J. comp. Physiol. 110, 271–286 (1976)

Journal of Comparative Physiology · A

Afferent Neurons Mediating Escape Swimming of the Marine Mollusc, *Tritonia*

Peter A. Getting

Department of Biological Sciences, Stanford University, Stanford, California 94305, USA

Received May 24, 1976

Summary. Intracellular recordings were made from afferent neurons with central cell bodies (S-cells) mediating reflexive withdrawals and escape swimming of the mollusc, *Tritonia diomedia*. Approximately 100 S-cells are located just under the somata of the trigger group neurons (TGN) in each pleural ganglion. S-cells are normally silent but respond phasically to tactile stimuli and tonically to noxious stimuli (NaCl solution or contact with the tube feet of *Pycnopodia*). Directly driven S-cell activity results in reflexive withdrawals and escape swimming; the initiation of swimming requires both a higher frequency and number of S-cells. Further evidence is provided that the group of cells known as the trigger group neurons (TGN) may be neither necessary nor sufficient for the initiation of escape swimming, but that S-cell activity is the first causal step in the trigger process.

Introduction

Escape swimming of several marine molluscs in the genus *Tritonia* is a fixed action pattern characterized by a general reflexive withdrawal of all body extremities followed by a series of alternating dorsal and ventral flexions (Willows and Hoyle, 1967; Willows, 1973; Willows and Dorsett, 1975). The "sign stimulus" for the release of escape swimming is epithelial contact with the tube feet of certain voracious sea stars (Mauzey et al., 1968) (*Pycnopodia helianthoides* is one of the most effective releasors), or in the laboratory, contact with salt crystals. A whole animal preparation has been developed which allows multiple intracellular recording from central neurons during swimming behavior (Willows and Hoyle, 1967; Willows, Dorsett and Hoyle, 1973a). The dorsal and ventral flexions of swimming are apparently driven by alternating bursts of activity in antagonistic motor units in the pedal ganglia. This patterned activity arises centrally and does not require sensory feedback (Dorsett et al., 1969, 1973).

The initiation of this central program has been attributed to the coordinated activity in a population of electrically coupled neurons called trigger group

neurons (TGN) located symmetrically on the dorsal surface of each pleural ganglion (Willows and Hoyle, 1969; Willows, Dorsett and Hoyle, 1973b; Willows and Dorsett, 1975). Recent evidence, however, suggests that TGN activity may be neither necessary nor sufficient for the initiation of escape swimming (Getting, 1975). In this paper, a population of afferent neurons with central cell bodies is described. The afferents (termed S-cells) respond phasically to tactile stimuli and tonically to noxious chemical stimuli (epithelial contact with salt crystals or with the tube feet of *Pycnopodia*). Directly driven activity in a minimum of 5 S-cells is required to initiate escape swimming. Further evidence is provided that S-cell rather than TGN activity is the initial step in triggering escape swimming.

Materials and Methods

Tritonia diomedia were obtained from Pacific Biomarine Supply, Venice, California, and maintained in a recirculating sea-water system at 13°C. All experiments were performed at 12–14°C in sea water.

Conventional intracellular recording techniques were employed with either 3 M KCl or 4 M KAc filled glass microelectrodes of resistances 10–60 megohms. Direct intracellular stimulation was accomplished by current injection through the recording electrode with appropriate bridge compensation. The whole animal and isolated brain preparations have been described elsewhere (Willows, Dorsett and Hoyle, 1973a). Responses were recorded on a 4-channel chart recorder (Brush Model 440).

The CNS of *Tritonia* is encased in a tough transparent sheath. Penetration of neurons with microelectrodes has therefore been limited mainly to the larger (>50 μm) cells. Desheathing of the CNS was initially performed by soaking the ganglia in 0.5% trypsin at room temperature for 10–20 min. The sheath was broken and carefully lifted free of the underlying neurons and glia. Due to the possible degradative effects of the trypsin treatment, more recent preparations were desheathed without the trypsin pretreatment. The only observed effect of the trypsin treatment was to decrease the firing rate of flexion neurons during escape swimming and was therefore discontinued. All experiments employing the trypsin treatment are so identified.

The newly identified afferent neurons (S-cells; Getting, 1975) are located just under the TGN populations on the dorsal surface of each pleural ganglion. In order to expose the S-cells the TGN cell bodies were carefully stripped off by suction applied to a small glass capillary.

Results

Failure of TGN Activity to Initiate Swimming

Previous studies on *Tritonia diomedia* and *Tritonia hombergi* suggest that the coordinated activity of the TGN's initiates escape swimming in both species (Willows and Hoyle, 1969; Willows, Dorsett and Hoyle, 1973b; Dorsett and Willows, 1975). For *Tritonia diomedia* this conclusion is based on the following observations: i) in response to natural stimuli or electrical stimulation of peripheral nerves TGN activity always precedes escape swimming; ii) stimulation of TGN activity via current pulses applied through a large suction electrode placed over the TGN cluster elicits swimming whereas similar stimulation in other regions of the CNS does not; iii) TGN's receive synaptic input from nearly all peripheral nerves and swimming can be elicited by natural stimuli

anywhere on the epithelium; and iv) rarely, swimming can be elicited by direct stimulation of a single TGN via an intracellular electrode (Willows and Hoyle, 1969; Willows, Dorsett and Hoyle, 1973b). Although this evidence establishes a correlation between TGN activity and initiation of swimming, only the last item indicates a causal link. Preliminary experiments performed on *Tritonia diomedia* from southern California suggested that TGN activity may be neither necessary nor sufficient for the initiation of swimming (Getting, 1975). Further experiments are reported here to substantiate that the TGN's may not be involved.

Two experiments were performed to test directly whether TGN activity is necessary and/or sufficient for escape swimming. These experiments rely on the fact that each TGN is electrically coupled via non-rectifying junctions to all other TGN's. Current injected directly into one neuron will therefore affect the activity of the population as a whole (Willows and Hoyle, 1969; Getting, 1974; Getting and Willows, 1974). To test whether TGN activity is necessary, four TGN's (two per pleural ganglion) were penetrated and large hyperpolarizing currents were passed to prevent spike activity throughout the network. As a monitor of general TGN activity, a fifth TGN was penetrated but received no direct current stimulation (Getting, 1975). Getting and Willows (1974) have shown that spike activity in a monitor TGN not receiving direct current stimulation represents mostly synchronous activity throughout the TGN network. Swim activity in response to electrical stimulation of cerebral nerve 2 (CN-2) was monitored by a sixth microelectrode in a pedal flexion neuron. Behavioral responses can not be observed with the isolated brain preparation but Dorsett et al. (1969, 1973) have shown that the isolated brain can generate burst activity in pedal flexion neurons (FN) which is similar to that during escape swimming of the whole animal. The results for one representative case of five isolated brain experiments is shown in Figure 1A and B. Figure 1A shows the response of the monitor TGN and flexion neuron to electrical stimulation of CN-2 at 5/sec between the arrows. "Swimming" is indicated by the typical burst pattern in the flexion neuron. In Figure 1B, sufficient hyperpolarizing current was passed into four TGN's (not shown) to hyperpolarize the monitor TGN indirectly by 38 mV. In response to the identical electrical stimulation of CN-2 swim activity is initiated without observable TGN spike activity. A comparison of the latency and number of swim cycles for control versus hyperpolarized TGN's is shown in Table 1 for five isolated brain preparations. No consistent change in either of these two swim parameters is observed when the TGN's are hyperpolarized.

The efficacy of blocking TGN spike activity by hyperpolarization is demonstrated in Figure 2. The response of the monitor TGN to the identical CN-2 stimulation is shown for progressively larger hyperpolarizations of four other TGN's. In Figure 2B the monitor TGN is indirectly hyperpolarized by 22 mV which is insufficient to block over-shooting spikes. In C, however, a hyperpolarization of 26 mV blocks overshooting spikes revealing small fast potentials (arrows) superimposed on the direct chemically mediated synaptic input from CN-2 stimulation. The fast potentials (arrows) are either electrotonically transmitted spikes from other TGN's which presumably are not hyperpolarized enough

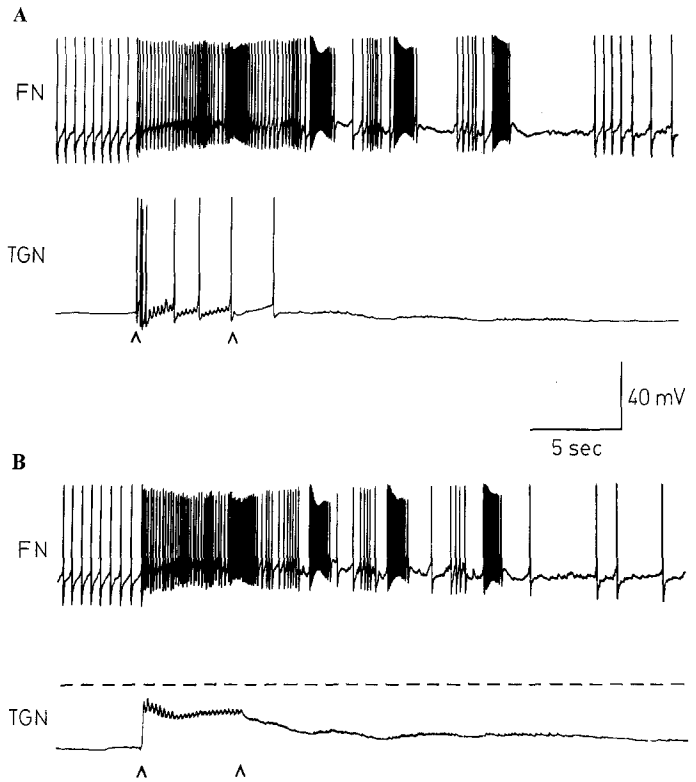

Fig. 1A and B. Necessary test of TGN activity for the initiation of swimming. Upper trace in each section is an intracellular recording from a flexion neuron (*FN*) in the pedal ganglion. "Swim" activity is clearly shown as repeated high frequency bursts. Lower trace is from the monitor TGN (see text) at rest potential **A** and during hyperpolarization of four other TGN's **B**. **A** "Normal" firing pattern of a TGN and FN is shown in response to electrical stimulation of CN-2 at 5/sec between the arrows. **B** The monitor TGN has been hyperpolarized indirectly 38 mV by current injection into four other TGN's (not shown) throughout the record. Despite no observable TGN spike activity, swim activity can be elicited by the same stimulus as in **A**. Rest potential is indicated by the dashed line. The depolarizing potentials in the TGN between the arrows are chemically mediated synaptic input to the TGN's from stimulation of CN-2

to totally prevent spike activity or are blocked axonal spikes (A-spikes). In either case, a hyperpolarization of 38 mV (D) blocks the fast superimposed potentials leaving only the slower synaptic potentials which correspond one-to-one with CN-2 stimuli. These slower synaptic potentials are chemically mediated input to the TGN network from stimulation of CN-2. This conclusion is based on the fact that the first PSP in each record grows progressively larger with increasing hyperpolarization. This is not expected for electrotonically transmitted spike activity. I, therefore, conclude that in this case a hyperpolarization of 38 mV is sufficient to block spike activity throughout the TGN network. In each case swim activity was initiated despite no observable TGN activity. Records A and D are the same as presented in Figure 1A and B.

Table 1. Comparison of latency and number of swim cycles with and without TGN's hyperpolarized. Latency was measured from the beginning of stimulation to the first FN burst

Prep No.		Latency (s)		Number of Swim Cycles	
		Control	TGN Hyp.	Control	TGN Hyp.
1		5	4.4	7	7
2		2.6	2.8	4	5
3	mean	9.7	10.5	5.6	6.5
	range	(8.4–12)	(10–11)	(4–7)	(6–7)
	N	3	2	3	2
4	mean	6.1	5.9	4.7	5
	range	(6–6.2)	(5.8–6)	4–5	5
	N	3	2	3	2
5	mean	4.5	4.4	5	5
	range	(4.2–4.8)	(4.2–4.6)	(4–6)	(4–6)
	N	3	5	3	5

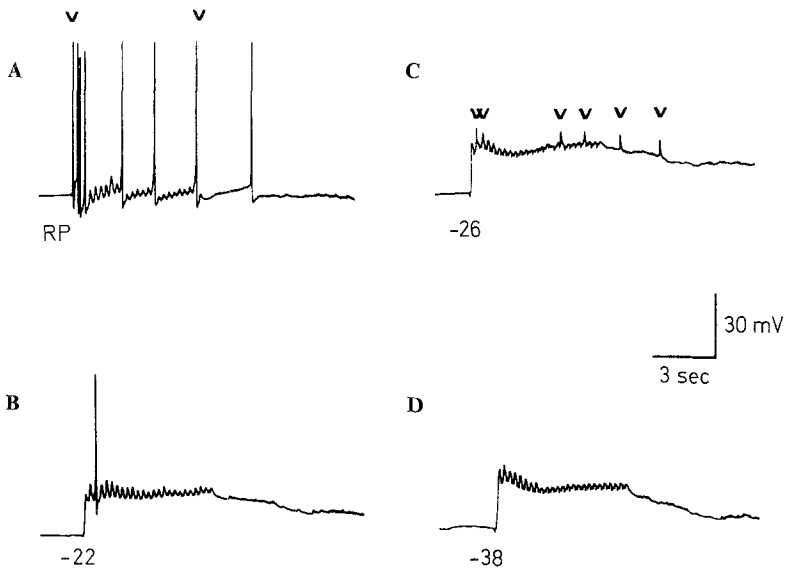

Fig. 2A–D. Efficacy of hyperpolarization in blocking TGN population activity. **A** Response of a monitor TGN at rest potential (*RP*) to stimulation of CN-2 at 5/sec between the arrows. **B** Response of the monitor TGN indirectly hyperpolarized by 22 mV. This level of hyperpolarization is insufficient to block over-shooting spikes in response to the identical CN-2 stimulation. **C** An indirect hyperpolarization of 26 mV is, however, sufficient to block over-shooting spikes revealing fast potentials (arrows) superimposed on the synaptic drive from CN-2 stimulation. These fast potentials are either electrotonically coupled spikes from other TGN's insufficiently hyperpolarized to block spike activity or represent blocked spikes (A-spikes) in the TGN axon. In either case a hyperpolarization of 38 mV **D** blocks the fast potentials leaving only the chemical synaptic input from CN-2. Since the entire TGN population is electrotonically coupled, spike activity anywhere in the population would show up as fast potentials superimposed on the synaptic input as in **C**. The absence of the fast potentials in **D** indicates blockage of spike activity throughout the TGN population, yet swim activity was initiated in this case. Records **A** and **D** are the same monitor TGN as shown in Figure 1A and B

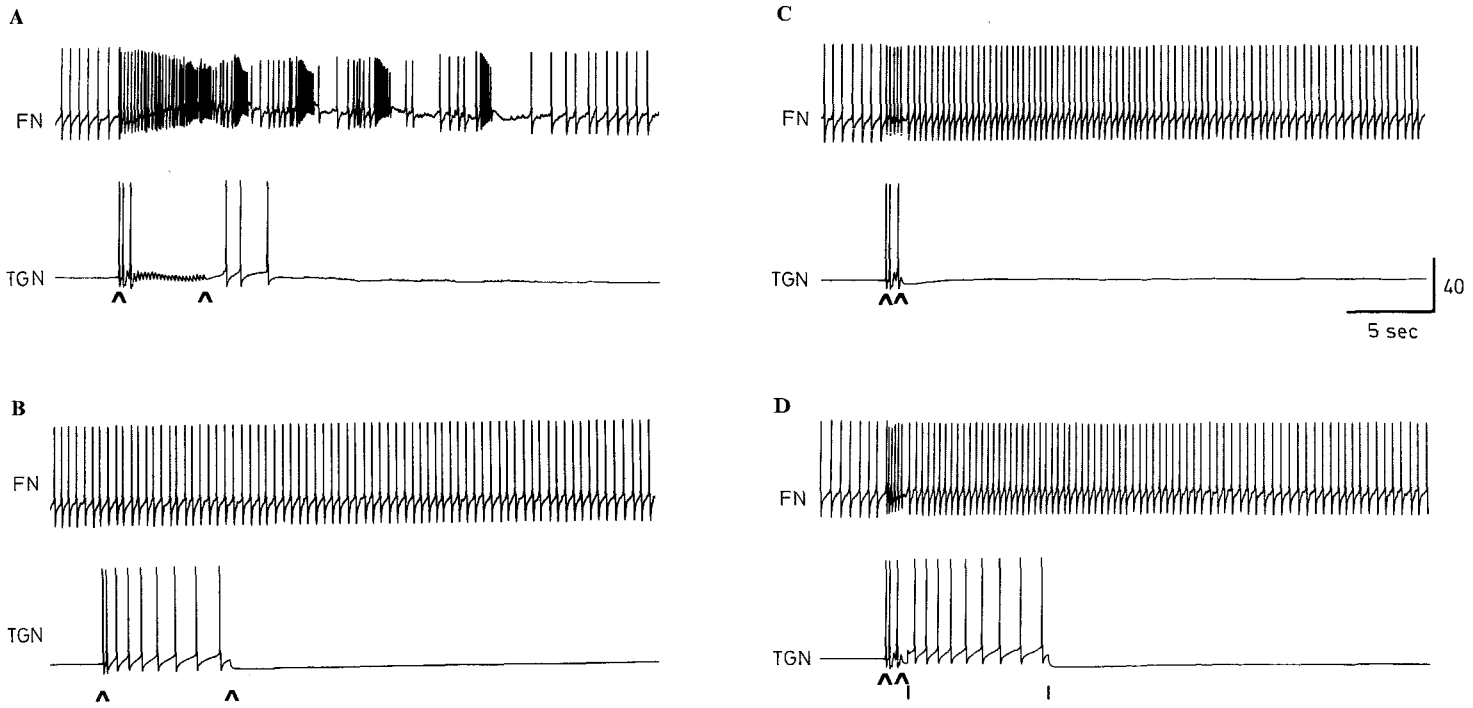

Fig. 3A–D. Sufficiency test for TGN activity. Cell notations are the same as in Figure 1. **A** "Normal" TGN and FN swim activity in response to electrical stimulation of CN-2 at 5/sec between the arrows. **B** Response of the FN to directly driven TGN activity by passing depolarizing current through four TGN's (not shown) between the arrows. Despite a higher overall TGN firing rate, no swim activity is initiated, nor is the spontaneous firing rate of the FN modified. Spikes recorded in the monitor TGN represent synchronous activity throughout the network (Getting and Willows, 1974). **C** Short stimulation of CN-2 (between arrows) synaptically drives a short TGN burst of identical structure as in **A** yet no swim activity ensues, only a slight increase in spontaneous FN activity. **D** Using a combination of short CN-2 stimulation ($\wedge - \wedge$) as in **C** and direct current stimulation (1–1) as in **B**, the identical initial TGN pattern is replicated with a higher overall TGN spike frequency as compared to the "normal" TGN pattern of **A**. No swim activity is initiated and the increase in FN activity can be attributed entirely to the effect of the brief CN-2 stimulation (compare FN activity in **C** to that in **D**). TGN activity, therefore, does not appear sufficient to trigger swimming

To test the sufficiency of TGN activity, depolarizing current was passed into four TGN's while the population response was monitored by the fifth TGN (Fig. 3B). Despite a higher over-all TGN spike frequency in 3B than in 3A, swim activity was not elicited nor was any modification of ongoing FN firing rate observed. As concluded previously (Getting, 1975), this experiment suggests that TGN activity is not sufficient.

Willows et al. (1973b) and Dorsett and Willows (1975) have suggested that an important property of the TGN network is the tendency to produce short high frequency bursts mediated by the electrical coupling. Directly driven TGN activity as in Figure 3B does not reproduce the same spike pattern as peripheral synaptic input (Fig. 3A). The initiation of swim activity in Figure 3A as opposed to 3B could, therefore, be due to the higher frequency of TGN firing at the beginning of stimulation. To test this possibility CN-2 was briefly stimulated to drive TGN activity synaptically in a similar initial pattern followed by directly driven TGN activity via intracellular current to four TGN's. Figure 3A shows flexion neuron (FN) and TGN activity during a "swim" elicited by a control electrical stimulation of CN-2 between the arrows. In Figure 3C a short stimulation of CN-2 produced the identical initial TGN activity but does not initiate swim activity. In Figure 3D short stimulation of CN-2 is followed immediately by directly driven TGN activity. The initial firing pattern of TGN activity is identical to the control (3A) while the total TGN activity is considerably higher; yet no swim activity ensues. The FN frequency increase can be attributed entirely to the effect of the initial nerve stimulation as in Figure 2B. A control stimulation of CN-2 immediately following the above sequence resulted in swim activity indicating the preparation was still capable of generating the swim pattern. This experiment in conjunction with previous evidence (Getting, 1975) strongly supports the interpretation that TGN activity is neither necessary nor sufficient for the initiation of swimming in *Tritonia diomedia.*

Characterization of S-Cells: Modality

Located just under the TGN cell bodies on the dorsal surface of each pleural ganglion is a population of approximately 60–80 small, (20–50 μm) darkly pigmented somata of afferents termed S-cells (Getting, 1975). The population runs diagonally across the pleural ganglion with the anterio-medial (A-M) margin near the central commissure and the posterio-lateral (P-L) margin near the exit of pleural nerve 1 (P1N-1). S-cells are multi-modal in that they respond phasically to mechanical stimuli and tonically to chemical stimuli of small areas of the dorsal epithelium. Figure 4 shows the response of a typical S-cell to A) mechanical deformation of the epithelium in its receptive field; B) a concentrated salt (NaCl) solution dropped within the receptive field; C) epithelial contact with the tube feet of the sea star *Pycnopodia* within and D) outside the receptive field.

Responses to mechanical stimuli are phasic with a prominent discharge at the onset and rapid adaptation. Repeated mechanical stimulation at once per sec fails to elicit a response in 3 to 4 trials. S-cell responses to salt or

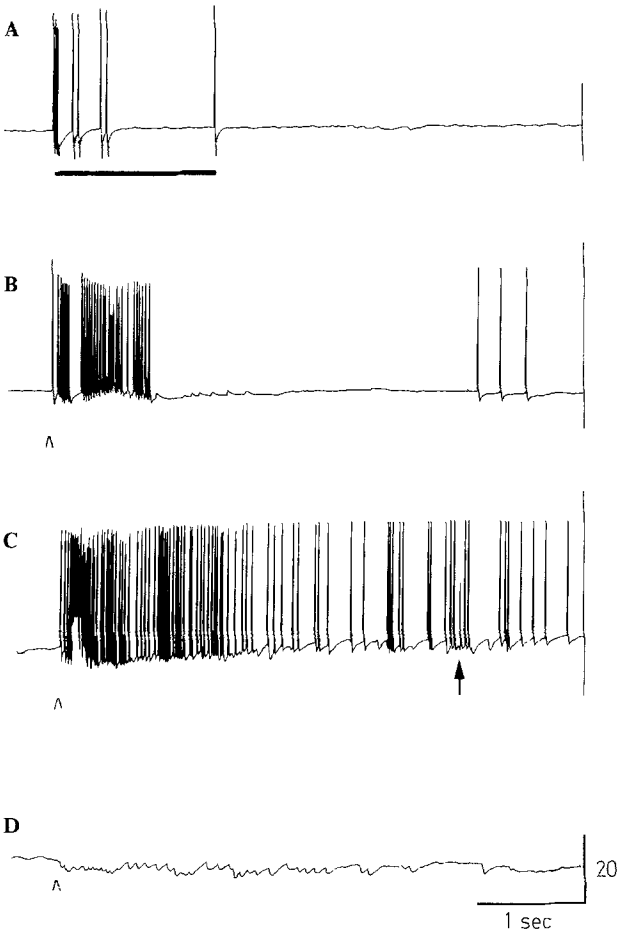

Fig. 4A–D. Typical responses of S-cell afferents to tactile and chemical stimuli of the epithelium in a whole animal preparation. **A** Response to a 2–3 mm depression of the epithelium by a sharpened probe in the receptive field to the afferent. The mechanical stimulus was maintained for the duration of the bar under the trace. A phasic response at both onset and termination of the stimulus is shown. **B** Response of the same S-cell to a drop of concentrated NaCl solution in the receptive field. The response is of higher frequency and more tonic than in **A**. The apparent EPSP's are reversed IPSP's due to leakage of Cl ions from the KCl microelectrode. Only hyperpolarizing potentials (IPSP's) are observed with KAc electrodes. **C** Response to maintained contact with several tube feet of the sea-star *Pycnopodia* within the receptive field. Note the initial high frequency discharge with maintained irregular firing throughout the stimulus. S-cell responses are typically bursty with periods of high frequency interrupted by IPSP's. **D** Response of the same S-cell to contact with *Pycnopodia* outside its receptive field. Note the prolonged barrage of IPSP's. The functional significance of these IPSP's is not clear, however, they are capable of blocking incoming spikes as shown by the arrow in **C**

sea star stimulation are generally more tonic and of higher spike frequency. This difference is shown dramatically in Figure 4C. When any of the above stimuli are applied outside the receptive field, S-cells receive volleys of IPSP's (Fig. 4D). This inhibition is mediated via an unidentified interneuron; no direct inter-afferent interactions have been observed.

Receptive Fields

Receptive fields of S-cells to mechanical stimuli vary between small areas of epithelium approximately 1/2 cm in diameter to elongated fields about $2 \times 1/2$ cm. Receptive fields were determined by displacing the epithelium by 2–3 mm with a sharpened probe. This method is not particularly sensitive especially when dealing with a soft-bodied animal (Olivo, 1970; Mellon, 1972), and more quantitative stimuli will be necessary to fully characterize receptive field size. The method does, however, allow preliminary characterization of receptive fields. Many fields overlap, and the highest density of fields lie in a strip about 1 cm on each side of the branchial tufts and on the dorsal surface of the oral veil. Several afferents have been found that have two or more distinctly separate receptive fields. This is consistent with the observation that most afferents have axons in more than one peripheral nerve (Getting and Slawsky, unpublished observation). Within the limits of my technique the receptive field for chemical (salt and sea star) is the same as for tactile stimuli.

Due to the difficulty in penetrating S-cells with the whole animal preparation, I have not as yet been able to quantify the extent of receptive field overlap. On several occasions, S-cells with elongated fields, however, have been observed to overlap with at least 3 other S-cells. It is, therefore, likely that a localized tactile or chemical stimulus activates numerous S-cells.

From recordings of over 50 receptive fields in 10 preparations, a somatosensory map correlating receptive field location with soma location is emerging. The soma of S-cells with anteriorly located receptive fields are located near the central commissure at the A-M third of the S-cell population. Progression towards the P-L margin in soma location is correlated with more posteriorly located receptive fields. Some notable exceptions have been encountered. Many S-cells have more than one distinct receptive field. In general, one receptive field can usually be located by soma position; the other may be almost anywhere on the ipsi-lateral epithelium.

Since an average of only 5 receptive fields per preparation have been characterized, the sample is not large enough to quantify the map in more detail. A new mounting procedure has been developed which will allow many S-cells per preparation to be characterized and should result in a quantitative analysis of receptive field overlap and somato-sensory mapping.

Identification as Afferents

Characterization of S-cells as afferents is based upon the following observations: i) each S-cell has one or more axon in a peripheral nerve, ii) spikes recorded in the soma rise smoothly from the baseline with no indication of a synaptic prepotential (Fig. 5A), iii) hyperpolarization of the soma blocks regenerative spikes revealing an "A-spike" that gets smaller with progressive hyperpolarization (Fig. 5B and C), and iv) only hyperpolarizing synaptic potentials are observed. Two additional observations indirectly implicate S-cells as an afferent pathway. Using electrical stimulation of peripheral nerves, synaptic potentials

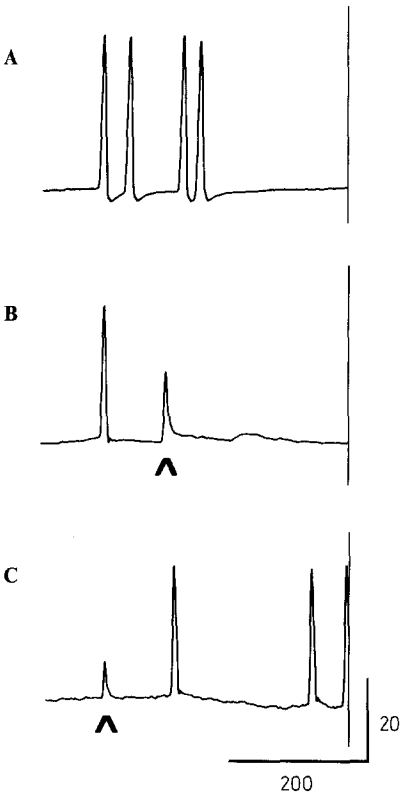

Fig. 5A–C. Progressive hyperpolarization of a S-cell demonstrating that orthodromic responses to concentrated salt solution represent afferent input to the CNS. **A** High speed recording of S-cell spikes recorded in the soma in response to stimulation of the epithelium in the receptive field. Each spike rises rapidly from base-line with no indication of a synaptic prepotential. **B** and **C** Progressive hyperpolarization of an S-cell showing blocked soma spikes (arrows) which decrease in size with hyperpolarization, and no evidence for synaptic prepotentials underlying the spikes. Anomalous rectification (Kandel and Tauc, 1966) is not a property of S-cells within the range of hyperpolarizations. In **B** a single depolarizing synaptic potential can be seen towards the end of the record. This is a reversed IPSP (Fig. 4D) due to the hyperpolarization

in a variety of central neurons including flexion neurons and TGN's are not evoked until S-cell threshold is reached. Furthermore, the latency of these evoked synaptic potentials is always longer than the arrival time of spikes in S-cell somata. Taken together, all these observations are consistent with S-cells being afferents primarily responsible for input associated with tactile and noxious chemical stimuli.

Responses to Centrally Driven S-Cell Activity: Behavior

In a whole animal preparation driving a single S-cell by intracellular current results in little or no behavioral response. In 3 of about 20 cases driven S-cell

activity has resulted in a local withdrawal of a small region of epithelium which corresponds generally to the receptive field location of the S-cell. The most common response is withdrawal of one or a few branchial tufts or oral veil regions. Due to poor visibility in the whole animal preparation, multiple penetrations of S-cells has proven difficult. To date a maximum of 4 S-cells have been simultaneously penetrated. In response to driving 2 to 4 S-cells a full reflexive withdrawal occurs including withdrawal of all branchial tufts, oral veil, rhinophores, and a general contraction of the body wall. Escape swimming has not yet been elicited in the whole animal by directly driving S-cells; it will be shown in the next section that this is due to inadequate summation of input.

Central Synaptic Distribution of S-Cells

The isolated brain can be better stabilized and illuminated to allow penetration of 5 or more S-cells. If S-cell activity is involved in initiating escape swimming, then directly driven S-cell activity should mimic the effect of peripherally applied stimuli. The criteria applied for the isolated preparation are that S-cell activity should cause i) modified FN activity during the initial reflexive withdrawal phase and elicit swim activity, and ii) increased activity in a number of identified pleural neurons suspected of participating in reflexive withdrawals preceding a swim. This later group includes pleural cells 1, 3, 6, 7, 8 and the TGN's (Willows et al., 1973a).

Figure 6 shows that centrally driven S-cell activity in a minimum of 5 S-cells is sufficient to initiate swim activity in flexion neurons. The upper trace shows

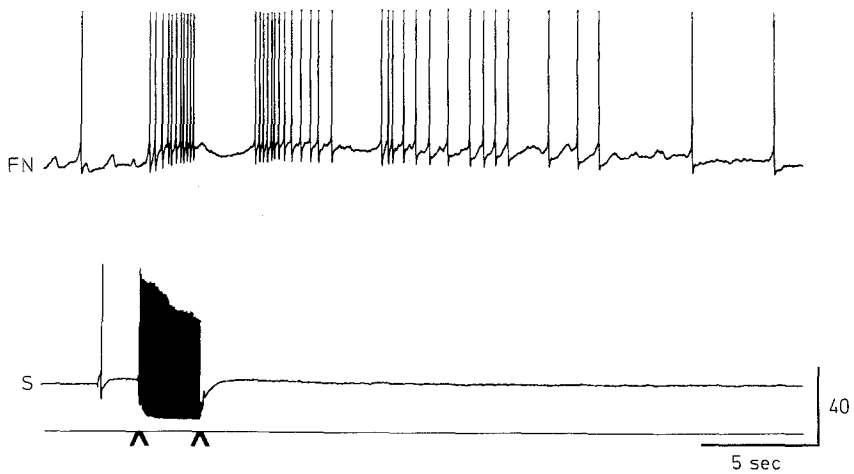

Fig. 6. Swim activity initiated by intracellularily driven activity in five S-cells (only one shown). Upper trace: intracellular recording of a pedal flexion neuron (*FN*) in an isolated brain preparation showing typical swim activity in response to high frequency activity in S-cells (lower trace). Duration of intracellular current injection into the S-cell is shown by the arrows. S-cell activity alone is sufficient to trigger escape swimming. Trypsin pretreatment was used in this case

a recording from a pedal flexion neuron while the lower trace is from one of five penetrated S-cells. Swimming is indicated by the typical burst activity in the FN. Trypsin pretreatment was used in this case to desheath the pleural ganglion. The lower frequency of flexion neuron activity (FN) during swim activity is typical of trypsin treated preparations. In each of three preparations driving 5 S-cells has initiated swimming. Swim activity resulted in 5 out of 7 attempts; the two cases in which a swim was not initiated may be due to decrement in the S-cell synaptic efficacy (see below). Driving 2, 3 or 4 S-cells results in a short-term increase or decrease in FN activity but is not sufficient to produce repeated bursts typical of swim activity. For this reason it has not been possible to elicit swimming in the whole animal preparation in which a maximum of 4 S-cells have been simultaneously penetrated.

No direct connections between the S-cells and flexion neurons (FN) have been observed but this effort has been hampered by the large ongoing synaptic activity in FN neurons. Compound PSP'S in FN-s to peripheral nerve stimulation have similar latencies to EPSP's in the TGN's which are driven by S-cells via an interneuronal pathway (see below). It is therefore believed that the S-cells synapse with the FN via as yet unidentified interneuronal pathway.

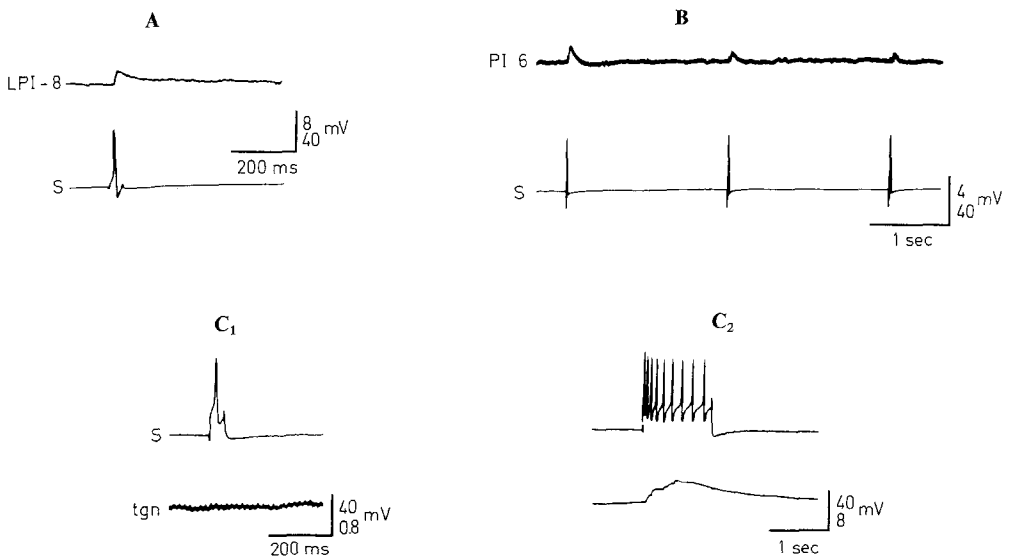

Fig. 7A–C. Central synaptic distribution of S-cell activity. **A** Intracellular recordings from a S-cell(s) and pleural ganglion cell (LP1–8) showing a single presumably monosynaptic EPSP from the S-cell. Monosynapticity is based upon i) a short constant delay, ii) and the EPSP follows high frequency S-cell activity despite significant decrement. **B** Decrement of S-cell EPSP in P1–6 during repetitive stimulation. Typically, an EPSP decrements from approximately 2 mV to 200–400 μV in 3 to 4 spikes at one per two sec. C_1 S-cells do not produce unitary PSP's in the TGN's neurons. C_2 Interneuronal connection between S-cells and TGN's is demonstrated by driving 3 S-cells (only one shown). This interneuronal pathway has not as yet been identified. Pleural cells 6, 7, or 8 do not comprise this interneuronal pathway as there is no interaction between these cells and the TGN's

Swimming is preceeded by a general withdrawal which is temporally correlated with activity in a number of identifiable pleural neurons. Many of these neurons are suspected of participating in this reflexive withdrawal phase (i.e. pleural cell 1 causes bilateral branchial tuft withdrawal). Most S-cells make monosynaptic connections with pleural cells 6, 7, 8 (Fig. 7A). A single afferent produces a 1–5 mV EPSP in each of these cells. With repeated activity the EPSP's decrement very quickly in amplitude (Fig. 7B). Two or more S-cells must be simultaneously active to elicit spikes in pleural cells 6, 7, 8. The motor function of cells 6, 7, 8 is not known. Figure 7C shows that a single S-cell spike does not produce a unitary EPSP in a TGN but that driving 3 S-cell (Fig. 7D) produces a compound EPSP mediated by an unidentified interneuronal pathway. Similarly, S-cells drive pleural cell 1 (bilateral tuft withdrawal) and cell 3 (general withdrawal and weak dorsal flexion) via a polysynaptic pathway. S-cell activity is therefore sufficient to account for most if not all observed activity in a number of pleural and pedal neurons known to participate in reflexive withdrawal preceeding escape swimming. Whether or not the S-cells represent all of the afferent input to the CNS used in the initiation of escape swimming is difficult to evaluate. If the S-cells in both pleural ganglia are removed, escape swimming cannot be elicited. This procedure is too disruptive to be conclusive as it results in considerable deformation of the pleural neuropile. Nevertheless, the observation that directly driven S-cell activity is sufficient to initiate swim activity suggests that S-cells carry a major portion of afferent activity responsible for reflexive withdrawals and initiation of escape swimming.

Discussion

Comparison to Other Central Afferents: Mechanoreception

S-cells of *Tritonia diomedia* share many features in common with presumed central sensory neurons of *Aplysia* (Byrne et al., 1973) including i) size (approximately 50 μm), ii) appearance (ringed by dark pigment), iii) variable receptor field size, iv) spontaneous activity (normally silent but with irregular firing pattern when stimulated), and v) decrementing synaptic connections. S-cells differ significantly from *Aplysia* cells in their response characteristics to mechanical stimulation. Whereas *Aplysia* sensory cells tend to respond tonically with slow adaptation, *Tritonia* S-cells respond phasically adapting quickly. In this respect S-cells more closely resemble phasic mechanoreceptors of the razor clam (Olivo, 1970) and T-cells of the leech (Nicholls and Baylor, 1968).

S-cells respond more tonically to presumed noxious stimuli including salt crystals and the tube feet of sea stars. Since these two particular stimuli are more or less unique to the study of *Tritonia* it is difficult to make direct comparisons with other afferents. Nociceptive (N) cells of leech (Nicholls and Baylor, 1968) also generate slowly adapting responses to prolonged stimuli but are not as sensitive to tactile stimulation. No information is available for either clam or *Aplysia* sensory neurons to similar stimuli.

Behavioral Function of S-Cells

S-cells appear to provide a major source of input underlying the initiation of reflexive withdrawal and escape swimming. Reflexive withdrawal occurs in response to mechanical as well as noxious (salt crystals or sea star) stimuli whereas swimming only occurs in response to noxious stimuli. It is interesting that the responses of S-cells show a similar dichotomy. S-cells respond phasically with a relative low frequency to mechanical stimuli but give prolonged high frequency activity to both salt crystals and sea stars.

Experiments using directly driven S-cell activity shed some light on this dichotomy. A minimum of five S-cells must be driven at high frequency in order to initiate escape swimming, however, two or more S-cells can elicit reflexive withdrawal, indicating the behavioral threshold for reflexive withdrawal is lower than escape swimming. On several occasions, behavioral responses were observed to result from activity in a single S-cell. In three cases a local withdrawal of the epithelium and a few branchial tufts in the receptive field of the S-cell resulted from a short (1 sec) train of spikes. In two other instances no local withdrawal occurred but in response to repeated trains of S-cell activity (0.5 sec duration, 1/sec for 10 sec) the animal turned away from the stimulated side. Both these responses can be observed in a brainless animal to tactile stimulation suggesting these responses may be peripherally mediated.

The general conclusions are i) reflexive withdrawals and escape swimming share a common input pathway (S-cells), ii) S-cells respond bimodally giving phasic low frequency responses to tactile stimuli and tonic high frequency responses to noxious stimuli, and iii) escape swimming has a higher threshold for initiation both in terms of the number of active afferents as well as afferent spike frequency.

Initiation of Escape Swimming

Willows (personal communication) has repeated several of these experiments on *T. diomedia* from Puget Sound with somewhat conflicting results. Despite simultaneous hyperpolarization of 5 TGN's (in 6 different preparations) with sufficient current to produce over a 35 mV hyperpolarization of a 6th monitor cell, TGN burst activity was not prevented. Nor was spiking blocked in the directly hyperpolarized TGN's. The fact that TGN activity could not be blocked in *T. diomedia* from Puget Sound but can be in *Tritonia* from Southern California, most probably resides in a difference in the synaptic drive to or electrical coupling between the TGN's in the two varieties. If TGN activity is blocked, then the initiation of swimming as tested by latency and number of swim cycles (Table 1) is not affected.

In addition, direct depolarization of the TGN's in both species produces a slowly adapting spike train (Willows, personal communication; Fig. 3B) rather than bursts as produced by synaptic drive. Likewise, direct depolarization with multiple electrode does not initiate escape swimming in either species. Willows et al. (1973b) suggest that the initiation of swimming is associated with discrete

bursts of TGN activity. If, however, the discrete bursts of TGN activity are reproduced by a combination of synaptic and direct stimulation (Fig. 3D), swimming is not initiated.

Experiments reported here and previously (Getting, 1975) suggest that the TGN's do not play a significant role in the initiation of escape swimming. They are excited by the S-cells via an unidentified polysynaptic pathway explaining the temporal correlation of TGN activity with escape swimming. Stimulation via a large suction electrode over the surface of the TGN's also can initiate escape swimming (Willows and Hoyle, 1969) but this is probably due to direct stimulation of the S-cells which lie directly underneath the TGN somata. Intracellular recording from P1–6 which receives monosynaptic excitation from S-cells show large EPSP's at the stimulus intensity required to initiate swimming via a suction electrode placed over the TGN's. Since there is no direct interaction between the TGN's and P1–6 this result strongly suggests direct stimulation of the S-cells. Further support for the non-involvement of TGN's is that swimming can be elicited when all TGN's are surgically removed from both pleural ganglia. Although this evidence is suggestive it does not rule out the possibility that the remaining processes of the TGN's may still contribute activity. The preponderance of evidence, therefore, favors the interpretation that the TGN's are not directly involved in triggering escape swimming but that S-cell activity is the initial step. Since the projection of S-cell activity to the swim network (DFN-GEN-VFN network; Willows, 1972) is not known in detail, speculation as to exactly how S-cell activity mediates the initiation of escape swimming is premature, however, a causal link has been established.

I thank Drs. D. Kennedy, J. Wine, and A.O.D. Willows for helpful comments during the research and on the manuscript, and special thanks to Robert Snow for identifying *Tritonia* from southern California as *Tritonia diomedia*. This work was supported in part by a grant from Research Development Fund, Stanford University and a NIH research grant #1-R01-NS-12529.

References

Byrne, J., Castellucci, V., Kandel, E.R.: Receptive fields and response properties of mechanoreceptor neurons innervating siphon skin and mantle shelf in *Aplysia*. J. Neurophysiol. **37**, 1041–1064 (1973)

Dorsett, D.A., Willows, A.O.D., Hoyle, G.: Centrally generated nerve impulse sequences determining swimming behavior in *Tritonia*. Nature (Lond.) **244**, 711–712 (1969)

Dorsett, D.A., Willows, A.O.D., Hoyle, G.: The neuronal basis of behavior in *Tritonia*. IV. The central origin of a fixed action pattern in the isolated brain. J. Neurobiol. **4**, 287–300 (1973)

Getting, P.A.: Modification of neuron properties by electrotonic synapses. I. Input resistance, time constant, and integration. J. Neurophysiol. **37**, 846–857 (1974)

Getting, P.A.: *Tritonia* swimming. Triggering of a fixed action pattern. Brain Res. **96**, 128–133 (1975)

Getting, P.A., Willows, A.O.D.: Modification of neuron properties by electronic synapses. II. Burst formation by electrotonic synapses. J. Neurophysiol. **37**, 858–868 (1974)

Kandel, E.R., Tauc, L.: Anomalous rectification in the metacerebral giant cells and its consequences for synaptic transmission. J. Physiol. (Lond.) **183**, 287–304 (1966)

Mauzey, K.P., Birkeland, C., Dayton, P.K.: Feeding behavior of asteroids and escape responses of their prey in the Puget Sound region. Ecology **49**, 603–619 (1968)

Mellon, DeF.: Electrophysiology of touch sensitive neurons in a mollusc. J. comp. Physiol. **79**, 63–78 (1972)

Nicholls, J.G., Baylor, D.A.: Specific modalities and receptive fields of sensory neurons in CNS of the leech. J. Neurophysiol. **31**, 740–756 (1968)

Olivo, R.F.: Mechanoreceptor function in the razor clam: Sensory aspects of the foot withdrawal reflex. Comp. Biochem. Physiol. **35**, 761–786 (1970)

Willows, A.O.D.: Behavioral acts elicited by stimulation of single identifiable nerve cells. In: Physiological and biochemical aspects of nervous integration (ed. F.D. Carlson), p. 217–243. Englewood Cliffs, N.J.: Prentice-Hall 1968

Willows, A.O.D.: Interactions between brain cells controlling swimming in a mollusc. In: Neurobiology of invertebrates (ed. J. Salanski), p. 237–247. Budapest: Akademiai Kiado 1973

Willows, A.O.D., Dorsett, D.A.: Evolution of swimming behavior in *Tritonia* and its neurophysiological correlates. J. comp. Physiol. **100**, 117–134 (1975)

Willows, A.O.D., Dorsett, D.A., Hoyle, G.: The neuronal basis of behavior in *Tritonia*. I. Functional organization of the central nervous system. J. Neurobiol. **4**, 207–237 (1973a)

Willows, A.O.D., Dorsett, D.A., Hoyle, G.: The neuronal basis of behavior in *Tritonia*. III. Neuronal mechanism of a fixed action pattern. J. Neurobiol. **4**, 255–285 (1973b)

Willows, A.O.D., Hoyle, G.: Correlation of behavior with the activity of single identifiable neurons in the brain of *Tritonia*. Symp. Biol. Hung. **8**, 443–461 (1967)

Willows, A.O.D., Hoyle, G.: Neuronal network triggering a fixed action pattern. Science **166**, 1549–1551 (1969)