetrated to make it profitable to study them in detail; however, favourable recording conditions were realized in many preparations, and some of the more marked characteristics of these responses could be seen. In general the synaptic potentials tended to comprise an early, large amplitude phase with a fast decay, and a later more prolonged phase; this feature is particularly noticeable in the records of Fig. 10. Since known efferent axons in the siphonal nerve are among the fastest present, large numbers of slower, presumably afferent, fibres must also be present (cf *f.* fig. 4), and a logical argument may be advanced that the later phase of postsynaptic excitation is driven by input in this slow pathway. Facilitated unitary EPSP's in central neurons of *Aplysia* can have a time course of 100 msec. or more (Kandel & Tauc, 1964). The decay of depolarization in such preparations may be governed predominantly by the kinetics of transmitter hydrolysis; however, the largest cells have time constants of 100–200 msec. so that the cable properties of the membrane can prolong excitatory influences to a considerable extent (Tauc, 1962*a*). The decay phase of both types of influence would be smooth functions of time, however, and considering the irregular time course of the present EPSP's it is simpler to assume that asynchronous input is the predominant factor. In a few cells, however,

extremely delayed responses were observed in addition to the normal prolonged responses; examples are shown in Figs. 8*e* and *f*, and 9*A*. The properties of this late activity were entirely unlike those of the earlier phases, and showed a marked instability in impulse pattern; compare, for instance, the records in *A* and *B* of Fig. 9. The latency of this delayed response is too long to be explained by even the slowest

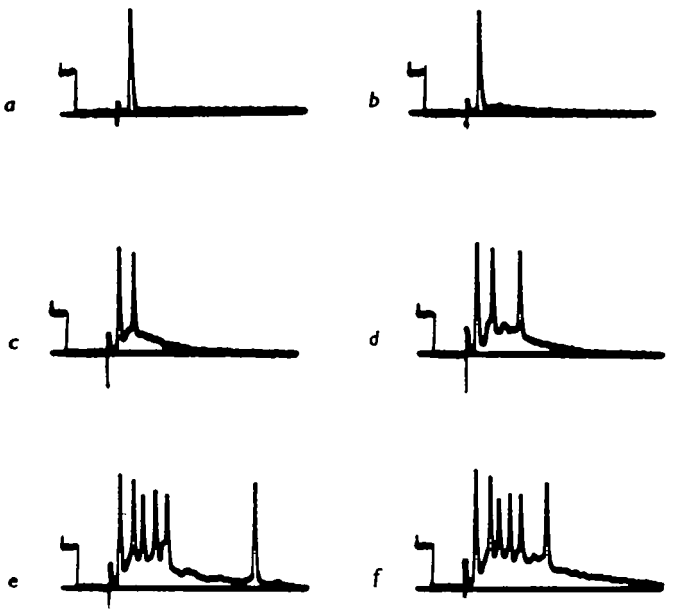


Fig. 8. Intracellular records of the response of a ganglion cell to an ipsilateral intensity series. An antidromic spike is evoked at low stimulus strength and is followed by prolonged orthodromic activity in the later frames. Compare the firing level of the extremely late spike in *e* with that of the first orthodromic spike in *c*. Calibration: 10 mv. and 10 msec.

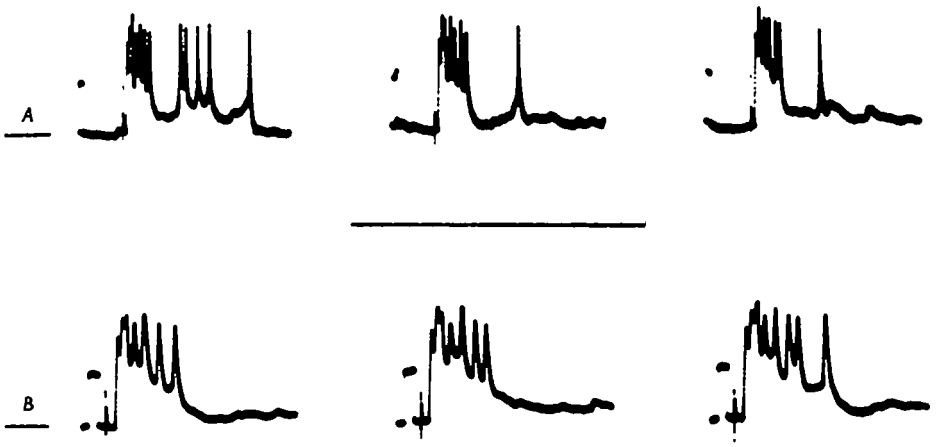


Fig. 9. Successive records of the response of a ganglion cell to maximal ipsilateral stimuli. Note the stability of firing pattern of the early response and the labile character of the delayed discharge (*A*). The records in *B* were photographed at a faster sweep speed to make details of the early response more visible. Calibration signals: 10 mV.; horizontal line indicates 600 msec. in *A*, 300 msec. in *B*.

fibres present in the pallial nerve; furthermore, the firing level for spikes evoked by this late synaptic activity is often different from that of the earlier phases. When the firing level for the first synaptic spike in Fig. 8*c* is compared with that for the late spike occurring in *e*, it will be seen that the late impulse is triggered at a significantly lower level of depolarization. This would seem to indicate that different synaptic loci (which are at unequal distances from the recording electrode and/or the spike-generating locus) are involved in generating the early and late responses.

The monosynaptic nature of the pathways involved in ipsilateral excitation of neurons in the pallial integrating centres is suggested by evidence from three separate lines of inquiry. First, in the records of Figs. 7–9 appearance of the antidromic

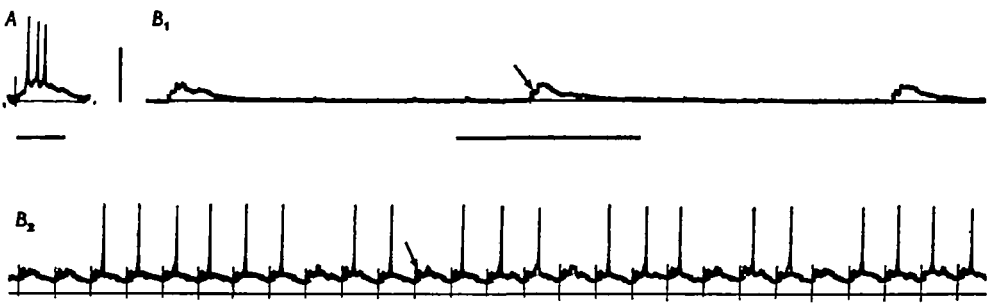


Fig. 10. Intracellular records from a ganglion cell. *A*, response to a suprathreshold ipsilateral volley. $B_1$, Three subthreshold responses evoked by electrical stimuli delivered to the ipsilateral incurrent nerve one second apart. $B_2$, Same stimulus strength as in $B_1$, but repetition rate increased to 10 per sec. Firing level for the spike is about 15 mV. depolarization. Voltage calibration: 25 mV.; horizontal lines represent 100 msec. in *A*, and 500 msec. in *B*. See text.

impulse coincides with the rising phase of the EPSP. Considering the temporal relationships in the records of Fig. 7 alone, we may assume, that the conduction velocity of the efferent axon is 0·7 m./sec., while that of the afferents responsible for the earliest phase of the EPSP is 1·5 m./sec. The peak latency of the antidromic impulse is 6·5 msec.; from these figures we can calculate that the afferent volley must have arrived fully 3 msec. *prior* to the first appearance of the EPSP. Over the 18–20° C. temperature range at which these records were obtained the delay at the fast relay synapse between the giant fibres of the second and third orders in the stellate ganglion of the squid is nearly 2 msec. (Bullock & Hagiwara, 1957); therefore, unless one is willing to postulate that synaptic delays in the *Spisula* central nervous system are shorter than those occurring in the squid—a possibility which somehow seems unlikely—the 3 msec. delay calculated for the preparation shown in Fig. 7 cannot involve more than one synaptic transfer. Indeed, if less extreme values are assumed for the conduction velocity of the afferent limb of the pathway, the central delay will be smaller, and the possibility of more than one synapse being involved becomes even more remote.

Additional evidence favouring monosynaptic activation of pallial neurons is provided by the temporal stability of the EPSP when driven repetitively at frequencies of up to 10 per sec. The prolonged and irregular time course of the EPSP, and the usual absence of noticeable unitary responses made observations of this nature somewhat difficult; none the less, the example shown in Fig. 10 indicates that the first

peak of the EPSP maintains constant latency and amplitude when driven repetitively. The second peak, though less reliable, is driven by temporal summation above the firing level of the cell, in response to more than 70% of the volleys.

Finally, the stability in waveform of the postsynaptic responses, is suggestive of a monosynaptic pathway. Peculiarities in spiking interval, which must be a function of variations of the amplitude of the underlying EPSP, are rigidly maintained when stimuli

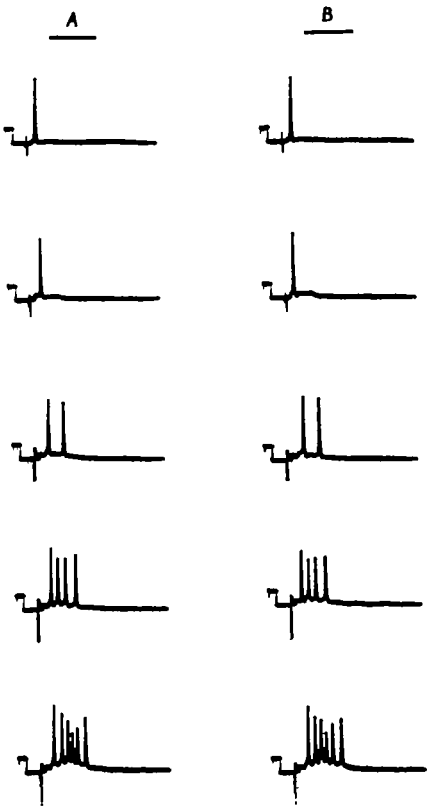


Fig. 11. Responses from a ganglion cell taken during two independent complete intensity series (*A* and *B*). Note stability of EPSP waveform and spiking interval at similar stimulus intensities. Calibration signal: 10 mV. and 10 msec.

of comparable amplitude are delivered to the input pathway on different occasions. This transfer stability, which is normally associated with monosynaptic pathways, arises from properties of electrically excitable membrane, with its greater temporal stability and constant velocity of propagation. An electrically evoked presynaptic volley results in a fixed sequence of arrival of ***single*** events occurring in a number of parallel pathways. The postsynaptic output, however, is usually composed of ***trains*** of high-frequency events in each pathway; if these trains impinge upon the temporally unstable mechanism at a second synapse, some variation in output pattern can be expected to occur in response to identical volley strengths at the first synapse. The records shown in Fig. 11*A* and *B*, are taken respectively from two different intensity series which were initiated about 30 sec. apart. Nearly identical responses were

obtained following stimulation at comparable stimulus intensities, not only in regard to the number of spikes evoked per volley but including details in timing of the spike intervals as well.

### (5) *Responses to contralateral excitation*

Responses of efferent cells in the pallial integrating centres to contralateral stimuli were consistently different from those observed following ipsilateral stimulation. For example, antidromically activated spikes were never observed in response to stimulation of the contralateral nerve branches. This was found to be true in the more than one hundred individual units which were observed. The cell bodies of bilaterally branched neurons probably do not occur in regions of the visceral ganglion examined in the present investigation.

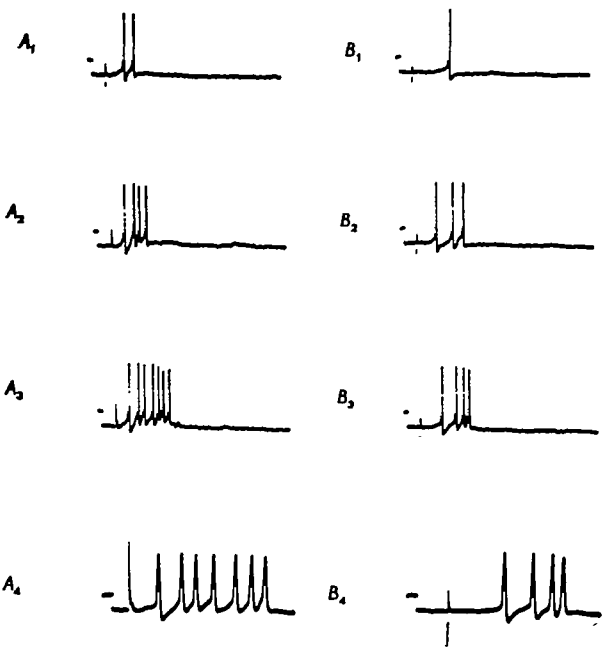


Fig. 12. Responses of an efferent neuron to (*A*) ipsilateral and (*B*) contralateral intensity series. Records in bottom row, taken with an expanded sweep, illustrate difference in latency of the response when initiated on the two sides. Calibration: 10 mV. and 10 msec.

Ipsilateral stimulation invariably evokes a stronger postsynaptic response from pallial neurons than can be achieved by way of contralateral pathways; in addition, the latency for the appearance of the first synaptically driven spike following stimulation of the contralateral side is almost twice as long as with comparable stimuli delivered to an ipsilateral pathway of identical length. The records of Fig. 12 were obtained from a preparation in which the stimulating cathodes on the incurrent siphonal nerves of either side were about 15 mm. distant from the ganglion. No antidromic response is present in this unit to obscure differences in latency. Since the additional distance through which afferent impulses initiated in the contralateral nerve would be required to travel must have been, at the most, 2 mm., or an increase of only

about 14%, the observed doubling in latency cannot be due to increased conduction time alone. Additional synapses in the afferent pathway would, of course, require more time; however, the same constancy in output pattern that is observed in response to ipsilateral excitation can also be seen when nerves on the opposite side are stimulated (Fig. 12$B_3$, $B_4$), and it is therefore tentatively concluded that contralateral excitation occurs via monosynaptic pathways involving afferent populations with medium to slow conduction velocities. Although postsynaptic excitation of contralateral neurons appears to have less temporal stability than pathways involving one side only, this is not necessarily incompatible with a proposed monosynaptic arrangement; Horridge (1958) observed that small fibres in *Mya* are temporally less stable than larger ones, and these findings have been supported by observations of a similar type in *Spisula* The temporal properties of any synaptic transfer may thus partially reflect those of the afferent fibres involved. In any case subthreshold postsynaptic excitation from the contralateral side can summate electrically with ipsilaterally evoked EPSP's to produce propagated impulses. Output to the siphonal muscles on the opposite side is therefore guaranteed, and though it lags by several milliseconds behind activity occurring in the ipsilateral pathways, the resulting asynchrony in the response on the two sides would almost certainly not be detectable by the unaided human observer.

## DISCUSSION

Observations of single postsynaptic units in the visceral ganglion of *Spisula* have confirmed and extended many of the findings obtained by Horridge (1958) in multi-fibred preparations of *Mya*. Efferent neurons in specified regions of the ganglion are excited through spatial summation by sensory neurons involving a broad receptive field; this seems to include the siphons and posterior areas of the mantle on the contralateral as well as the ipsilateral sides of the animal. EPSP's evoked by electrical stimulation of these pathways have a long-lasting time-course which probably results from temporal dispersion among arriving impulses. Pronounced temporal summation occurs even at stimulation frequencies of less than 10 per sec.; simultaneous repetitive discharges evoked by natural stimulation in parallel input pathways must therefore result in the prolongation of the EPSP's and provide the basis for the after discharges in postsynaptic fibres which were described by Horridge.

Although axons of this particular efferent population apparently do not branch to the contralateral side of the animal, the highly convergent input to these units can account for the symmetrical response in the muscles which they separately supply on the two sides. This arrangement is analogous to that which controls the movements of the parapodia in *Aplysia* (Hughes & Tauc, 1962). The function of the bilaterally branched axons, whose presence has been surmised in *Mya* and this preparation as a result of extracellular recording methods, is unknown. The high conduction velocity of such units places them in the same size category as the large unilateral efferent fibres; moreover, since the fastest afferent pathways appear to be distributed asymmetrically—to the ipsilateral efferent neurons alone—it seems unlikely that the branched axons are sensory. If they are efferent in nature,their somata must be located in other regions of the visceral ganglion, since antidromic activity has never been recorded by intracellular methods in response to contralateral stimuli.

*Spisula* evidently differs from *Mya* with respect to the peripheral control of the

siphon-withdrawal reflex. Horridge (1958) claims that symmetrical withdrawal occurs in response to tactile stimulation of the tentacles which ring the openings even after all nervous connections with the visceral ganglion have been cut. Responses of the isolated *Spisula* siphons to tactile stimuli are strictly local and by no means involve contralateral regions. Moreover, stimulation of the pallial nerve on one side results in contraction of the retractor muscle of that side only. Thus, there is no evidence for the occurrence in *Spisula* of the type of peripheral branching of sensory and motor fibres which must be postulated to account for the response of the isolated siphons in *Mya*.

No evidence was obtained which bears particularly on the problem of sustained nervous activity. Late synaptically evoked impulse discharges were observed on several occasions from cells in the pallial regions of the visceral ganglion; the simple monosynaptic pathways described above cannot be responsible, however, for the activity occurs long after volleys in even the slowest conducting pathways would have arrived at the ganglion. Whatever their origin, these late discharges are not nearly sufficiently prolonged to account for the nervous activity which controls closure. Since the present observations were confined to isolated visceral ganglia no conclusions can be drawn about the possible involvement of pathways within the cerebrovisceral connectives and the anterior regions of the central nervous system. The axons of efferent neurons in the pallial integrating centres have high conduction velocities and must therefore be relatively large. Pumphrey's (1938) findings in *Mya* indicate that, although fast efferent pathways are responsible for fast twitch-like activity in the adductor muscles, nerve traffic concerned with prolonged activity involves only small fibred pathways; an extension of this principle to involve the *Spisula* siphon retractor muscles must lead to the conclusion that pathways made up of small fibres, and, therefore, the closure response, are activated in regions of the ganglion other than those examined in the course of the present investigation.

## SUMMARY

1. Neural pathways controlling reflex withdrawal of the siphons have been examined in the surf clam. Conduction-velocity measurements, coupled with intracellular recordings of postsynaptic responses, indicate that the pathways involved are among the fastest in the relevant nerve trunks.

2. Cell bodies of neurons in the efferent limb of the reflex are located in specific paired regions of the visceral ganglion. Their axons are distributed to *ipsilateral* effectors via branches of the posterior pallial nerve and input to these cells is derived from a wide area of the periphery involving *both* sides of the animal. This pronounced sensory convergence can adequately account for symmetrical siphon withdrawal.

3. It is concluded from several lines of evidence that fast reflex pathways within the ganglion are monosynaptic in nature. In addition, the prolonged time course of the postsynaptic response to single volleys, and the capabilities of the synaptic contacts for spatial and temporal summation, make it possible to account for after discharges in the efferent pathways lasting a second or more. Prolonged activity from these cells has not been observed in isolated visceral ganglion preparations, and it is possible that the sustained closure is controlled by pathways involving different groups of neurons.

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