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A NEURAL CORRELATE OF BEHAVIOURAL STIMULUS INTENSITY DISCRIMINATION IN A MOLLUSC

BY DAVID J. PRIOR*

Department of Biology, University of Virginia, Charlottesville, Virginia 22903

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INTRODUCTION

Much of molluscan behaviour is stereotyped and therefore more amenable to analysis than the highly complex and variable behaviour of phylogenetically more advanced animals. Several types of molluscan behaviour have been studied, varying from sequential swimming and digging patterns (Dorsett, Willows & Hoyle, 1969; Mellon, 1969; Trueman, 1967) to simple withdrawal responses (Mellon, 1965; Peretz, 1970; Prior, 1970). In most cases tactile stimulation has been employed to elicit the behavioural responses under study. Tactile stimuli applied to the skin of a mollusc usually evoke muscular contractions near the site of stimulation. These responses can be mediated by local reflex pathways.

In their natural habitats molluscs receive a rather steady stream of weak tactile stimuli, due to debris such as stirred-up sand and floating weeds. The responses to such stimuli need be no more than siphon closure or local contractions. Thus, responses mediated by local reflexes constitute a fine level of behavioural activity capable of simple defensive manoeuvres such as siphon closure or gill withdrawal.

In molluscs sequential behavioural responses to tactile stimulation frequently involve activity mediated by both peripheral and central nervous (CNS) systems. To decide what portion of such a response is due to the CNS and what portion due to peripheral neural elements is often difficult. This difficulty must be dealt with because the failure to include local reflexes in an analysis of a behavioural sequence would constitute the deletion of a component of the neural organization involved. One method of approaching the problem is to first define and analyse the effects of local and central reflexes separately and then study their combined effects on the behaviour.

In the siphon closure and withdrawal responses of the surf clam, *Spisula*, the activity due to local reflexes can be isolated from the activity mediated by the CNS, thus presenting a good opportunity for studying the role of local reflexes in a behavioural sequence. Local reflexes are activated by an intensity of tactile stimulation lower than that necessary to evoke siphon retraction (which is centrally mediated). This simple behavioural discrimination between weak and medium stimulus intensities enables the animal to respond minimally to lesser stimuli and more defensively to threatening stimuli, thereby preventing activation of the complete process of siphon retraction in response to every tactile input. When the stimulus is strong (i.e. a jab

* Present address: Department of Biology, Princeton University, Princeton, New Jersey, 08540.

to the siphons with a stylus), the clam responds by adducting its shell valves. The behavioural sequence examined has three components: (1) Siphon closure and local contractions. (2) Siphon withdrawal. (3) Adduction of valves. These three components are elicited in a fixed order following increasing stimulus intensity. The present report describes neural correlates of the first two components and presents a possible mechanism for the observed stimulus-intensity discrimination.

METHODS AND MATERIAL

The specimens of *Spisula solidissima* used in this study were kept in circulating artificial sea water at 10–15 °C. All experiments were performed at 15 °C. Standard extracellular and intracellular recording techniques were used. Measurements of membrane electrical properties were made by passing pulses of current, using a bridge circuit, through the recording micro-electrode. One channel of an oscilloscope was used to monitor the current-pulse amplitude and duration while a second channel monitored the coincident voltage changes. Use of a single micro-electrode and a bridge circuit were necessary because of the small size of the cells studied (diameter 20–30 μm).

The major drawback in the use of a bridge circuit is the difficulty which is encountered in balancing the bridge properly so that the potential changes may be ascribed to current passing through the membrane resistance alone. Although several balancing methods are available, the following techniques were used in the present experiments (R. Llinás, personal communication). Following penetration of a cell and establishment of a steady resting potential, a pulse of current 0·7 to 1·0 msec, 0·1 to 0·3 nA was passed through the recording micro-electrode. Within this brief time span, the current pulse does not charge the membrane capacitance significantly. A resultant voltage drop is indicative of bridge imbalance. In this situation, the bridge was adjusted until no voltage drop was observed between the capacitative charging artifacts. When no voltage drop is observed the electrode resistance is counterbalanced by the bridge resistance, and any subsequent voltage drop observed during a current pulse is due to current passage through the membrane resistance.

To facilitate balancing the bridge, the capacitative charging artifacts ought to be kept as short as possible. The following procedures were employed to minimize the shunt capacitance (which contributes to the charging time) of the electrode and electrode holder: (1) The outer surface of the electrode shaft was dried. (2) Only a small length of the tip of the micro-electrode was submerged below the surface of the saline bathing the preparation. (3) Only the shank of the microelectrode was filled with KCl solution. Contact between the KCl solution in the electrode and the amplifier was made with a chlorided silver wire.

During the measurements made on each cell the current pulse direction was repeatedly reversed to avoid polarization of the electrode tip, and the bridge was rechecked several times for proper balance.

As a check on the balancing method, amplitudes of directly evoked action potentials (by passing depolarizing current through the micro-electrode) and antidromically or synaptically evoked action potentials were compared. Assuming the electrode to be close to the synaptic region, the two methods ought to evoke action potentials of the

same amplitude. A detailed discussion of the bases for this assumption will be presented with the results. Measurements were made only when the comparison of spike heights indicated the bridge was balanced.

RESULTS

In its natural situation *Spisula* is found buried in the substrate with only its siphons protruding. Touching one tentacle of the incurrent siphon with a fine glass probe evokes reflex closure of the excurrent siphon or occasionally both siphons. If a similar weak tactile stimulus is applied to the siphon wall or to the mantle, there occurs at the site of stimulation a localized muscular contraction coupled with closure of the siphons. The siphon wall musculature is composed of both longitudinal and circular muscle layers, whose contractions give rise respectively to shortening or dimpling of the wall and closure of the siphon apertures. It has been shown that these responses are mediated by local reflexes (Prior, 1972). It is interesting that siphon closure can be evoked by stimulation of areas other than the siphon aperatures. This is indicative of a peripheral neural organization beyond that of mere restricted local reflexes. Touching the siphons more firmly causes not only local reflex activity but also retraction of the siphons into the mantle cavity. This action is effected by the paired siphon retractor muscles. These muscles, which adhere to the inner surfaces of the valves, are composed of parallel bundles of muscle fibres oriented so that their contraction results in withdrawal of the siphons into the mantle cavity. The retraction process (which is centrally mediated) is graded, increasing with increased stimulation of the siphons. A strong jab to the out-stretched siphons results in local contractions, siphon retraction and finally, adduction of the shell valves. The motoneurones involved in adduction have been described elsewhere (Mellon, 1967; Mellon & Prior, 1970).

To study the muscle activity further, electromyograms (EMGs) were recorded from both the siphon retractors and siphon wall musculature during tactile stimulation of the siphons. With the visceral ganglion intact, the retractor muscles respond with co-ordinated bursts of potentials superimposed on smaller more diffuse activity. The frequency of bursts increases with more widespread stimulation of the siphon margins. In contrast, electrical responses of the siphon wall musculature are smaller and more dispersed. Although the recorded responses of the wall musculature were little affected by removal of the visceral ganglion, the responses of the retractor muscles were considerably altered. Removal of the visceral ganglion abolishes the co-ordinated bursts of potentials and the concomitant increases in muscle tension that are recorded from the retractor muscles with the CNS intact (Fig. 1). The diffuse potentials recorded from both the wall musculature and retractor muscles are mediated by peripheral neural elements. The effects of local reflexes are therefore distinguishable from those mediated by the CNS. In these experiments it has been possible to separate the ressponses due to local reflexes from those centrally mediated. The results show that centrally mediated muscle responses can be superimposed on those due to local reflexes.

A population of neurones located in the pallial lobes of the visceral ganglion of *Spisula* have been implicated in the siphon withdrawal reflex (Mellon, 1965). Fig. 2 shows intracellular records from pallial neurones (PN) in response to electrical and tactile stimulation. These ganglion cells receive convergent tactile sensory input from most regions of the siphons and mantle. Their axons are found in the ipsilateral

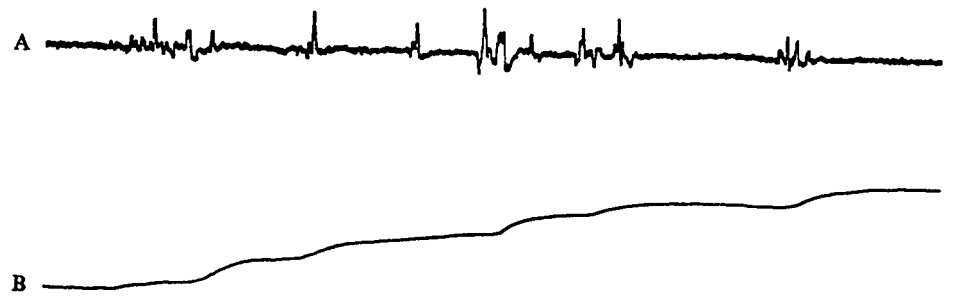

Fig. 1. Simultaneous recordings of electrical activity (A) and tension (B) generated by the retractor muscle in response to tactile stimulation of the siphons. There is an increase in muscle tension concomitant with each burst of electrical activity in the muscle. Following removal of the visceral ganglion, no increases in tension or co-ordinated bursts of electrical activity have been recorded from the retractor muscles. Time calibration: 250 msec.

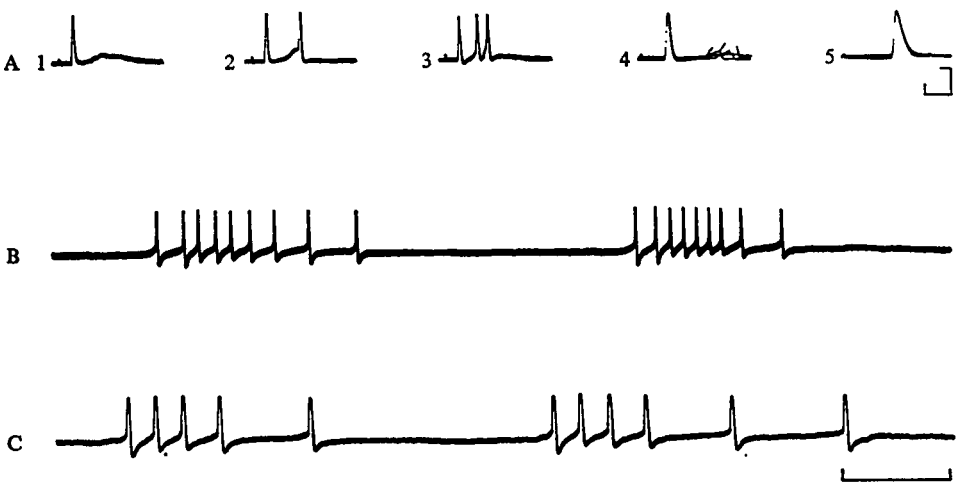

Fig. 2. Intracellular records illustrating the responses of pallial neurones to electrical stimulation of a nerve branch and tactile stimulation of the siphons. The responses to progressively greater electrical stimulation of a nerve branch (containing the pallial neurone axon) are seen in A_1–A_3. Antidromic activation of the soma is followed by synaptic activity that in A_3 is strong enough to generate two action potentials. Repetitive stimulation (10/sec in A_4 is 25/sec in A_5) shows the constant latency of the antidromic spike and lability of the synaptically mediated spike (A_4). Upper calibration mark: 30 mV/20 msec for A_1, A_2, A_3; 30 mV/10 msec for A_4; 30 mV/5 msec for A_5. Tactile stimulation of the siphons evokes from pallial neurones bursts of synaptically mediated action potentials (B, C). Note that each action potential is preceded by a slowly rising depolarization. Lower time calibration: 400 msec for B; 200 msec for C.

pallial nerves. These neurones, which are efferents to the retractor muscles, respond to tactile stimulation of the siphons with bursts of synaptically mediated action potentials (Fig. 2B, C). The results of the following experiments indicate close coupling between these pallial neurones and retractor muscle activity. It can be hypothesized that pallial neurones are motoneurones to the retractor muscles.

EMGs were recorded from retractor muscles while recording intracellularly from pallial neurones; the responses to tactile stimulation of the siphons are seen in

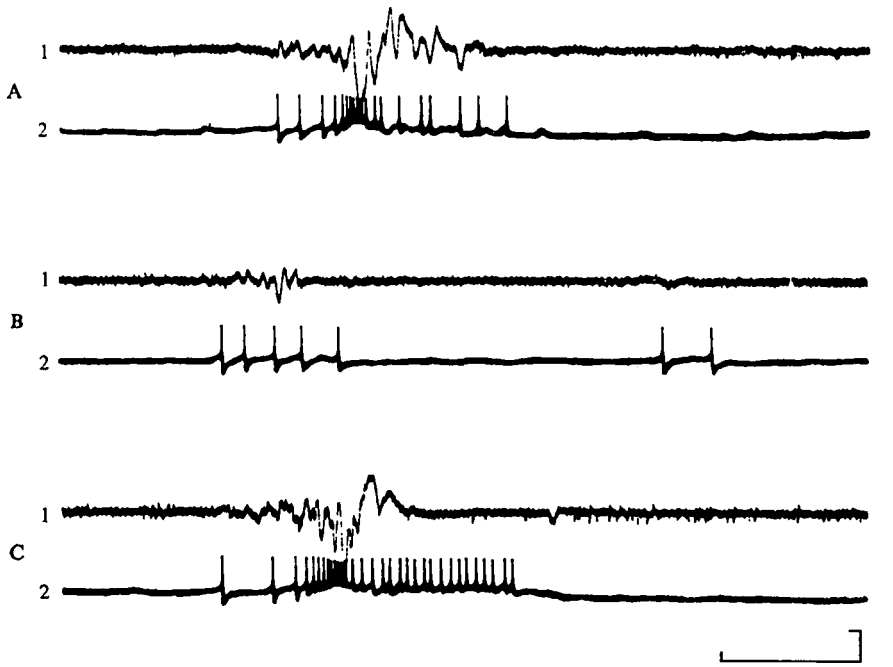

Fig. 3. A, B and C are continuous simultaneous recordings, intracellular from a pallial neurone and electromyographic from the ipsilateral retractor muscle in response to tactile stimulation of the siphon. In A, B and C the upper trace is the EMG and the lower the intracellular record. The large co-ordinated bursts of retractor muscle activity correspond with high frequency bursts of pallial neuron action potentials. The smaller electrical responses recorded from the retractor muscle in B correspond with the generation of small (two to five spikes), low-frequency bursts of action potentials by the pallial neurone. Calibration: 250 msec, 35 mV.

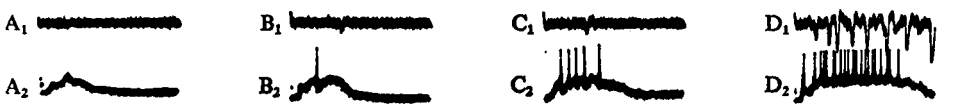

Fig. 4. Simultaneous recordings of the electrical activity of the retractor muscle (EMG) and a pallial neurone (intracellularly recorded) in response to electrical stimulation of the contralateral pallial nerve trunk. The upper trace in each set of data is the EMG. A to D inclusive shows the responses to increasingly higher stimulus intensity. At an intensity sufficient only to generate an EPSP in the pallial neurone (A_2) no electrical activity is recorded from the muscle (A_1). As the stimulus intensity is increased (B–D) the increase in number of action potentials generated in the pallial neurone (B_2, C_2, D_2) corresponds with an increase in EMG activity; in B_1 and C_1, an increase in amplitude of a recorded potential and in D_1 an increase in number of potentials are recorded.

Fig. 3. The bursts of activity from the retractor muscle which correspond to contractions, follow closely the bursts of action potentials elicited from the pallial neurone. Fig. 4 shows the responses of the retractor muscle and a pallial neurone to electrical stimulation of the contralateral pallial nerve (PN axons leave the ganglion in the ipsilateral pallia nerve). The number of synaptically evoked action potentials recorded from the pallial neurone corresponds with the intensity of retractor muscle activity. This evidence, although circumstantial, is indicative of the pallial neurones being

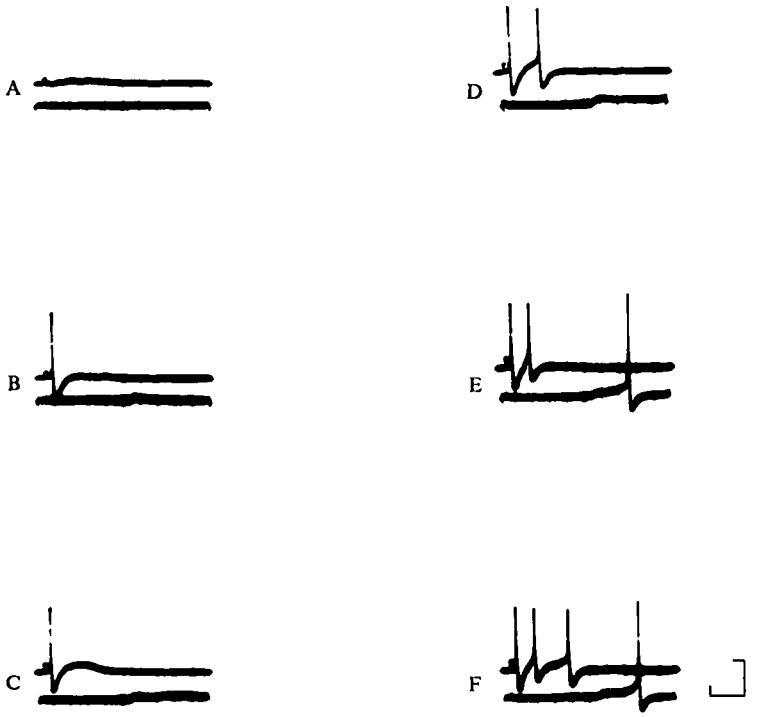

Fig. 5. Simultaneous intracellular records obtained from cluster cell-pallial neurone pairs in response to stimulation of an afferent pathway. A fine nerve-branch perpheral to the cluster cells was electrically stimulated. In A to F inclusive, the upper trace is the recording from the cluster cell (CC) and the lower is from the pallial neurone (PN). A to F inclusive are recordings of the activity in response to progressively increasing stimulus intensities. At very low stimulus intensity (A) only a small EPSP in the CC is observed. With increased stimulus intensity (B and C) an antidromic spike along with increased amplitude EPSPs are evoked from the CC. At this stimulus intensity only EPSPs are recorded from the pallial neuron. In D a synaptically mediated action potential is evoked from the cluster cell at a stimulus intensity great enough to elicit only an EPSP from the pallial neurone. At greater stimulus intensities (E and F) there was evoked from the pallial neurone a synaptically mediated action potential and from the cluster cell increased synaptic activity (characteristic latency shift of first synaptically mediated spike and generation of a second). Calibration: 20 mV/40 msec.

motoneurones inervating the retractor muscles. As shown in the preceding paper, peripherally located cluster cells (CC) are efferents to the siphon wall musculature and are presumably involved in local reflexes.

The relative accessibility of these two distinct neurone populations provides an opportunity to study the possible neural basis of the first two components of the behavioural sequence described above. The former of the two components, local reflex activity, is evoked at stimulus intensities lower than those necessary to elicit siphon withdrawal. A neural correlate of this stimulus-intensity discrimination was obtained by simultaneous intracellular recordings from cluster cell-pallial neurone pairs. The responses of such a pair to electrical stimulation of a fine nerve branch peripheral to the cluster cells are shown in Fig. 5. At low stimulus intensity (Fig. 5B–C) an EPSP was generated along with antidromic activation of the cluster cell (the axon of the CC was in the nerve branch stimulated). At a sufficient stimulus intensity (Fig. 5D) a synaptically mediated action potential is evoked from the cluster cell,

whereas only an EPSP is generated in the pallial neurone. A latency shift of the synaptically evoked cluster-cell action potential occurred as stimulus intensity was increased (Fig. 5E). Finally, in response to a strong enough stimulus (Fig. 5E, D) an action potential was also evoked from the pallial neurone. With but one exception, in over thirty cluster cell-pallial neurone pairs from which records have been obtained, lower stimulus intensities have been required to generate an action potential in the cluster cells than in pallial neurones. These findings provide a correlate of the behavioural observations that peripherally mediated local reflex activity is evoked at a lower stimulus intensity than that required to cause centrally mediated siphon retraction. To further investigate their responses, simultaneous intracellular records were obtained from cluster cell-pallial neurone pairs during tactile stimulation of the siphons. The stimulation elicited from both cell types characteristic bursts of synaptically mediated action potentials, but no observable direct correlation (i.e. one-for-one) was found between the firing patterns of the cells.

Several possible mechanisms for the differential sensitivity of cluster cells and pallial neurones have been tested and some eliminated. It has recently been shown that the order of recruitment of motoneurones in the lobster swimmeret system is correlated with the sizes of the neurone somata (Davis, 1971). This sort of correlation does not apply in the present case. The mean soma diameter of cluster cells ($n = 50$) is 26·5 μm and of pallial neurones ($n = 50$) is 25·0 μm. These measurements were made on 1·0 μm thick sections of epon-embedded tissue. The largest diameter of each cell (sectioned through the nucleus which is centrally located) was measured.

The architectures of the two cell types have been studied with conventional light microscopical, Nomarski interference-contrast and electron-microscopical techniques and have been found to be very similar. Both cell types are monopolar (without infolding of the soma membrane) each with a relatively large axon, the diameter being occasionally half the diameter of the soma. Axo-somatic synapses (as indicated by the presence of synaptic vesicles and membrane specialization) have been observed on the somata of both cluster cells (Prior & B. Lipton, unpublished observations) and pallial neurones (J. Ross, personal communication). Thus the morphology of the cells provides no distinct differences that could account for the differences in sensitivity. Synaptic efficacies and total number of synapses per cell have not, however, been determined and therefore must remain as possible differences between the two cell types.

The visceral ganglion, containing pallila neurones, is at least 2·0 cm further central than the nerve junctions containing cluster cells. The tactile afferents (which synapse on CCs and PNs) in the pallial nerve trunks have various conduction velocities, ranging from 65 to 164 cm/sec (Mellon & Mpitsos, 1967). The possibility of temporal dispersion of input volleys accounting for lower-amplitude PSPs in pallial neurones was tested by comparing PSPs resulting from separate stimulation of the pallial nerve trunk at two sites 3·0 cm apart. No consistent difference in pallila neurone PSP amplitudes was recorded, thus eliminating temporal dispersion as a possible explanation.

Another possible basis for the differential sensitivities is differences in membrane properties of the two cell types. The electrical properties of cluster cells and pallial neurones were determined by passing pulses of current through the cell membranes

Table 1. *Membrane electrical properties of cluster cells*

Cell	Measured Eth (mV)	I Rheob (nA)	Rinput (MΩ)	Calculated Eth (mV)
1	8·0	0·113	59·1	6·7
2	7·0	0·113	50·9	5·8
3	10·0	0·250	48·8	12·2
4	4·0	0·100	36·0	3·6
5	5·0	0·110	61·7	6·3
6	6·0	0·175	32·5	5·7
7	6·6	0·212	38·5	8·2
8	5·9	0·100	56·4	5·6
9	6·0	0·200	83·6	16·7
10	6·0	0·125	75·5	9·4
11	5·9	0·063	74·1	4·7
12	5·5	0·112	57·3	6·4
13	10·0	0·100	60·6	6·1
14	5·0	0·100	32·7	3·3
15	5·0	0·060	62·1	3·7
16	8·0	0·080	92·7	7·4
17	10·0	0·260	28·1	7·3
18	5·0	0·095	64·7	6·1
19	6·0	0·080	63·6	5·1
20	5·0	0·080	56·0	4·5
21	5·0	0·140	28·0	3·9
22	6·0	0·062	72·2	4·5
23	6·0	0·175	30·7	5·4
24	7·0	0·070	66·5	4·7
25	6·0	0·063	158·0	9·9
26	5·0	0·112	95·0	10·6
27	5·0	0·100	39·8	4·0
28	5·0	0·115	27·4	3·2

using single micro-electrodes with a bridge circuit (see methods and materials). The hyperpolarizing current pulses used for the measurements were less than 1·0 nA, a range in which neither neurone type shows any indication of rectification (Graph 1).

The results of these measurements (Tables 1, 2, 3) show that cluster cells have a lower spiking threshold (Eth) than the pallial neurones. Furthermore, the amount of current (passed through the recording electrode) required to directly evoke an action potential (Irheob) was found to be less for cluster cells than for pallial neurones. This is due, at least in part, to the higher input resistance of the cluster cell. It is concluded from these data (Table 3) that the 'critical firing level' of cluster cells is lower than that of pallial neurones.

A check on the reliability of the measurements was made by calculating Eth for each of the cells (Rin X Irheob). As seen in Tables 1 and 2, the values of calculated Eth and measured Eth for most cells are similar. However, there are some exceptions where values for Irheob greatly differ from the mode, resulting in discrepancies between calculated and measured Eth (Table 1, cells 9, 26; Table 2, cells 1, 13, 26). Due to the non-normal distribution of Irheob values, medians of the sets of data have been presented rather than mean values (which are applicable to normal distributions).

Valid measurements of potential changes can be made with a single micro-electrode and bridge circuit only if the bridge is accurately balanced. The balancing technique described in the methods section was checked by comparing the amplitudes of action potentials generated antidromically or orthodromically with those directly

Table 2. *Membrane electrical properties of pallial neurones*

Cell	Measured Eth (mV)	I Rhoeb (nA)	Rinput (MΩ)	Calculated Eth (mV)
1	11·0	0·312	57·0	17·8
2	7·0	0·100	53·7	5·4
3	8·0	0·175	51·0	8·9
4	8·0	0·340	24·7	8·4
5	14·0	0·600	23·0	13·8
6	10·0	0·163	46·5	7·6
7	10·0	0·125	98·0	12·3
8	9·0	0·063	130·5	6·5
9	10·0	0·125	92·0	11·5
10	15·0	0·312	61·0	19·0
11	10·0	0·500	22·2	11·1
12	10·0	0·175	63·4	11·0
13	9·0	1·012	25·5	25·8
14	10·0	0·150	73·0	11·0
15	9·0	0·250	31·2	7·8
16	9·0	0·500	23·3	11·6
17	9·0	0·225	37·6	8·5
18	10·0	0·313	44·7	13·9
19	8·0	0·325	22·3	7·2
20	6·0	0·150	27·0	5·5
21	7·0	0·240	21·8	5·2
22	10·0	0·387	25·2	9·8
23	9·0	0·212	33·3	7·0
24	10·0	0·637	23·6	15·0
25	11·0	0·362	32·9	11·9
26	12·0	0·512	106·5	54·5
27	12·0	0·262	40·2	10·5
28	12·0	0·250	29·1	7·3

Table 3. *Summary of membrane electrical properties of cluster cells and pallial neurones (from data in Tables 1 and 2)*

	Rm (Ω.cm^2)	Mean soma diameter (μ)	Median Rin (MΩ)	Median measured Eth (mV)	Median I rheob (nA)
Cluster cells (n = 28)	1530	25·0	58·2 (50·5–62·4)	6·0 (5·4–6·0)	0·105 (0·100–0·113)
Pallial neurones (n = 28)	1030	26·5	35·4 (28·7–47·4)	10·0 (9·0–10·0)	0·256 (0·222–0·315)

evoked by passing depolarizing current through the micro-electrode. Action potentials activated synaptically or directly ought to be the same amplitude if the bridge is properly balanced. Several lines of evidence indicate this to be the case when recording intrasomatically from pallial neurones or cluster cells.

With the bridge properly balanced (Fig. 6A_1) the amplitudes of synaptically (first spike in A_1) and directly evoked (second spike in A_1) action potentials are identical. Closer examination of the rising phases of synaptically (Fig. 6A_2) and directly evoked (Fig. 6A_3) action potentials from the same neurone reveals identical spike threshold values (11·0 mV.). Only the recording electrode being close to the synaptic region can account for this observation. Further indications of the contiguity of the intrasomatic

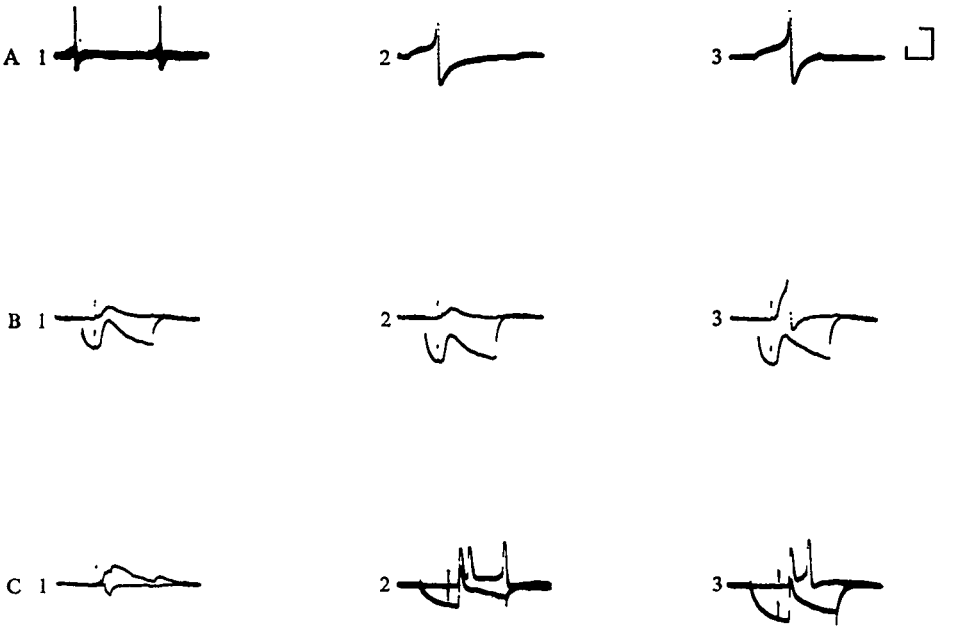

Fig. 6. Intrasomatic responses from cluster cells and pallial neurones indicative of the recording electrode being near the region of synaptic contacts. In all of the following illustrations electrical stimulation of a nerve branch was used to elicit synaptic activity. When the bridge circuit is balanced a synaptically evoked action potential (first spike in A_1) has the same amplitude as an action potential evoked by passage of current through the electrode (second spike in A_1). The action potentials evoked by these two methods have identical spike thresholds. The spike threshold value for both A_2 (synaptically evoked) and A_3 (directly evoked) is 11·0 mV.
EPSP amplitude can be enhanced ($B_{1,2}$) and action potentials suppressed (B_3) by passing a pulse of hyperpolarizing current into the neurone soma. A conductance change occurs during an EPSP as shown by reduced amplitude of a test pulse of current during the EPSP (C_1). Electrical stimulation of a nerve branch containing the axon of a cluster cell as well as afferents generated an antidromic action potential and synaptically mediated action potentials. Hyperpolarization of the soma suppressed the synaptically mediated action potentials but not the antidromically evoked action potential ($C_{2,3}$). Calibration: $A_{2,3}$ are 10 mV/40 msec; $B_{1,2,3}$ and C_1 are 5 mV/40 msec; $C_{2,3}$ are 20 mV/40 msec.

recording site and synaptic region (in CCs and PNs) are (i) recording a membraned conductance change during the EPSP (Fig. 6C_1), (ii) enhancement of EPSP amplitude (Fig. 6$B_{1,2}$) and (iii) suppression of synaptically evoked action potentials (Fig. 6B_3) by hyperpolarization of the cell.

Hyperpolarization of the neurone soma does not affect the capability of the soma membrane to conduct an action potential. This was shown by electrically stimulating a nerve trunk containing a cluster cell axon and afferents to the cluster cell during passage of a hyperpolarizing pulse of current through the micro-electrode (Fig. 6C_2, C_3). The antidromic activation of the soma is not impeded whereas generation of the synaptically mediated action potentials is blocked.

In order to arrive at values for specific membrane resistance (Rm), an estimate of the area of the neurone soma and initial segment was made by assuming the cell to be a sphere with an attached cylinder. The mean Rms of cluster cells and pallial neurones are given in table 3.

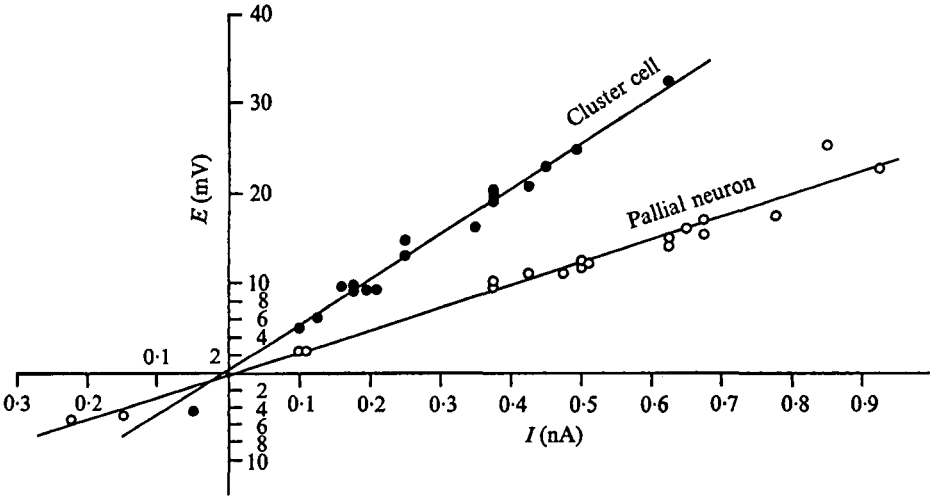

Fig. 7. The current-voltage relationships of a cluster cell and a pallial neurone are plotted. The data were obtained by passing pulses of current through a recording micro-electrode, via a bridge circuit, and measuring the amount of current passed and the voltage change. The slopes (R input) of the lines are linear up to at least hyperpolarizing current pulse of 1·0 nA. In response to depolarizing current there is variation in the slope due to conductance changes in the membranes as spike threshold is approached. The regression lines were plotted by the method of least squares. The correlation coefficient for the cluster cell data is 0·992 and for the pallial neurone data, 0·974.

DISCUSSION

The behavioural and electromyographic observations presented above describe a three-component response to tactile stimulation of the siphons. The three components (local siphonal contractions, siphon withdrawal and valve adduction) occur in a fixed sequence according to stimulus intensity. The ability to discriminate between weak stimuli and medium-intensity stimuli is useful to the animal in that weak tactile stimuli which usually represent little or no danger, can be essentially 'ignored' by the CNS. In contrast, more threatening (i.e. stronger) stimuli cause the siphons to be withdrawn for protection, a process mediated by the CNS. By means of this discrimination the animal can avoid frequent unnecessary interruptions of the normal flow of water through the mantle cavity that would necessarily occur were the siphons withdrawn in response to every slight stimulus. The third component of the sequence, adduction, which is the most efficient defensive manoeuvre, occurs in response to serious threats, such as strong tactile stimulation applied by a predator.

Type I ganglion cells (Mellon & Prior, 1970) which mediate valve adduction receive a combination of inhibitory and excitatory synaptic input. The response of type I cells to weak stimuli (tactile or electrical) is a long (several seconds) hyperpolarization due to the inhibitory synaptic input. In contrast, strong stimulation elicits sufficient excitatory synaptic input to overcome the inhibition and generate action potentials in the type I cells, thereby activating the posterior adductor muscle. Therefore, by means of a combination of excitatory and inhibitory inputs this cell type is 'wired' to generate action potentials only in response to vigorous stimulation.

The present experiments describe a neural correlate for the first two components of the response sequence involving two populations of efferent neurones (CCs, PNs), each mediating one of the responses.

Several alternate explanations for the differential sensitivity have been examined. It has been found that temporal dispersion of afferent volleys does not cause significant reductions in PSP amplitudes of pallial neurones, hence is not responsible for the differential sensitivity. Other than the identification of axo-somatic synapses on the somata of both cell types, no data were obtained regarding the synaptic arrangements (i.e. number of synapses or areas of their concentrations). Although variations of these factors would undoubtedly affect neurone sensitivities, no conclusive evidence is currently available.

When recording responses of cluster cell-pallial neurone pairs to electrical stimulation of an afferent pathway, possible effects of differences in numbers of afferent fibres were minimized by selecting for stimulation the finest peripheral nerve branch possible.

The data from the analysis of membrane properties (Rin, Irheob, Eth) of the two cell types supports the hypothesis that differences in these parameters could account for the differential sensitivities. Initially it was established that the cluster cells have a lower spike threshold (Eth) than the pallial neurones (Table 3). It was further shown that less current (passed through the recording electrode) is required to directly generate an action potential in CCs than in PNs (Irheob in Table 3). Before considering these data, the following alternative had to be eliminated.

If the resting membrane potentials (Em) of the two cell types were different, they could still have identical absolute threshold potentials and yet, due to measuring Eth from the resting potential (taken as zero), appear to have different threshold potentials. It was possible that this was the cause of the observed differences between Eth of CCs and PNs. But as seen in Table 3, the mean resting membrane potentials of cluster cells and pallial neurons are not significantly different, thereby eliminating this as a possibility. Therefore it can be concluded that cluster cells do indeed have a lower 'critical firing level' that could account for their greater sensitivity to synaptic input.

Although Eth differences alone might account for the observation that cluster cells are more sensitive than pallial neurons, there is still the difference between Irheob values to be considered.

Measurements indicate that CCs have a significantly (95% confidence level) higher input resistance than PNs (Table 3). This difference is also seen by comparing the slopes (Rin) of the two current-voltage curves on Graph 1. With a linear relationship such as this, the current required for a potential change (i.e. Eth) is inversely proportional to input resistance. The intrinsically lower threshold potential coupled with the higher input resistance (resulting in lower Irheob) of cluster cells provides a basis for the order of recruitment of CCs and PNs in response to synaptic input.

The following data indicate that the measurements made from CC and PN somata accurately reflect the properties of the synaptic regions. Several characteristics of a synaptic region have been demonstrated while recording intrasomatically: conductance change during the EPSP, spike suppression and EPSP enhancement by hyperpolarization of the soma membrane. These data along with the electronmicroscopic observations of axo-somatic synapses on somata of both cell types indicate that intrasomatic electrodes are close to the synaptic region.

Simultaneous intracellular recordings were made from pairs of touch-sensitive neurones (TSN; see preceding paper) and CCs or PNs. These experiments were un-

Table 4. *Summary of neurone types and muscles involved in the three-component behavioural sequence*

Stimulus intensity	Response	Muscles	Neurone types
Weak	Local reflexes	Siphon wall Musculature	Cluster cells
Medium	Local reflexes	Siphon wall	Cluster cells
	Siphon retraction	Siphon retractors	Pallial neurones
Strong	Local reflexes	Siphon wall	Cluster cells
	Siphon retraction	Siphon retractors	Pallial neurones
	Valve adduction	Adductors	Type I ganglion cells

dertaken to analyse the effects of input from a single sensory cell on the efferent neurones. No interaction has been observed between the pairs in response to applied tactile stimuli or direct activation (by passing current through the electrode) of either member of a pair (15 CC-TSN pairs, 6 PN-TSN pairs). Several possible interpretations of similar negative results were discussed in the preceding paper. Attempts to eliminate any of the alternative explanations have met with little success, leaving the lack of obvious interaction an open question.

The following represents a working hypothesis of the neural bases of the three-component response of *Spisula* to tactile stimulation of the siphons (Table 4).

Weak tactile stimulation (i.e. touching a single siphonal tentacle with a glass stylus), sufficient to activate cluster cells but not pallial neurones, results in local contractions of the siphon at the site of stimulation and closure of the siphon apertures. Medium-intensity stimulation (i.e. stroking the siphons with stylus) results in siphon withdrawal due to activation of pallial neurones as well as local contractions. The increased level of stimulation elicits bursts of action potentials from pallial neurones and concomitant bursts of retractor muscle contractions. When a strong tactile stimulus (i.e. a jab with the stylus) is applied to the siphons, there occur all three components of the sequence; local contractions, siphon withdrawal and adduction of the valves. Adduction involves contraction of the posterior adductor muscle which is mediated by type I ganglion cells.

The present results indicate that the process of discriminating between weak and medium stimulus intensities is mediated by the cluster cells and pallial neurones directly rather than involving 'decision making' interneurones. Therefore the behaviourally observed stimulus-intensity discrimination can be explained on the basis of differences between the cluster cells and pallial neurons in the basic electrical properties of their membranes.

SUMMARY

1. A behavioural sequence of *Spisula* is described that involves local reflexes, siphon retraction and valve adduction. The response to low-intensity tactile stimulation is locally mediated reflex activity of the siphon wall musculature. In response to medium-intensity stimulation, the siphon retractor muscles are activated along with local reflex activity.

2. A neural correlate of this behavioural discrimination between low and medium stimulus intensities was obtained by simultaneous intracellular recordings from pairs

of efferent neurones, one neurone type involved in local reflexes and one efferent to the siphon retractor muscles. The peripherally located neurones of the siphon wall musculature (cluster cells) were found to have a lower spike threshold than the efferents to the siphon retractor muscles (pallial neurones).

3. Several lines of evidence are presented that support the assumption that intrasomatic recordings from these neurones accurately reflect the activity of the synaptic region.

4. The membrane electrical properties (i.e. input resistance, rheobasic current, threshold potential) of the two cell types were measured by passing current through single intrasomatic micro-electrodes. From these measurements it was found that the 'critical firing level' of the cluster cells is significantly lower than that of the pallial neurones. By way of their differential sensitivities to synaptic input, the stimulus-intensity discrimination could be mediated by cluster cells and pallial neurones directly.

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