PHYSIOLOGY OF WATER MOTION DETECTION IN THE MEDICINAL LEECH

By W. OTTO FRIESEN

Department of Biology, University of Virginia, Charlottesville, Virginia 22901, U.S.A.

(Received 12 September 1980)

SUMMARY

1. Neuronal activity resulting from stimulation by water waves occurs in ventral nerve cord–body wall preparations of the medicinal leech, Hirudo medicinalis. In segmental nerves, this activity consists of afferent compound action potentials with graded amplitudes resulting from simultaneous action potentials in many small sensory axons. Afferent input impinging on one segmental ganglion activates neuronal activity along much of the ventral nerve cord.

2. Previously identified tactile mechanoreceptors are insensitive to low-amplitude wave stimulation. Touch-cell impulse activity can be evoked by moderate or strong wave stimulation, but these impulses appear to arise near the cell body, not from the peripheral receptor endings.

3. The transduction sites for wave stimulation are localized at or very near the segmental sensilla. Because of their location and modality the receptors were named ‘sensillar movement receptors’ (SMR).

4. S cells (Rohde’s fibre) receive suprathreshold excitatory input during SMR activation without concomitant activity in the tactile mechanoreceptors.

5. The annulus erector motor neurones contralateral to the afferent SMR inflow are inhibited by SMR activation. This inhibition is also observed in ganglia adjacent to the ganglion receiving the afferent input and provides a neuronal basis for reflexive smoothing of the leech body wall.

6. Two neurones in the anterior median packet of segmental ganglia receive powerful synaptic input during SMR activation. One, cell 202, receives 10 mV excitatory potentials while the other, cell 201, receives 10 mV inhibitory potentials.

INTRODUCTION

The neuronal mechanisms generating swimming movement in the leech are understood well at several levels. At the output level, the motor neurones have been identified, their activity cycles described and their interconnexions mapped (Kristan, Stent & Ort, 1974a, b; Ort, Kristan & Stent, 1974). The findings are that eleven pairs of segmentally repeated inhibitory and excitatory ganglion cells innervate the longitudinal muscles of the body wall, and that the approximately antiphase activity cycles of these dorsal and ventral motor neurones give rise to the alternate contractions and distensions of the dorsal and ventral body wall muscles, which in turn
produce the undulations comprising the leech swimming movements. It has been shown that, while the electrotonic connexions between these motor neurones could serve to synchronize synergists and while the inhibitory connexions linking inhibitors to exciters could phase the latter, the neural origin of the swimming rhythm must lie elsewhere. Following the demonstration that the swimming rhythm could be obtained in the isolated ventral nerve cord (Kristan & Calabrese, 1976) an ensemble of oscillatory interneurones was identified in ganglia of the ventral nerve cord with the requisite activity patterns and output connexions that would largely account for the motor neurone activity patterns (Friesen, Poon & Stent, 1978; Poon, Friesen & Stent, 1978; Stent et al. 1978). Modelling of the circuit formed by the interactions between these oscillatory interneurones demonstrated that this neuronal circuit could largely account for the rhythmic activity of the interneurones and hence for the origin of the leech swimming rhythm (Friesen & Stent, 1977).

At another level, namely that of control — initiation, maintenance and termination — the swimming rhythm is less well understood. While mechanical manipulation of the body wall can clearly modify the swimming pattern (Kristan, 1974; Kristan & Calabrese, 1976, see also Gray, Lissmann & Pumphrey, 1938) neither the peripheral sensory receptors stimulated by these manipulations nor their central targets have yet been identified. Within the ventral nerve cord an interneurone (cell 204) whose depolarization reliably leads to swimming activity has now been identified (Weeks & Kristan, 1978), but despite repeated searches no connexions to the identified motor neurones or oscillatory interneurones have as yet been discovered (J. C. Weeks and W. O. Friesen, unpublished results).

Swimming activity in the semi-intact leech preparation (anterior and posterior body ends connected via the ventral nerve cord) can be initiated by light stroking of the ventral body wall (Kristan et al. 1974a). In the isolated nerve cord, swimming activity can be initiated via electrical stimulation of segmental nerves (Kristan & Calabrese, 1976). It is not known which receptors, in the case of mechanical stimulation, nor which axons, in the case of electrical stimulation, are activated to induce the swimming activity. The effective cells are unlikely to be the previously identified tactile mechanoreceptors (Nicholls & Baylor, 1968) because no connexion between these cells and the swimming circuit has ever been identified, nor does direct stimulation of these mechanoreceptors lead to swimming activity. What then are the sensory processes responsible for initiating leech swimming activity and how does their activation interact with the rhythm-generating circuit?

It has been known since the last century that leeches have specialized sensory structures called sensilla. Fourteen sensilla, which appear as small, light spots, are distributed circumferentially over the middle annulus of each mid-body segment. Segments in the head and tail regions appear to have fewer sensilla (Whitman, 1886). Recordings from sensillar nerves have recently confirmed the earlier morphological suggestion that the sensilla mediate photoreception (Kretz, Stent & Kristan, 1976). Photomicrographs reveal that filiform processes project from the sensilla through the cuticle of the body wall (Mann, 1962). The similarity of these processes to the structures known to mediate nearfield reception in other animals (Markl, 1977) suggests that the sensilla may be sensitive to this form of stimulation also.
Motion detectors in leeches

It has been shown recently (Friesen & Dedwylder, 1978; W. O. Friesen, S. Young & R. D. Dedwylder, in preparation) that medicinal leeches respond to water waves by initiating swimming movements. These are directed movements in that the leeches direct their swimming course towards the source of the waves. While not a simple reflex, i.e. the leech response to the waves appears to depend on a ‘readiness state’, the swimming response can be reliably obtained in selected animals which are in some, as yet ill-defined, excited state. At present this state can be best obtained by handling the leech or by inducing swimming activity shortly before a behavioural trial. Wave stimulation therefore provides a ‘natural’ means of evoking swimming movements in the leech.

This paper is a report on experiments designed to elucidate the mechanisms by which the stimulus provided by wave stimulation is detected and processed in leech mid-body segments. Experiments were carried out to determine (1) the nature of the sensory stimulus, (2) the sensory transduction loci, (3) the path of the sensory information flow from the external body wall to the ventral nerve cord and (4) the identity of target neurones in ventral cord ganglia. These experiments revealed the existence in the medicinal leech of a previously undescribed sensory modality (sensitivity to water movements) that is mediated by receptors located at the segmental sensilla. Neuronal impulses are conducted from these receptors via segmental nerves to neurones of segmental ganglia. An abstract of some of the results presented here has been published earlier (Friesen & Dedwylder, 1978).

MATERIAL AND METHODS

Medicinal leeches, Hirudo medicinalis, were obtained from a commercial distributor and maintained in small aquaria at 20 °C for periods of up to one year. The animals were fed four times a year on frogs or rabbits.

Several physiological preparations were employed for these studies: (1) a body-wall preparation consisting of a flap of body wall with attached segmental nerves; (2) a nearly isolated ventral nerve cord preparation consisting of a chain of segmental ganglia one or more of which innervated a flap of body wall (Fig. 1), and (3) a more nearly intact preparation consisting of some segments of intact leech attached to a chain of segmental ganglia. The procedures for obtaining these preparations have been described previously (Kristan et al. 1974a; Kristan & Calabrese, 1976). Extracellular records were obtained with suction electrodes, and intracellular recordings were obtained with standard glass microelectrodes. The recording chamber was filled to a depth of 0.5–1.0 cm with leech physiological saline (115 mM-NaCl, 4 mM-KCl, 1.8 mM-CaCl₂, 2 mM-MgCl₂, 10 mM Hepes buffer).

The nomenclature of Ort et al. (1974) is used in this paper to identify segmental nerves and their branches. Thus the large nerve branch arising from the posterior root of segmental ganglia to innervate the dorso-lateral body wall is labelled as the DP (dorsal-posterior) nerve, and the second branch arising from the DP nerve is labelled DP: B2. The seven pairs of segmental sensilla are numbered sequentially from the most ventral to the most dorsal body aspect according to the scheme of etz et al. (1976). The labelling of somata positions in the anterior median packet of the ventral aspect of segmental ganglia was adapted from the diagram of Weeks &
Fig. 1. Body wall–nerve cord preparation. Several variations of this preparation were employed for these experiments. The specific preparation shown consists of a flap of body wall extending several segments, from the ventral to the dorsal midlines. The body wall is innervated by the segmental nerves of a ventral cord ganglion, the ventral aspect of which is uppermost. The black dot to the left indicates the impact point for falling water drops and is the origin for radiating surface water waves. This preparation permits recordings of nerve impulses to be obtained en passant by suction electrodes while intracellular potentials are obtained by microelectrode penetrations of nerve cell somata. Other preparations employed in this study differed from the one illustrated in the number of body wall segments included, the length of the ventral nerve cord included, the number of innervated body wall segments, the number of nerves connecting the body wall to the ventral nerve cord and the location of the drop impact point with respect to the body wall. The drawing is not to scale.

Kristan (1978, Fig. 1 B), which indicates the approximate positions of soma profiles.

Water waves (to stimulate the leech body wall) with controlled amplitudes were created in the saline-filled experimental chamber, after the technique of Mistick (1978), by allowing water drops (volume per drop: 0.04 ml) to fall from a controlled height at a predetermined distance from the leech body wall flap. The waves generated by the drop impact propagate as a packet in which the wavelets have neither uniform amplitude nor equal velocity. Measurements were made to determine the maximum amplitudes above the quiescent water level of the wavelets for several dropper heights and for two distances from the drop impact point to the innervated body wall. To obtain these amplitudes, a microelectrode attached to a calibrated micromanipulator was lowered until it just made contact with the quiescent saline. The setting of the manipulator was noted and the electrode raised. Wave activity was then initiated and the microelectrode lowered again until it just recontacted the saline. The difference between this manipulator setting and the setting for the quiescent saline gives the maximum wave amplitude. These measurements yielded a maximum wave amplitude of nearly 600 µm for a drop falling through 12 cm, 2 cm from the measuring point. The lowest wave amplitude of 40 µm was obtained with a dro
Motion detectors in leeches

Bling through 0.2 cm, 10 cm from the measuring point. Rough estimates of wave amplitudes between the measured values were obtained by interpolation. The values obtained for wave amplitudes can yield no more than a qualitative measure of stimulus intensity since water depth, which is a crucial factor in determining the amplitude of water movements at the leech body wall, could change by several millimetres during an experiment. In addition wavelet frequency, which was not measured, could also be an important factor determining stimulus efficacy. Although difficult to measure precisely (because each wavelet travels at a slightly different velocity), the velocity of the wave packet was determined to be in the range from 25 to 33 cm/s.

The occurrence, but not the precise timing, of the waves was monitored with a light-phototransistor system. A small beam of light was directed at the water at a glancing angle, the reflected light from the saline surface being directed at a phototransistor. Waves deflect the beam, thereby producing a variable light signal at the phototransistor. Because the light was not focused to a point and because the locus at which the beam strikes the water changes as the saline level changes, this technique for monitoring wave motion does not allow for precise timing measurements to be obtained.

Electrical signals obtained from these experiments were recorded with an 8-channel FM tape recorder (Crown-Vetter) and later reproduced at a fourfold reduction in tape speed on a 4-channel Brush chart recorder. Used in this manner the frequency response of the system is adequate to record both intracellular and extracellular signals without significant distortion.

RESULTS

Neuronal response to surface-wave stimulation

Previous behavioural experiments have shown that intact leeches can respond to the water movements created with wave stimulation by initiating swimming movements (Friesen & Dedwylder, 1978; W. O. Friesen, S. Young & R. D. Dedwylder, in preparation). Since the transduction sites for this stimulus might be anywhere on the leech, experiments were carried out using a body-wall-nerve preparation (see Methods) with suction electrodes attached to segmental nerves to detect and map the neuronal signals evoked by wave stimulation. These experiments revealed that neuronal spiking activity resulting from this type of stimulation occurs in both the anterior and posterior roots of segmental ganglia in all segments of the ventral nerve cord assayed so far; i.e., from the second abdominal segment to the seventeenth abdominal segment. Specifically, neuronal spiking activity associated with wave stimulation occurs in the anterior (A) nerve root and the dorsal branch (DP) and the posterior branch (PP) of the posterior nerve root, although spiking activity in the PP nerve could only be detected in two experiments. Records obtained from several of these experiments can be seen in Fig. 2, where the lower trace of each panel is the output of a phototransistor (Ph Tr) monitoring the waves (created by fallen drops), while the remaining traces are records of electrical activity obtained from suction electrodes placed on the nerves indicated.

The left portions of records of Fig. 2(a–c) show that spiking activity is evoked
Extracellular recordings of nerve spikes evoked by wave stimulation. 

(a) Top traces were obtained from suction electrodes attached to the cut ends of the posterior nerve (PP), dorsal-posterior nerve (DP), and the anterior root (A) (attached to the body wall), respectively, in three experiments. The lower trace, marked Ph Tr in this and all succeeding figures, shows the output of a phototransistor and indicates the occurrence of surface water waves by upward or downward deflexions. The left portions of these three panels demonstrate that spiking activity is evoked by wave stimulation in all three electrode recordings obtained from three different body segments. The right portions are recordings from the same nerves after the nerves had been crushed at a point between the recording site and the body wall. Note the absence of spikes in all records following this procedure. 

(b) The top two traces are recordings from the connectives between the sixth and seventh segmental ganglia (Con (6, 7)) and between the twelfth and thirteenth ganglia (Con (12, 13)) in a preparation in which the only nerve innervating the body wall was a DP nerve of the ninth segmental ganglion. The records show that stimulation produces spiking activity in a considerable length of the ventral cord. The time calibration applies to all traces.

With each series of waves detected by the phototransistor, the waves persist for a variable time following their creation (the later waves result from multiple reflexions at the walls of the chamber). Likewise the spiking activity evoked by these waves continues for a variable time span, from several hundred ms to several s. Under favourable recording conditions the spikes can have a maximum amplitude of up to 150 µV while the smallest spikes have amplitudes only slightly larger than amplifier noise. Durations of the individual spikes also show a great deal of variability for reasons that are discussed below.

To eliminate the possibility that the electrode spikes are mere artifacts resulting from the effects of water movements on the suction electrodes, the nerves were crushed between the electrode and the body wall, thereby interrupting neuro
Motion detectors in leeches

Fig. 3. Spike activation latencies. Each panel shows four records obtained simultaneously from the cut ends of nerves innervating body wall segments 7, 11, 14 and 17. (a) The drop was released anterior to the body wall giving rise to waves travelling in the posterior direction; (b) the drop was released posterior to the body wall with the waves travelling in the anterior direction. The arrows at the first and last traces indicate the onset of SMR activity in DP (R, 7) and DP (R, 17). The calculated velocity of the excitation source (distance between segments divided by spike onset latency) is approximately equal to the propagation velocity of the surface waves. Calibration applies to both panels a and b.

Impulse traffic. As can be seen in the right portions of Fig. 2(a–c) this procedure effectively ended all recorded spiking activity in each electrode record. In another experiment, not illustrated here, the spiking response to drop stimulation was similarly ended by exposing the preparation to 15% ETOH. Both procedures demonstrate that the electrode spikes are not simply movement artifacts; rather, they are neuronal activity evoked by wave stimulation.

Extracellular recordings from nerve cord connectives reveal that the nerve impulse activity evoked by drop stimulation can spread from a single ganglion to most of the ventral nerve cord. Clearly, the water waves recorded in the lower, phototransistor trace evoke spiking activity in nerve cord at least two segments anterior and three segments posterior to the innervated body wall. In other experiments not illustrated here, spiking activity evoked in the ninth segment was detected in the ventral nerve cord connectives both nine segments anterior and nine segments posterior to the innervated segment. Thus spiking activity evoked in one segment can spread throughout much of the ventral nerve cord.

What is the nature of the sensory stimulus? Although it is apparent that the nerve spikes illustrated in Fig. 2 are causally related to the wave stimulation, it remains to be shown whether the effective stimulus is the sound waves (velocity, approximately 1500 m/s) or the surface water waves (velocity, approximately 0·3 m/s). In a previous study to examine the effects of wave stimulation on leech touch cells and Rohde’s fibre, Mistick (1978) concluded that the immediate stimulus was the slowly travelling surface waves. In the present experiments, also, the surface wave is unequivocally the effective stimulus as shown by the following experiment. The entire segmental body wall, excluding the head and tail, was dissected from a leech. The DP nerves from body segments 7, 11, 14 and 17 were dissected free and, with the body wall pinned out lengthwise in a Silgard-covered dish, suction electrodes were attached simultaneously to these four nerves. Water drops were then released from a height of
Fig. 4. Evoked spikes in DP:B3 (R, 8). Each trace is the response evoked by a separate but identical presentation of wave stimulation. The records are lined up so that all have the correct temporal relationship to the stimulus shown by the bottom trace (Ph Tr). Note the variability in the size, shape and duration of the large spikes in the top four records. These spikes are compound action potentials resulting from the summation of many small spikes whose unit amplitude is near that of the amplifier noise.

7 cm anterior or posterior to the body wall. With the drop released anterior to the body wall (Fig. 3a), spike activity is seen in the DP nerves with progressively greater latencies, anterior to posterior (the arrows in Fig. 3 indicate the beginning of activity). Conversely, with drops released to the posterior (Fig. 3b) spike activity appears first in the most posterior DP nerve record and only later in records from more anterior DP nerves. The large latency differences for the initiation of spiking activity in the four DP nerves are apparently due to the low conduction velocity of the stimulus wave. The velocity of this wave can be determined approximately by dividing the distance between segment 7 and segment 17 (7.0 cm) by the latency difference for spiking activity in segments 7 and 17. With waves travelling towards the posterior this latency is 280 ms and the calculated wave velocity is 0.25 m/s. For waves travelling towards the anterior the latency is 290 ms, yielding a wave velocity of 0.24 m/s. By each measure the stimulus velocity is therefore about 0.25 m/s. The velocities of the surface waves in the dish, measured directly, ranged from 0.25 m/s to 0.33 m/s. Evidently, the spikes in the DP nerves are evoked by the low-velocity surface waves and not the much higher velocity sound waves.
Two lines of evidence point to the conclusion that the nerve spikes resulting from wave stimulation are compound action potentials resulting from the summed electrical currents generated by a large number of small nerve impulses. First, recordings made during repeated stimulation reveal no large, constant-amplitude waveforms. Instead, as Fig. 4 illustrates, the large spikes evoked by each stimulus differ from the others substantially, both in duration and in amplitude. If these large spikes represent individual units there would have to exist a relatively large number of axons with markedly different spike durations and wave forms. It is more likely that the differences in large-spike shapes and amplitudes are due to the summation of many small spikes occurring nearly simultaneously. Candidates for these small, unit spikes are the smallest spikes occurring in the records of Fig. 4. Summation of about ten to fifteen of these unit spikes could generate the largest recorded spikes. The record of Fig. 5b resembles the records obtained from sensillar nerve recordings by Kretz et al. (1976, fig. 12A). The spikes in their records, which were evoked by photic stimulation, are evidently due to the summed activity in a number of photoreceptor cell axons. The amplitudes of the individual photoreceptor axon spikes and the unit spikes evoked by wave stimulation appear to have nearly equal amplitudes.

Second, the nerve response amplitude is graded with changes in stimulus amplitude. Small waves with maximum amplitudes of about 50 μm evoke nerve activity consisting of small spikes only (Fig. 5a). Progressively larger wave amplitudes (100, 200 and 500 μm) evoke progressively larger spikes, as illustrated in Fig. 5b, c, d. Since the records contain no recognizable unit spikes larger than those of very small size, it appears likely that the larger spikes evoked by the larger waves reflect the near-synchronous activity of more units rather than larger units with higher thresholds. (The largest spike, occurring once for each wave stimulation, trace d, is a touch cell impulse. The true recorded amplitude of the touch cell spikes was actually greater than shown here since they were clipped by the recording equipment.) The large spikes recorded in segmental nerves in response to wave stimulation are then most likely the result of simultaneous impulse activity in many (at least ten) axons with a range of activation thresholds.

Previously identified mechanoreceptors do not mediate response to wave stimulation

Touch cells in the leech can respond to water movements (Nicholls & Baylor, 1968) or, more specifically, to leech body-wall deformations resulting from wave stimulation (Mistick, 1978). Using this form of stimulation, Mistick found that impulses could be reliably evoked both in touch cells directly innervating a flap of body wall (via sensory endings in the body wall) and in touch cells whose axons did not innervate the body-wall flap (indirectly, presumably via synaptic input). These experiments suggested that the leech swimming response to wave stimulation might also be mediated by touch cells. Evidence presented above (Fig. 5) demonstrates that, while touch cells can be activated by wave stimulation of sufficient intensity, less intense stimulation evokes spikes in axons other than those of the touch cells. More direct evidence that the low-threshold nerve impulses are not those of any of the previously identified mechanoreceptors was obtained with intracellular recordings.
Fig. 5. Graded nature of the response to wave stimulation. These records were obtained from a suction electrode placed on the DP (R, 4) nerve attached to a flap of body wall. The maximum amplitude of the surface wave at the innervated body wall was about 50 μm for panel (a), 100 μm for panel (b), 200 μm for panel (c) and about 500 μm for panel (d). Each panel shows the SMR response from two stimulus presentations (onset at arrows). The maximum spike amplitudes increase with increasing stimulus amplitudes until in panel (d) a touch cell spike (much larger than the other spikes) is evoked by each stimulus presentation. The calibration applies to all traces.

from a nearly isolated nerve-cord preparation. Intracellular records were obtained from all fourteen previously identified mechanoreceptors both from those ipsilateral and those contralateral to the innervated body wall flap during periods of wave stimulation. Of all the mechanoreceptor cells examined, the touch cells were the only ones ever to respond to wave stimulation with impulse activity. In the pressure and nociceptive cells, this form of stimulation gave rise, at most, to small membrane potential changes (Fig. 6b, c).

The record shown in Fig. 6a was obtained while recording from a touch cell ipsilateral to the innervated body-wall flap during three presentations of wave stimulation. The first stimulus (maximum wave amplitude about 200 μm) evoked an impulse in this cell, while subsequent stimuli did not. Each stimulus presentation did, however, produce a depolarization of about 3 mV, which at the first presentation evidently exceeded threshold to evoke the action potential. Notice that this action potential was preceded by a depolarizing prepotential, unlike the two action potentials evoked by touching the body wall, at the right end of the record. These latter potentials arise directly from the baseline with no prepotential. In numerous recordings from
Motion detectors in leeches

Fig. 6. Recordings from previously identified mechanoreceptors during wave stimulation and touch. The preparation consisted of a flap of body wall innervated by the eighth segmental ganglion. The left portion of each panel shows the effects of three presentations of wave stimulation while the right portion shows evoked or spontaneous intracellular action potentials. All three recordings shown here were obtained from mechanosensory neurones ipsilateral to the innervated body wall. Panel (a) The left portion of this record shows that wave stimulation can depolarize cell T sufficiently to give rise to an action potential. Impulses evoked by light touch to the body wall, right portion of panel (a), rise from the base line with no prepotential. Panel (b) The left portion of this record shows that cell P is unaffected by wave stimulation. It does respond briskly to pressure applied to the innervated body wall (right portion of the record). Panel (c) The left portion of this record shows that no impulse activity is evoked in cell N from wave stimulation. The right portion shows spontaneous impulse activity following the injection of hyperpolarizing current. The time calibration applies to all traces.

(touch cells during wave stimulation, action potentials without the depolarizing prepotentials were not observed, except in those experiments in which the saline drops were allowed to fall directly on to, or within a few millimetres of, the innervated body wall. It seems likely, therefore, that the touch cell action potentials evoked by wave stimulation arise from excitatory synaptic input rather than from direct stimulation of the sensory endings.

The extracellular recording shown in Fig. 5d demonstrates that spikes in touch cell axons innervating the major receptive field (the largest spikes in this record) are not those of the low-threshold axons responding to wave stimulation. The touch cell axons innervating the minor receptive fields, on the other hand, give rise to small nerve spikes and therefore could contribute significantly to the nerve response to wave stimulation (Yau, 1976). This is not likely to occur, however, because there is no evidence that the minor fields are more sensitive than the major fields to sensory stimulation. In addition, the number of touch cell axons in any nerve innervating the
Fig. 7. Location of maximum sensitivity to wave stimulation. Records obtained from the cut ends of DP (R, 9) innervating a flap of body wall. The hatched bars on the left indicate the timing of the stimulation of the body wall with a vibrating water jet, while the solid bars on the right indicate the timing of light touch applied to the body wall with the vibrating nozzle. (a) Stimuli applied to annulus immediately anterior to the middle annulus of body wall segment 9. Note that no spikes are evoked by water jet stimulation (left) but that light touch evokes several touch cell spikes (right). (b) Stimuli applied to middle annulus of body wall segment 9. The response is vigorous and continues without apparent adaptation (left) while the response to light touch evokes both small spikes and the much larger touch cell spikes (right). (c) Stimuli applied to annulus immediately posterior to the middle annulus of body wall segment 9. Again, a response occurs only to light touch. The calibration applies to all traces.

minor fields is no more than four, a number that is too small to account for the nerve activity evoked by wave stimulation.

Transduction of wave stimulus occurs at the sensilla

The identity of the low-threshold mechanosensory structures remains to be determined. The location of these receptors was investigated in both body wall preparations and with nearly isolated ventral nerve cord preparations. Recordings were then obtained from either the cut ends of nerves via suction electrodes or from neuronal somata via intracellular electrodes, while the body flap was stimulated with a fine jet of saline. This saline jet could be aimed selectively and precisely at any part of the body wall flap. In all of these experiments the force of the jet was controlled so as to prevent activation of the touch cells. A constant saline jet proved to be a relatively ineffective stimulus for activation of the water-movement-sensitive receptors. Imposing low-amplitude lateral vibrations on the nozzle producing the jet (by gentle tapping), however, greatly increased its efficacy and allowed for reliable mapping of the receptive field for water movements.

The body area most sensitive to saline jets, excluding the head and tail regions which were not studied, is the middle annulus of the five annuli that ring each mid-body segment in Hirudo; that is, the annulus bearing the sensilla. Fig. 7 illustrates the results of one experiment in which a vibrating jet was aimed at the middle annulus (b) and the two adjacent annuli (a, c) of a body wall segment. With the jet
Fig. 8. Localization of the sensory receptors. Records obtained from the cut ends of indicated nerves attached to the body wall of segment 10. (a) Records obtained during two presentations of wave stimulation with sensilla S6 and S7 intact: both nerves respond vigorously. (b) Records obtained with both S6 and S7 excised, showing that no response to wave stimulation remains. (c) Records obtained immediately after those in panel (b), showing that touching the body wall (at arrows) can still evoke spiking activity in these nerves following excision of the sensilla, thereby demonstrating that this procedure does not damage the nerves. The calibration applies to all traces.

aimed at the middle annulus, bursts of large spikes, evoked by each vibration of the jet, were recorded from the cut end of the DP nerve, while few or no spikes are evident in records obtained when the jet was aimed at the two adjacent annuli. The right portions of the three records show touch cell impulses elicited in the DP nerve when the jet nozzle, a broken micropipette, was allowed to contact the body wall. As in other records, these touch cell spikes are large and of uniform amplitude; they can therefore be easily distinguished from the smaller and non-uniform spikes evoked by water movements. Note that the receptive field for the touch cell (in this case the dorsal touch cell) extends over all three annuli while the receptive field for the receptors sensitive to the saline jet is limited to the middle annulus. Moreover, in experiments not illustrated here, it was found that the portions of the central annulus giving the most vigorous responses were specifically those containing one of the 7 pairs of sensilla. These mapping studies show that the receptor sites are at or near the segmental sensilla.

Further experiments to locate the transduction sites for wave stimulation were carried out in surgically reduced body wall preparations. The aim of these experiments was to find the areas of the body wall that are essential for the transduction of wave stimulation. First, spiking activity was obtained from the segmental nerves (or their branches) to reveal the normal response to wave stimulation. Fig. 8a shows the normal response of two nerves from one body wall preparation: branch 1 of the DP nerve (DP: B1) innervating sensillum 6 and the surrounding body wall; and branch 3 of the DP nerve (DP: B3), innervating sensillum 7 (Kretz et al. 1976) and its surrounding body wall, during wave stimulation. The responses in both nerve branches are reliable and vigorous. Next, after the normal activity had been obtained, the sensilla were carefully excised from the body wall with fine scissors. Recordings
showed that excision eliminated the response to wave stimulation (Fig. 8b) but not to touch (Fig. 8c). These experiments show that intact sensilla are essential for obtaining impulse activity by wave stimulation.

A second surgical experimental procedure, in which the body wall surrounding a sensillum was removed in a stepwise manner, revealed that the vigour of the nerve response to wave stimulation does not depend on the body wall surrounding a sensillum: as the body wall is cut away the response amplitude remains constant. However, the nerve response depends critically on the presence of an intact sensillum, for once this is removed no further detectable response to wave stimulation remains. The conclusion that the sites of transduction are located at the sensilla, and evidently at all of the segmental sensilla, seems inescapable. Because these movement receptors are found only at the sensilla they are hereafter referred to as 'sensillar movement receptors' (SMR).

Cell S activation by SMR impulses

Mistick (1978) has reported that wave stimulation of the leech body wall evokes impulse activity in Rohde's fibre, the largest axon in the leech ventral nerve cord. The specific function of this multisegmental fibre remains obscure (Frank, Jansen & Rinqvist, 1975), even though its cell bodies (cell S in each segmental ganglion) and some of its connexions with other neurones have been documented. In particular, it has been shown that Rohde's fibre is monosynaptically activated by touch cells (Gardner-Medwin, Jansen & Taxt, 1973). Because of these known connexions between touch cells and Rohde's fibre, Mistick concluded that activation of this axon by wave stimulation is mediated by touch cells. There is however another possible pathway for excitatory drive to Rohde's fibre from wave stimulation; namely, the SMRs. To test whether Rohde's fibre can in fact be activated by SMR activity, an S cell soma was penetrated with a microelectrode (experimental preparation shown in Fig. 1) and the flap of body wall subjected to wave stimulation. The amplitude of the waves was below the level that had led to touch cell activation in previous experiments (see Fig. 5). As illustrated in the records of Fig. 9, cell S is depolarized by each series of waves (indicated by arrows) and these depolarizations can lead to impulse activity. The expanded and amplified trace in panel a shows that each spike arises from a depolarization of about 7 mV. In panel b the soma was hyperpolarized with an injected current of 2 nA, blocking impulse activity and thereby revealing some of the individual components contributing to the wave-evoked depolarization. Evidently this depolarization depends on the summed activity of several input elements and occurs because of SMR, and not touch cell activity.

AE neurones are inhibited by SMR activity

In addition to the excitatory effects on cell S, SMR activity reliably leads to inhibition of the annulus erector (AE) motor neurones, whose activity causes the body annuli to be raised into sharp ridges (Stuart, 1970). Activity of AE during leech swimming movements is therefore incompatible with the hydrodynamic requirements of a smooth body profile. For the experiment illustrated in Fig. 10, the preparation consisted of the fourth segmental ganglion attached to a flap of body wall by nerve DP (R, 4). The figure shows simultaneous records obtained from an intrac...
Motion detectors in leeches

Cell S (8)

Fig. 9. Cell S response to SMR activity. The preparation was a chain of ventral cord ganglia of which the eighth segmental ganglion innervated the right body wall of segment 8. (a) The top trace shows the membrane potential of cell S during wave stimulation (drops falling at 2 Hz, indicated by arrows). Each stimulus evoked a depolarization in cell S which frequently led to an action potential. The second trace shows the cell S potential at a higher gain and on an expanded time scale. Note that each spike rises from a depolarizing potential of about 7 mV. (The spikes are clipped because of the high gain.) (b) This record was obtained from the same cell as that of panel a. Cell S has been hyperpolarized by intracellular injection of 2 nA current. Note that the depolarizing response to wave stimulation (at arrows) remains while action potentials have been abolished.

cellular penetration of AE (L, 4) (contralateral to the innervated body wall), from an en passant extracellular electrode monitoring activity in DP (R, 4) and from the phototransistor monitoring wave motion. The intracellular trace indicates that each wave packet recorded by the phototransistor is accompanied by inhibition of AE (evident in the intracellular electrode trace both from hyperpolarization and from interruption of impulse activity). This interruption of AE impulse activity is evident also in the extracellular recording trace. (Note the 1–1 correspondence of the large spikes in DP (R, 4) with those in AE (L, 4).) In addition, the extracellular trace reveals the occurrence of SMR spikes, preceding and coincident with the interruption in AE spiking activity. The extracellular trace shows also that no large axons from touch cells were activated by the wave stimulation.

No wave-induced inhibition was ever observed in AE ipsilateral to the innervated side; it seems likely therefore that the pathway from the SMRs to AE is entirely crossed. In addition, wave-induced inhibition was evident in contralateral AE located one ganglion anterior and one ganglion posterior to the innervated segment. This intersegmental pathway is therefore also crossed, and could be one of the pathways giving rise to the wave-evoked activity observed in recordings from the ventral cord connectives (Fig. 2). It should be noted that the intersegmental inhibition of AE is neither as reliable (it was not observed in all preparations) nor as strong as the homoganglionic inhibition. Visual observation of the body flap during wave stimu-
Fig. 10. AE response to SMR activity. The preparation was a chain of five ventral nerve cord ganglia of which the fourth segmental ganglion innervated the body wall flap via DP (R, 4). The intracellular record from AE was obtained simultaneously with an *en passant* record of DP (R, 4) activity. The spikes in this intracellular record match 1:1 with the large spikes in the extracellular record. Each of the three presentations of wave stimulation results in AE inhibition, as is evident from the temporary cessation of impulse activity in both traces. In the expanded record (the second stimulus presentation) it can be seen that SMR activity in DP (R, 4) (small spikes) precedes the hyperpolarization observed in AE.

Despite repeated experiments, no clear, reliable effects of SMR activity have yet been observed in swim-related neurones of the ventral nerve cord. Motor neurones innervating the ventral and dorsal longitudinal muscles have, in a few preparations, exhibited very weak, mixed excitatory and inhibitory membrane potential fluctuations in response to SMR activation, but these have not been reproducible. Recordings from interneurones have yielded little more positive information; while several recordings from cell 204, whose depolarization reliably elicits swimming activity in the ventral nerve cord, have also yielded no clear-cut results. In fact, there has as yet been no success in eliciting swimming activity with wave stimulation in a dissected preparation.

Additional neurones linked to the SMRs

Several survey experiments have been completed in search of neurones in segmental ganglia which receive excitatory or inhibitory input from the SMRs. Although...
no neurones have as yet been shown definitively to receive monosynaptic synaptic input, several candidate cells have been identified. Records from two such cells, located in the anterior median packet, are shown in Fig. 11. The records in Fig. 11a were obtained from a cell that has been designated cell 202 to indicate its approximate location within the anterior median packet (numbering according to Weeks & Kristan, 1978). This cell is strongly depolarized by SMR activity. As is most clear in the expanded trace, a train of spikes rising from rapid depolarizations with amplitudes of up to 10 mV occurs with each series of waves. The activity illustrated in the figure is very reliable and occurs at the lowest wave amplitudes feasible with the present apparatus. Hyperpolarization of cell 202 (not shown) blocked the spikes and dramatically increased the amplitude of the subthreshold depolarization. This indicates that the input to cell 202 may be chemically mediated. Because of its pronounced activity in response to wave stimulation, this cell is a candidate cell linking the SMR activity with the effects observed in previously identified neurones.

Another previously undescribed neurone, whose cell body also lies in the anterior median pocket near cell 202 (and therefore designated cell 201), is strongly and reliably inhibited by SMR activity. As can be seen in Fig. 11b, each series of waves (occurrence indicated by arrows) produces a prolonged hyperpolarization whose maximum amplitude is nearly 10 mV. The duration of the inhibition parallels the duration of the wave pocket, both lasting about 0.5 s.

As for cell 202, the input of cell 201 from the SMRs is probably mediated by
chemical synapses. Whether this link is direct or via undiscovered interneurone remains to be investigated. The reliability of the responses of both cells 202 and 201 to wave stimulation suggests that while the link between these cells and the SMR may well be polysynaptic the number of intervening synapses is small.

**DISCUSSION**

*Analogy to lateral line receptors*

The ability of animals to detect surface water waves and other sources of media displacements is widespread (see review by Markl, 1978). In the lower invertebrates, the receptors mediating this sensory modality are presumed to be ciliated sensory cells, while in higher invertebrates, such as crustaceans and insects, cuticular sensory hairs have been conclusively identified as the receptor organs (Markl, 1978; Wiese, 1976). Among the vertebrates, the receptors for media displacements have been most extensively studied in the amphibian and teleost fish, in which the receptors have been identified as the lateral line system (Dijkgraaf, 1962).

The notion that another mechanosensory modality might occur in leeches, in addition to the previously described tactile senses, has surfaced more than once (Laverack, 1969; Nicholls & Baylor, 1968; Weeks & Kristan, 1978). This additional modality was postulated when it appeared that the observed activity (or inactivity) of the tactile mechanoreceptors could not account for observed physiological results. The experiments described in this paper demonstrate that leeches do indeed possess additional mechanosensory receptors which respond specifically to water movements and that even the most sensitive of the tactile mechanoreceptors, the touch cells, are relatively insensitive to this modality. The insensitivity of the touch cells to wave stimulation is not really surprising. As was recently shown (Blackshaw & Nicholls 1979), the endings of the touch cells are club-like, and are buried in the body wall. While exquisitely sensitive to body wall deformations, such receptors are not morphologically suitable for detecting water currents directed tangentially to the outer aspect of the body wall.

Physiological mapping experiments described in this report revealed that the transduction sites for wave stimulation are at or near the segmental sensilla. The filiform processes projecting from the sensilla are likely candidates for the transducing structures. Examination with the scanning electron microscope (Y. S. DeRosa and W. O. Friesen, in preparation) reveals that there are about 80 such processes, 0.3 μm in diameter and 4-10 μm long at each segmental sensillum. These processes are also found on the head and tail segments. Bending or displacement of the filiform processes, as in the lateral line system of vertebrates or the hairs of invertebrates, could be the first step in the sensory activation of the sensillar movement receptors.

*Annulus erection reflex*

The discovery that wave stimulation leads to reflexive relaxation of the annulus erector muscle provides us with an example of two competing reflexes involving one pair of motor neurones. It has previously been demonstrated that activation of leech tactile mechanoreceptors can synaptically drive the AE, thereby commanding annulus erection.
Muscle contractions which throw the body surface into sharp ridges (Muller & Nicholls, 1974). The adequate stimulus for this reflex is strong tactile stimulation, a form of stimulation that ensures activation of all three classes of tactile sensory neurones. Wave stimulation, thanks to its strong inhibitory effects on the annulus erector motor neurones, leads to a smoothing of these annular ridges leaving a smooth body surface suitable for swimming behaviour. The SMRs, therefore, mediate a reflex which is the opposite of the one mediated by the tactile sensory receptors, and the two reflexes cannot be simultaneously evoked. Since stimulation which leads to activation of all the tactile receptors will also lead to activation of the SMRs, the expression of the annulus erection reflex clearly takes precedence over its negation. The neuronal mechanisms by which this precedence is achieved remain as yet unexplored.

**Role of SMR for swimming movements**

Activation of the SMRs occurs in response to stimulation by waves in dissected preparations, the same stimulus that evokes swimming activity in intact leeches (W. O. Friesen, S. Young & R. D. Dedwylder, in preparation). In dissected preparations, SMR activation can occur without concomitant suprathreshold activity in touch-sensitive cells; indeed, touch-cell impulse activity occurs only in response to moderate or intense wave stimulation. On the basis of these findings, it seems reasonable to conclude that during the behavioural experiments on intact leeches, leech swimming behaviour was elicited via a neurone chain whose first link is the SMRs, not by the tactile mechanosensory neurones.

Swimming activity was never elicited in a dissected preparation by wave stimulation nor were reliable responses observed in any neurones known to play a role in generating the leech swimming movement. One inference which can be drawn from these negative results is that the neural chain joining the SMRs to the oscillatory neurones generating the swimming rhythm (Friesen et al. 1978) may have several intervening links. Because of the finding, during behavioural experiments, that swimming movements can no longer be elicited by wave stimulation in a leech whose anterior brain has been surgically disconnected from the ventral nerve cord, it seems reasonable to conclude that the anterior brain plays a role in the initiation of swimming movements by wave stimulation. (Disconnexion of the posterior brain, on the other hand, did not greatly interfere with the initiation of swimming movement (W. O. Friesen, S. Young & R. D. Dedwylder, in preparation)). It is also possible that SMRs located in the head region, which were not examined in this study, play a critical role in the initiation of swimming movements by wave stimulation in intact leeches.

How can leeches use the sensory cues provided by wave stimulation to direct their swimming activity into the waves? As measured in these experiments the stimulus wave propagation velocity is about 30 cm/s. For a 10 cm long leech, the interval between activation of the most anterior receptors and the most posterior SMRs by a wave travelling parallel to the body axis would be about 300 ms, while the intersegmental time lags (a mid-body segment spans approximately 5 mm) would be about 15 ms. Similarly, the intrasegmental time lag for a wave travelling orthogonally
to the body axis (the leech width is about 1 cm) would be about 30 ms. These intervals are sufficiently long for temporal intersegmental comparisons, as well as intrasegmental comparisons between left and right SMR activation times, to be accomplished by synaptic interactions. Thus alignment of the body axis with the propagation direction of the surface wave could be accomplished by body movements that minimize the differences in the intrasegmental response onsets and maximize anterior-to-posterior response delays.

This research was supported by the UVA-NIH Biomedical Science Support Program and by NIH grant NS 14965. I wish to express my thanks to Drs Block, Calabrese, Kristan, Mellon, Muller and Stent for their many helpful comments.

REFERENCES


Motion detectors in leeches


