

A SENSORY SYSTEM INITIATING SWIMMING ACTIVITY IN THE MEDICINAL LEECH

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SUMMARY

1. Water-wave stimulation, which was previously shown to elicit swimming in intact leeches, can initiate swimming in a semi-intact leech preparation *via* activation of the sensillar movement receptors (SMRs), provided that 50 μ -serotonin is added to the physiological saline.

2. The neuronal responses resulting from near-field stimulation of the leech body wall with a vibrating probe were recorded in peripheral nerves and in nerve-cord connectives. The response in the dorsal posterior nerve to a single vibratory pulse consists of a graded compound action potential. The units contributing to this action potential have a much lower threshold for near-field stimulation than do touch cells. They appear to be the same sensory units, the SMRs, that mediate leech sensitivity to water waves.

3. The frequency domain of the SMR sensitivity extends as low as 1 Hz. Thus, leeches could receive self-stimulation from the water vibrations created by their own swimming movements.

4. Leech physiological saline containing 20–40 m-Mg²⁺ does not eliminate the SMR response to near-field stimulation recorded in the DP nerve; however, elevated Mg²⁺ concentrations do eliminate the neuronal responses in the nerve cord connectives. Thus, while no chemical synapse occurs between the peripherally situated SMRs and nerve cord ganglia, a synapse may be interposed between the SMRs and the intersegmental neurones activated by near-field stimulation.

5. The swim-facilitating action of serotonin occurs at unidentified sites within the ventral nerve cord, since serotonin does not alter the sensitivity of the SMRs.

INTRODUCTION

A complete characterization of the neuronal basis of animal behaviour must include a description of the central pattern generator, and of the central and sensory mechanisms that initiate, modulate and terminate the movement sequences. In the medicinal leech, major components of the central neuronal oscillator generating the swimming rhythm have been identified (Friesen, Poon & Stent, 1978; Poon, Friesen & Stent, 1978) together with the motor neurones which are driven by the central oscillator and in turn command the muscle contractions (Kristan, Stent & Ort,

1974*a,b*; Ort, Kristan & Stent, 1974). However, several newly described candidate oscillator neurones (Weeks, 1982*c*; Friesen, 1982), whose interactions with the other oscillator neurones and with motor neurones remain incompletely explored, must be included in the description of the central pattern generator of leech swimming.

Swimming activity in the leech can be initiated by a variety of methods but few specific excitatory inputs to the central pattern generator are known. Recently, new insights into neuronal mechanisms of swim initiation in the leech have been obtained from the observation that depolarization of a set of ventral cord interneurones (cells 204 and 205) reliably initiates swimming oscillations (Weeks & Kristan, 1978; Weeks, 1982*a,b*). These intersegmental interneurones receive excitatory synaptic inputs throughout swim episodes; thus they could be part of the system maintaining swim activity as well as part of the central swim initiating system. In contrast, the tactile mechanosensory cells (T, P and N; Nicholls & Baylor, 1968), which also can initiate swimming activity when activated either by intracellular or by cutaneous stimulation (Debski & Friesen, 1982; Kristan, McGirr & Simpson, 1982), receive no such central excitatory input, and hence cannot act to maintain swim oscillations in the isolated nerve cord.

In intact leeches, one method by which swimming can be initiated is through stimulation by surface water waves (Young, Dedwylder & Friesen, 1981). This form of vibratory mechanical stimulation does not activate the tactile mechanoreceptors, but does activate the sensillar movement receptors (SMRs; Friesen, 1981), which are sensory receptors localized at the sensilla of the medicinal leech (DeRosa & Friesen, 1981). Of the three hair cell types found at the sensilla, the unciliated cells with 12 μm long apical hairs are the best candidates for transducing vibratory water stimulation (Phillips & Friesen, 1982; Markl, 1978). The details of the mechanism of swim initiation by water vibrations are at present unknown. To investigate fully the pathway from the SMRs to the central oscillator neurones, conditions must be found such that swimming activity can be initiated by water vibration stimulation while recording from neurones of the leech ventral nerve cord.

Recently, Willard (1981) demonstrated that leech physiological saline containing serotonin (0.5–100 μM) produces recurring spontaneous swimming episodes in isolated ventral nerve cords. In addition, he found a correlation between the levels of serotonin in the sinus blood and the frequency of spontaneous swimming episodes in intact leeches. We report, first, that a semi-intact leech preparation (in which some ventral nerve cord ganglia are exposed for intracellular recording and the posterior end of the animal remains nearly intact) will undergo swimming activity in response to water vibrations when serotonin is present in the bathing saline. Second, we show that water vibrations resulting from the pulsatile movements of a small probe stimulate the SMRs, provided that the SMRs are in the near-field of this stimulus source. The water oscillations near the probe provide a discrete and precisely controllable form of vibratory stimulation that we employ to investigate further the physiology of water vibration detection by the SMRs. Finally, we show that the swim-facilitating action of serotonin occurs in the ventral nerve cord and not at the sensory transduction sites of the SMRs.

MATERIALS AND METHODS

Medicinal leeches, *Hirudo medicinalis*, were purchased from a commercial supplier and maintained in aquaria at 20°C for periods of up to one year. The leeches were fed four times a year on frogs.

Three experimental preparations, similar to those described by Friesen (1981), were used in this study. One preparation consisted of an isolated body wall flap attached to one of the segmental nerves, the dorsal posterior nerve (DP). The length of the body wall flap was three to five segments, and its width extended from the dorsal midline to the lateral edge of the leech body wall. Kretz, Stent & Kristan (1976) have shown that only sensilla 6 and 7 (S6 and S7), the two most dorsal sensilla, have axons in the DP nerve. Thus, only the neuronal responses of S6 and S7 to near-field stimulation were characterized in this study. The remaining sensilla, S1–S5, are innervated by the anterior root (Kretz *et al.* 1976). A second preparation consisted of a body wall flap attached to an isolated ventral nerve cord by one or more DP nerves.

For swim initiation, we employed a third preparation, consisting of the ventral nerve cord extending from segmental ganglion 3 to ganglion 18, with ganglia 3 to 9 detached from the body wall. (Segmental ganglia are numbered sequentially, anterior to posterior, beginning with the first ganglion posterior to the head brain; see Kristan *et al.* 1974a.) For some experiments, body wall flaps identical to those described above were dissected and left attached to ganglion 7 *via* the DP nerve. The posterior end of the leech, including ganglia 10 to 18, was intact; however, the connectives were severed posterior to ganglion 18 to disconnect the tail brain. This preparation is referred to as the posterior semi-intact leech preparation.

Extracellular records were obtained with suction electrodes, and intracellular recordings were obtained with glass microelectrodes. Permanent records of the electrical signals were obtained either by photographing an oscilloscope screen or by the use of an FM tape recorder. The tape records were later reproduced at a two-fold reduction in tape speed on a four-channel chart recorder.

The sensilla on the leech body wall were stimulated by two methods. In some experiments, surface water waves were used to stimulate the sensilla. The water-drop procedure of Friesen (1981) was used to generate surface waves. The amplitude of the waves is controlled by the height above the surface of the recording chamber from which the drops fall, and by the distance from the drop impact point to the preparation. Wave packets travel approximately 0.3 ms⁻¹ (Friesen, 1981). However, with surface water waves, precise control over such stimulus parameters as intensity, frequency, duration and localization of the stimulus is impossible. In order to characterize the physiological properties of the SMRs in greater detail, more accurate control over these stimulus parameters is necessary. To accomplish this, a glass probe was mechanically coupled to the diaphragm of a 6 cm speaker which, in turn, was connected to a function generator. The probe was driven either with square pulses or sinusoidal signals. The resonant frequency of this stimulus system was about 80 Hz. The tip diameter of the glass probe was approximately 120 µm, twice the diameter of the dorsal sensilla. Measurements of probe displacement were made by observing the probe movement through a calibrated ocular. Probe displacement is approximately linear between 10 µm and 130 µm for applied voltages between 0.55 V and 0.8 V respectively.

Leech physiological saline for most experiments had the following composition: NaCl, 115 mM; KCl, 4 mM; CaCl₂, 1.8 mM; MgCl₂, 2 mM; 10 mM-Hepes buffer. For some experiments, saline contained increased concentrations of Mg²⁺ (20–40 mM), with an appropriate reduction in Na⁺ concentration.

RESULTS

Swim initiation in dissected leeches

The SMRs found in all body regions of the leech are activated by water vibrations, and leech swimming can be evoked by water vibrations in intact animals. Nevertheless, previous attempts to elicit swimming *via* SMR stimulation in dissected animals have proved unsuccessful (Friesen, 1981). We show here that if serotonin is added to the saline solution, SMR stimulation will evoke swimming in dissected preparations.

Dissected leeches swim in response to surface waves

In the posterior semi-intact leech preparation, the number of spontaneous swimming episodes increased in the presence of 50 μ M-serotonin, usually within 30 min of application. The frequency of these spontaneous episodes subsequently declined, reaching a level of one or two per 10 min within 90 min after serotonin application. At this time, wave stimulation reliably evoked the motor programme for swimming, as monitored by rhythmic bursts of cell 3 in a DP nerve (Fig. 1A). As many as 22 swimming episodes could be initiated by waves in 10 min. Once wave stimulation ceased, the rate of spontaneous swimming episodes again was only about one per 10 min. Two observations indicated that the wave-induced motor activity corresponded to swimming. First, the period of the cell 3 bursts was between 400–1500 ms, the period range of normal leech swimming, and second, the intact posterior body wall

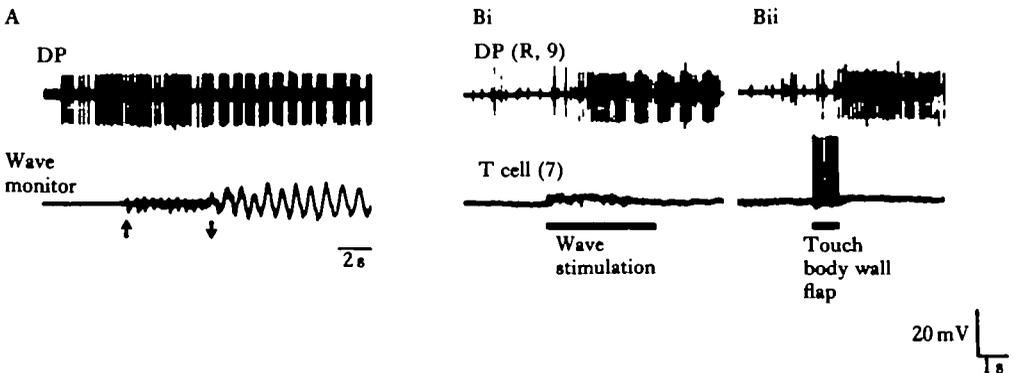


Fig. 1. Swimming initiation in posterior semi-intact preparations bathed in physiological saline containing 50 μ M-serotonin. (A) DP nerve swim bursts resulting from wave stimulation (applied between arrows, lower trace). The oscillations seen in the latter portion of the lower trace resulted from waves generated by the swimming movements of the intact body wall. (B) Simultaneous recording from a DP nerve and the dorsal touch cell. (Bi) Wave stimulation (bar) sufficient to initiate swimming does not evoke impulses in this cell. However, Bii, direct touch (bar) of the innervated body, elicits a barrage of impulses.

of the leech underwent the sinusoidal oscillations characteristic of swimming leeches. With serotonin present in the saline, 70% (22/31) of the posterior semi-intact leech preparations swam in response to wave stimulation, demonstrating that surface waves can initiate the motor programme for swimming even in a dissected leech. The ability of serotonin to facilitate the initiation of swimming by wave stimulation lasts 1–2 h.

There was considerable variability in the latency of swim initiation following wave stimulation. For most preparations, the onset latency was 2–5 s; however, in some preparations swimming ensued immediately following the arrival of the first drop-evoked wave packet at the intact body wall. In addition, swimming activity almost always continued for several cycles after stimulation was discontinued. Thus, like stimulation of cell 204 and the tactile mechanosensory cells (Weeks & Kristan, 1978; Debski & Friesen, 1982), activation of the SMRs acts as a trigger to initiate swimming, but maintained stimulation is not essential for prolonged swimming. The most obvious candidate neurones for mediating SMR-evoked swimming activity are cells 204 and 205, which have their cell bodies in posterior ganglia of the ventral nerve cord (Weeks & Kristan, 1978; Weeks, 1982*a,b*). However, repeated experiments have failed to reveal any consistent membrane depolarizations or increased impulse activity in these swim-initiating neurones resulting from SMR stimulation (Friesen, 1981; E. A. Debski, P. D. Brodfuehrer & W. O. Friesen, unpublished observations).

In the absence of serotonin, we were able to initiate swimming in three posterior semi-intact leech preparations from leeches which had just arrived from our commercial supplier and which were extremely active swimmers in the holding aquarium. Leeches that are considered 'frequent swimmers' have been shown to have elevated levels of serotonin in their sinus blood (Willard, 1981). Thus these three leeches may have had elevated serotonin levels and therefore when dissected swam in response to wave stimulation without requiring the addition of serotonin.

Touch cells do not mediate wave-induced swimming in serotonin

Touch cells are normally insensitive to low-amplitude surface waves (Friesen, 1981). However, if touch cells were sensitive to surface waves in the presence of serotonin, then swimming could be activated by them, since the stimulation of even an individual touch cell can induce leech swimming (Debski & Friesen, 1982). To determine if serotonin increased the sensitivity of touch cells, we recorded intracellularly from touch cells innervating body wall flaps attached to ganglion 7 of the posterior semi-intact leech preparation. No spiking activity occurred in the touch cell during the wave stimulation period, yet swimming was initiated (Fig. 1Bi). The touch cell could be stimulated, however, by light touch of the body wall flap (Fig. 1Bii). Thus, serotonin did not increase significantly the sensitivity of the touch cells, a result which is consistent with previous observations (Willard, 1981). We conclude that swimming activity in response to surface waves in serotonin does not occur *via* the most sensitive of the tactile mechanosensory cells, touch cells, but most probably is mediated by the SMRs.

Near-field stimulation

To characterize the role of the SMRs in the initiation of leech swimming by water waves, the physiological properties of the SMRs were investigated. We show here that

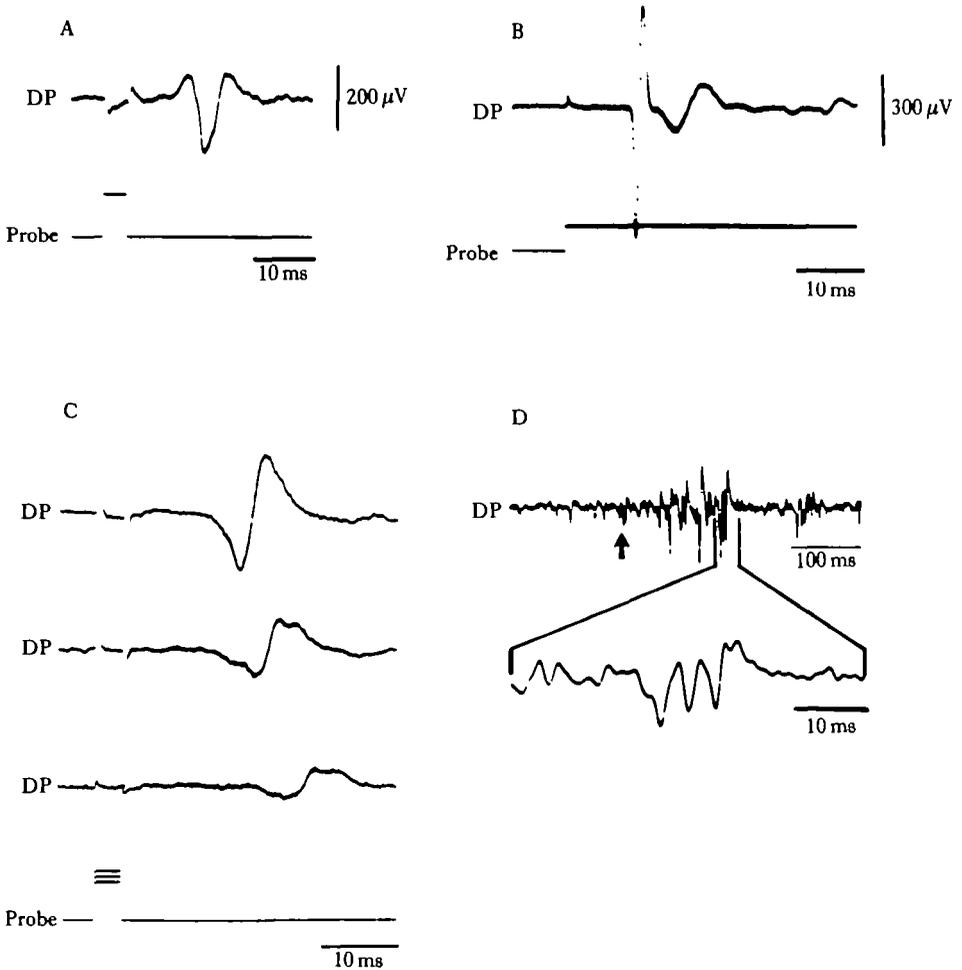


Fig. 2. DP nerve responses to near-field and wave stimulation. (A) Triphasic response evoked by a brief pulse. (B) DP nerve response obtained when vibrating probe is allowed to touch the sensillum. The large spike is a touch cell impulse. Because of its greater conduction velocity, it precedes the smaller, broader SMR spike. (C) SMR responses to three different probe displacements, ranging from 10 μm to 110 μm . Note the graded nature of the response amplitude and latency. (D) DP nerve response to wave stimulation. The lower trace shows a 10-fold expansion of a small section of the upper trace. The arrow indicates the onset of wave stimulation. These records were obtained from DP nerve recordings from four different isolated body-wall preparations. In parts (A–C) the 'Probe' trace indicates the timing and relative amplitude of the near-field stimulation.

the water vibrations in the near-field of a vibrating probe activate the SMRs, and evoke the same types of neuronal responses as stimulation by water waves or jets. Probe stimulation was then used in further characterization of the SMRs.

Near-field stimulation activates the SMRs

The neuronal responses to near-field stimulation of the leech body wall were analysed in a preparation consisting of an isolated body wall flap attached to the D

Nerve. Near-field pulse stimulation with the vibrating probe positioned within about $200\ \mu\text{m}$ of either sensillum 6 or 7 (S6 or S7) produced a relatively long duration (8–12 ms) biphasic or triphasic spike in the DP nerve (Fig. 2A). Two observations indicated that the electrical activity recorded in the DP nerve was not an artifact of the stimulation procedure. First, excising one of the sensilla from the body wall abolished the neuronal response to near-field stimulation of the excised body wall region. However, near-field stimulation of the other, intact sensillum remained effective. Second, moving the probe 500–1000 μm away from either S6 or S7 eliminated the neuronal response in the DP nerve during near-field stimulation. These results indicate that the receptors transducing near-field stimulation, like the receptors for wave stimulation (Friesen, 1981), are located at the sensilla. Thus the neuronal response evoked by vibrational stimulation of either S6 or S7 will be referred to as an 'SMR response'. In addition, these results show that the stimulus provided by the probe is ineffective at distances of 1 mm or greater. Since the distance between S6 and S7 is about 1.2 mm (DeRosa & Friesen, 1981), these two sensilla can be stimulated independently of each other.

The SMR response illustrated in Fig. 2A is easily distinguished from touch (T) cell spikes evoked by light touch of the sensillum with the probe (Fig. 2B). The SMR response has a lower amplitude (180 μV) than the T-cell spike (950 μV); the SMR response has a greater duration (10 ms) than the T-cell spike (3 ms); and the SMR response has a greater latency than the T-cell spike. The greater SMR response latency is due, in part, to the lower conduction velocity in SMR nerve fibres. Measured with paired suction electrodes on the DP nerve, the conduction velocity for SMR fibres was $0.34\ \text{m s}^{-1}$ (s.d. = 0.09, $N = 9$) compared with $0.89\ \text{m s}^{-1}$ (s.d. = 0.16, $N = 4$) for the conduction velocity of T-cell axons in the DP nerve.

The SMR response was a compound action potential (CAP) resulting from the summed currents generated by numerous axons, as indicated by its graded nature in response to stimulation (Fig. 2C). As probe displacement was increased from 15 μm to 110 μm , the peak-to-peak amplitude of the SMR response increased from 100 μV to 330 μV while the latency decreased from 20 ms to 13 ms. Although only three traces, and therefore only three CAP amplitudes, are shown in this figure, the CAP amplitudes are almost continuously graded when the probe displacement is changed by small increments.

Although both wave stimulation from fallen water drops and near-field stimulation provided by the vibrating probe activated the SMRs, the evoked electrical response recorded in segmented nerves was not identical. Because wave stimulation consists of a series of wavelets, it is evident that each wavelet evokes one or more CAPs (Fig. 2D). Thus, waves provide stimulation equivalent to a series of rapid, non-uniform probe vibrations. This stimulation is much more intense than that provided by a single vibration of the probe.

Intact leeches have been shown to respond to water waves, which provide vibratory stimulation at about 10 Hz, by initiating swimming movements (Young *et al.* 1981). To examine whether the SMRs could be stimulated by the 1–2 Hz oscillations characteristic of leech swim undulations, we applied single, constant-amplitude, sinusoidal wave-forms rather than square pulses to the vibrating probe. As revealed in Fig. 3, SMR activity was detectable at stimulation frequencies as low as 1 Hz: stronger

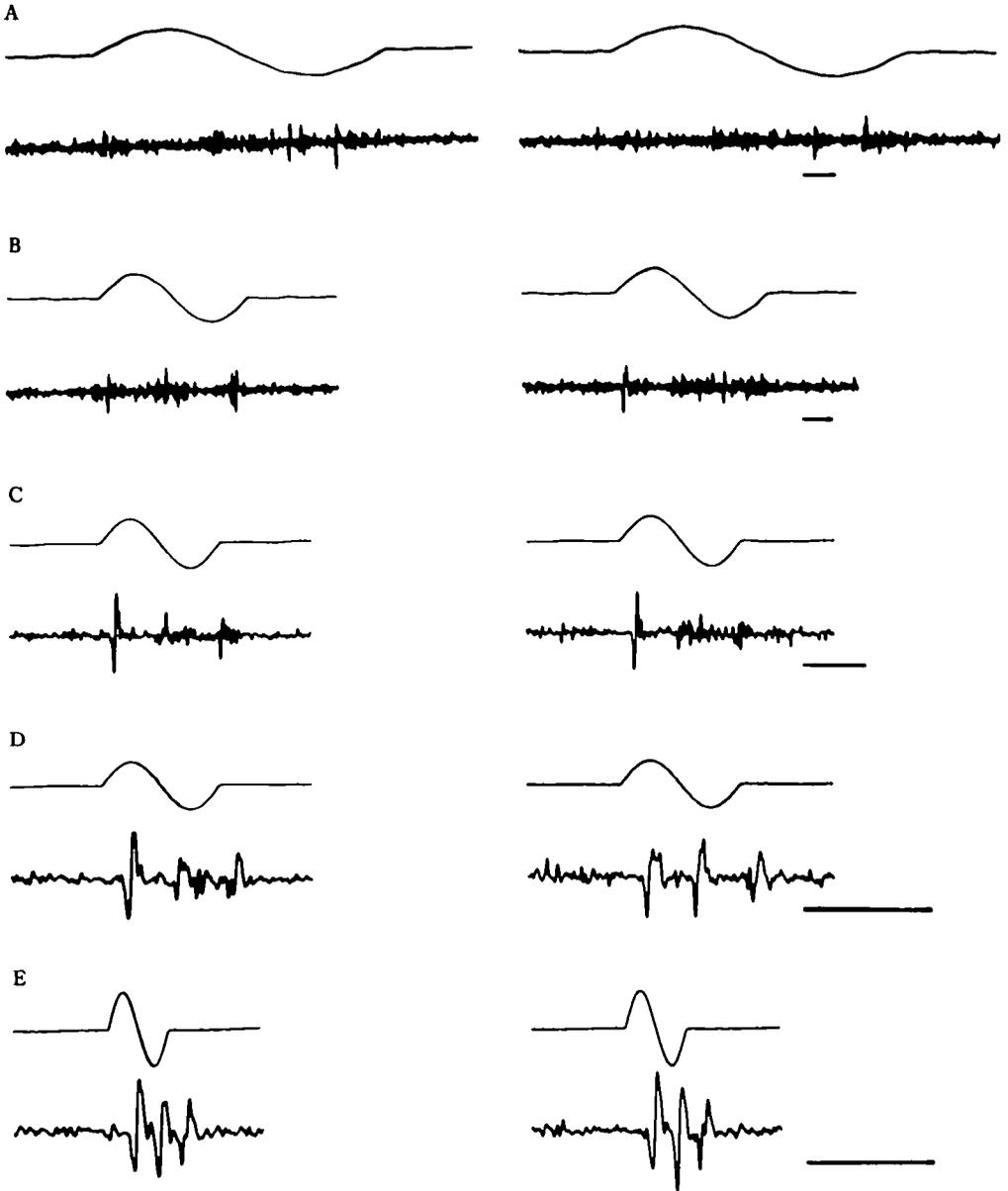


Fig. 3. DP nerve activity evoked by constant-amplitude sinusoidal stimulation. In each part of the figure, the upper trace shows the single-sine-wave movement of the probe, while the lower trace shows the CAPs recorded from the DP nerve. The frequencies employed are: (A) 1 Hz; (B) 2 Hz, (C) 5 Hz, (D) 10 Hz and (E) 20 Hz. The amplitude of the probe movement was about $75 \mu\text{m}$ at all frequencies. An isolated body wall flap from segment 14 was used in this experiment. The time calibration bars denote 100 ms in each part of the figure.

responses occurred at 2 and 5 Hz; while at 10 and 20 Hz the responses resembled a short segment of the wave-evoked response (compare Fig. 2D with Fig. 3D, E). A single sine wave with frequency greater than 40 Hz (not shown) produced a response similar to that produced by a square pulse. In addition, continuous sinusoidal

■ stimulation at 10 Hz produced activity similar to the neuronal response to wave stimulation. This type of stimulation, however, did not initiate swimming even in the presence of serotonin. Because SMR sensitivity does extend to frequencies as low as 1 Hz, leeches could experience self-stimulation resulting from their movements through the water.

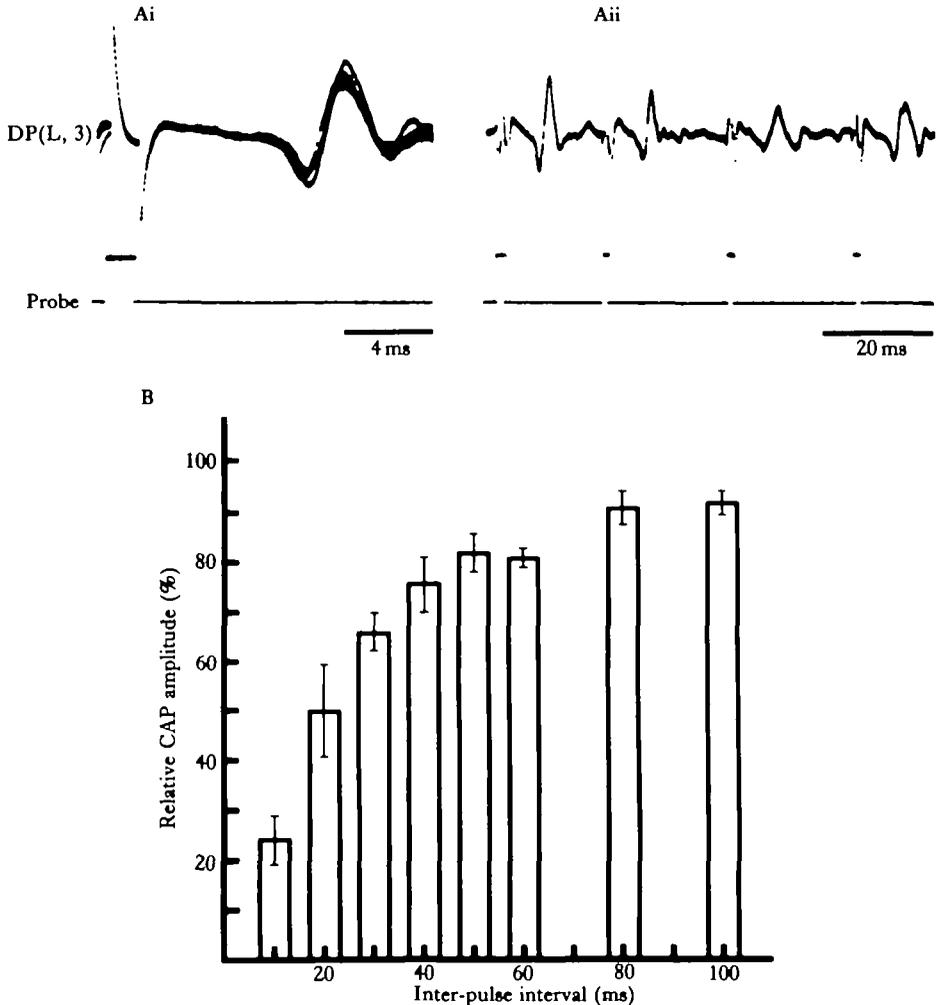


Fig. 4. SMR response to repetitive near-field stimulation. (Ai) Stimulation frequency 0.1 Hz. Five superimposed traces are shown. (Aii) 50-Hz stimulus train. Note that the response amplitude to the first stimulus in the train is larger than subsequent responses. The deflections in the DP nerve records coinciding with probe displacements are stimulus artifacts. (B) Graph of SMR response decrements obtained from two-pulse experiments. The ordinate is the ratio of the second to the first SMR responses, multiplied by 100. The standard deviation for each of the measurements is shown. The data for this figure were obtained from isolated body wall flaps.

The SMR response is usually variable

The SMR response to constant intensity near-field stimulation pulses was constant only at stimulation frequencies of less than 1 Hz (Fig. 4Ai). At high stimulation frequencies (25 Hz or greater), response amplitude was reduced and duration increased (Fig. 4Aii). At intermediate frequencies, no consistent changes in amplitude or duration were observed; however, the shape of the CAPs fluctuated considerably. Thus, during repetitive stimulation at rates above 1 Hz the synchrony of units comprising the CAP was reduced, and at high frequencies (above 25 Hz) not all units were activated by each stimulus, perhaps due to receptor adaptation. The decrement in CAP amplitude resulting from short inter-pulse intervals was examined by supplying paired electrical pulses to the stimulus probe. At inter-pulse intervals greater than 100 ms, the CAP amplitude is close (0.92) to the control value, in agreement with results from repetitive stimulation experiments (Fig. 4B).

There is no chemical synapse intervening between the SMRs and the axons mediating the DP nerve CAP

This was determined by applying high Mg^{2+} physiological saline (20–40 mM- Mg^{2+} , 1.8 mM- Ca^{2+}), which blocks chemical synapses centrally in the leech (Nicholls & Purves, 1970), to an isolated body wall preparation. The presence of high Mg^{2+} saline did not eliminate the SMR response in the DP nerve; however, high Mg^{2+} saline reversibly eliminated the neuronal activity evoked in the anterior and posterior connectives (Fig. 5). High Mg^{2+} saline did have a slight effect, however, on the SMR response in most preparations, in that high Mg^{2+} saline tended to decrease the amplitude and to increase both the latency and the duration of the SMR response in the DP nerve.

Site of action of serotonin

We show here that serotonin does not increase the sensitivity of the SMRs to near-field stimulation, but that serotonin does increase the DP nerve responsiveness to wave stimulation.

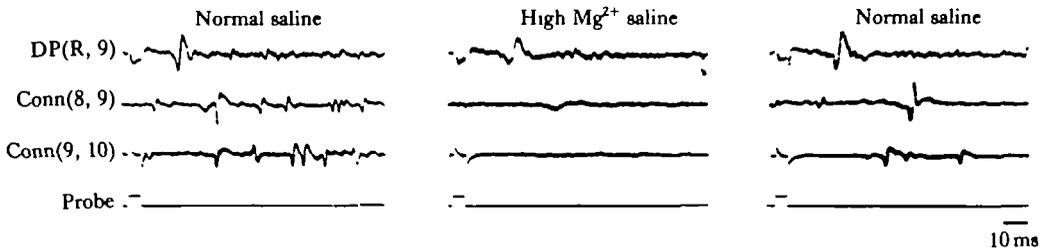


Fig. 5. Effects of high Mg^{2+} saline on the neuronal responses to near-field stimulation. The first and last panels show responses obtained in normal saline, while the middle panel shows the response obtained in 40 mM- Mg^{2+} saline. Note that the elevated Mg^{2+} did not block the DP nerve response, but reversibly eliminated evoked activity in the connectives anterior [Conn (8,9)] and posterior [Conn (9,10)] to the innervated ganglion (number 9). The preparation for this experiment consisted of a body wall flap attached to segmental ganglion 9 by DP (R, 9). The connective recordings are from the cut ends of the segmental connectives while the DP record is from an *en passant* suction electrode.

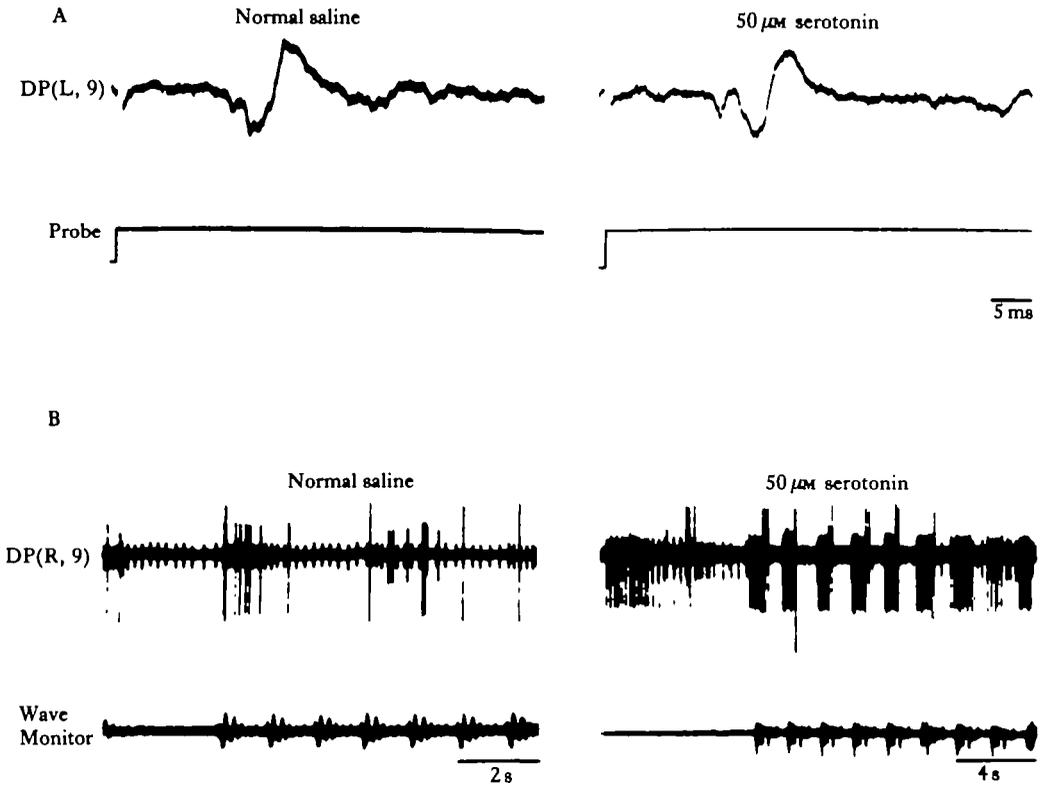


Fig. 6. Effects of serotonin on DP nerve activity. (A) SMR responses to near-field stimulation in normal saline (left traces) and in saline containing $50 \mu\text{M}$ -serotonin (right traces). No large difference can be observed between the two records. (B) DP nerve responses to repeated wave stimulation. The largest spikes in the DP nerve traces are impulses from a dorsal excitator neurone (cell 3). In the absence of serotonin, only occasional cell 3 impulses are elicited by each wave packet (lower trace), while bursts of cell 3 impulses are evoked by each wave packet when $50 \mu\text{M}$ -serotonin is added to the saline. The records in A were obtained from an isolated body wall flap, while those of B were obtained from a non-swimming, posterior semi-intact preparation.

Serotonin does not increase the sensitivity of the SMRs to near-field stimulation

In four of five experiments, the presence of $50 \mu\text{M}$ -serotonin altered neither the amplitude nor the duration of the DP nerve CAP (Fig. 6A). In the remaining experiment, serotonin produced a small (about 25%) increase in the CAP amplitude. In addition, both the threshold sensitivity of the SMRs and the variability of the DP nerve responses at high stimulation rates appeared to be unchanged. Thus, serotonin had little or no effect on the transduction of vibratory stimulation by the SMRs. Furthermore, serotonin had no consistent effect on the evoked activity in the connectives to near-field stimulation (not shown). However, serotonin generally produced an increase in the spontaneous activity of the connectives. Thus serotonin must be acting centrally in the ventral nerve cord.

The motor neurone response to wave stimulation increases in serotonin

In normal saline, SMR activation has been shown to produce no reliable effect on the motor neurones involved in swimming (Friesen, 1981), as can be seen in cell 3 activity recorded in the DP nerve (Fig. 6B, left traces). When serotonin was added to the preparation, surface waves evoked bursts of cell 3 spikes (Fig. 6B, right traces). The observed cell 3 response in the DP nerve was not a wave-induced swimming episode because: (1) the interval between cell 3 bursts could be much greater than 2000 ms and (2) upon cessation of the wave stimulation the cell 3 bursts discontinued. Thus in the presence of serotonin there was a strong excitatory input to cell 3 from the SMRs that was not evident in normal saline.

DISCUSSION

Swim initiation via SMR stimulation

Even with serotonin present in the saline, swimming in response to water vibrations occurs only in response to wave stimulation and not to near-field stimulation of one or two sensilla. The most likely explanation is not that wave stimulation is more intense but, rather that it is much more wide-spread (up to 130 sensilla may be stimulated by each wave packet in a semi-intact preparation). Since near-field stimulation does activate the SMRs, this form of controllable stimulation can be used to identify neurones receiving input from wave stimulation.

The specific central mechanisms mediating water-vibration-initiated swimming activity remain undiscovered. Two conclusions can be drawn from these experiments, however. First, neither the head nor the tail ganglia are required for swim initiation by the SMRs. Second, because swim initiation frequently requires the presentation of several wave packets, there must exist central neuronal mechanisms capable of summing the sensory excitatory input over a span of several seconds. The means for such prolonged summation is likely to involve an excitatory network rather than to depend on an individual neurone. The swim facilitating action of serotonin may act on such a network to change synaptic efficiency, membrane excitability or to prolong either synaptic or membrane events. Although several neurones that receive strong excitatory input from SMR stimulation have been identified, their role in swim initiation has not been determined (Friesen, 1981; W. O. Friesen, E. A. Debski & P. D. Brodfuehrer, unpublished observations).

The physiological role of serotonin in intact leeches is unknown. However, there is a strong correlation between the probability of spontaneous swimming in both intact leeches and isolated ventral nerve cords and the level of serotonin bathing the nerve cord (Willard, 1981). In addition, we have shown that swim initiation by SMR stimulation in dissected leeches is facilitated by the addition of serotonin to the saline. There are two aspects of the role of serotonin in the initiation of swimming that are common to both studies. First, serotonin acts centrally in the ventral nerve cord, since neither mechanosensory nor SMR sensitivity is altered in the presence of serotonin, and second, serotonin has a long-term effect on increasing the probability of swimming. However, unlike the negligible effect serotonin has on responses to tacti-

mechanosensory stimuli (Willard, 1981), serotonin induces pronounced changes in the response to SMR stimulation in the segmental ganglia. Thus, it appears that serotonin modulates sensory effects on swimming from the SMRs but not from the tactile mechanoreceptors.

Surface waves and near-field stimulation activate the SMRs

A comparison of the neuronal activity evoked in the DP nerve by wave and near-field stimulation indicates that both methods of producing rapid water vibrations are activating the same receptors. First, the site of sensory transduction is localized at the sensilla by both stimulation procedures. Second, the neuronal responses evoked in the segmental nerves by wave and near-field stimulation are compound action potentials. Third, the maximum amplitude and duration of the spikes evoked by wave stimulation are 150 μV and 10–15 ms respectively (Friesen, 1981 and Fig. 2D). These values are similar to the maximum amplitude and duration of the neuronal activity evoked by near-field stimulation. Fourth, nerve impulses are evoked in the connectives by both near-field and wave stimulation and this activity travels both anteriorly and posteriorly along the nerve cord. Lastly, near-field stimulation activates the same identified segmental neurones as wave stimulation (E. A. Debski & W. O. Friesen, unpublished observations). Therefore, near-field stimulation activates the same receptors, the SMRs, as wave stimulation. Of the morphologically identified structures at the sensilla, the uniciliate cells (S-hairs) (DeRosa & Friesen, 1981; Phillips & Friesen, 1982) are the best candidates for transducing water vibrations.

Do SMR axons project to the ventral nerve cord ganglia?

The experiments demonstrating that elevated Mg^{2+} does not block the SMR response provide additional evidence that the SMRs are primary sensory neurones that have a direct (without synapse) input to neurones of segmental ganglia. Previous morphological examinations of segmental sensilla at the ultrastructural level (Phillips & Friesen, 1982) have failed to reveal any morphologically identifiable synapses associated with the ciliated receptor cells. Our physiological experiments, while they do not rule out the presence of an electrical junction in the pathway from SMRs to the ventral nerve cord, indicate that no chemical synapse intervenes. Since the potentials recorded in the DP nerve follow stimulation activity at rates up to 10 Hz and, with some amplitude fluctuations, up to 50 Hz even with elevated Mg^{2+} concentrations, the electrical synapses, if present, must act as faithful relay points. Thus, physiologically, it would appear to be of little consequence if there are electrical synapses between the SMRs and the axons in the DP nerve. However, there may be a synapse between the SMRs and ventral nerve cord axons since SMR evoked nerve cord impulses were blocked by elevated Mg^{2+} .

Variability of the DP nerve CAP

We have demonstrated two types of variability in the amplitude of the SMR response. First, when the intensity of near-field stimulation is varied, the evoked DP nerve CAP amplitude appears to be continuously graded. We propose that the size of the CAP reflects the summed response of some fraction of the approximately 40

uniciliate receptors found at dorsal sensilla (DeRosa & Friesen, 1981; Phillips & Friesen, 1982) and that this fraction is a function of stimulus intensity. The CAP appears to be continuously graded because the response amplitude of a single uniciliate receptor is only $7.5 \mu\text{V}$, less than the quiescent noise level of our recording system (about $10 \mu\text{V}$). The unit response amplitude was calculated by dividing the maximum CAP amplitude ($300 \mu\text{V}$) by the number of uniciliate receptors observed at dorsal sensilla (about 40). Second, variability in the CAP amplitude was observed at moderate to high stimulation rates. We believe that this variability reflects threshold fluctuations in individual uniciliate receptors during repetitive stimulation. Since the threshold range for near-field stimulation is quite restricted (a seven-fold change in probe displacement from $15 \mu\text{m}$ to $110 \mu\text{m}$ produces the whole range of CAP amplitudes from minimally discernible to the maximum SMR response) even small changes in receptor adaptation could cause large CAP fluctuations.

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