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Water movement sensitive cells in leech CNS

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SUMMARY

The medicinal leech, *Hirudo medicinalis*, responds to surface waves by first orientating and then swimming towards the source of the disturbance. Leeches deduce the source of water motion from both the movement of the waves over the surface of the body and from the movement of light bars produced by differential reflection of light from the surface of the waves. Water movement is detected by motion sensitive cilia distributed over the entire body surface. We describe here a bilaterally distributed population of cells (CBW cells) located in each segmental ganglion, which are sensitive to water movement. These cells have a characteristic morphology within the ganglion in which their cell bodies lie but their axons may project to the adjacent ganglion through the anterior contralateral connective, through the posterior connective or in some cases in both directions. These cells are depolarized by water movement on both sides of the body. The CBW cells are also depolarized by ipsilateral touch cells through electrical synapses, by ipsilateral pressure cells through chemical synapses and are inhibited by contralateral touch cells. The CBW cells thus do not distinguish between ipsilateral touch or bilateral water movement. The role of the CBW cells is discussed.

1. INTRODUCTION

Water motion is important to *Hirudo medicinalis*. To a hungry leech it signals the arrival of potential food. Surface waves initially orientate the leech, this is followed by crawling or swimming movements towards the source of the disturbance. Information about the position of the source is obtained from the direction of wave motion (Young *et al.* 1981) and from the movement of light bars caused by the differential reflection of sunlight from the wave surface (Dickinson & Lent 1984). Light is detected by five pairs of eyes on the head as well as by photoreceptors buried beneath the 14 neural sensilla distributed around the central annulus of each of the 21 mid-body segments (Phillips & Friesen 1982). Additional cells on the surface of the neural sensilla bear single cilia, sensillar movement receptors, which have been identified as the transduction site for water movement (Friesen 1981). Each neural sensillum carries between 40 and 90 single cilia (Derosa & Friesen 1981). An adult leech carries a minimum of 19000 cells on its skin that respond to water movement and more than 1000 photoreceptors. Peterson (1983, 1984*b*, 1985*a*, *b*) has described a number of visual interneurons in the first segmental ganglion and the suboesphageal ganglion that respond to changes of illumination falling on the eyes. Friesen (1981) showed that the previously identified mechanoreceptor cells (Nicholls & Baylor 1968) do not mediate the response to low amplitude water waves although the peripheral terminals of the touch cells can be stimulated by large amplitude waves (Mistick 1978). Within each segmental ganglion the unpaired S cell (Frank *et al.* 1975) responds to touch and water motion. Two other cells have been identified which also respond to water movement (Friesen 1981). Cell 202 is depolarized by surface water waves, whereas cell 201 is inhibited. Apart from this little is known about interneurons which process sensory information obtained from water movement despite the importance of this sensory modality to the leech. We would like to know how sensillar movement receptor activity initiates and controls the direction of swimming. How is the information from the sensillar movement receptors in one segment integrated with that from neighbouring segments? How does the leech use this information to make deductions about the location of the wave source? Is directionality of response encoded in individual neurons or does the activity of a large number of neurons have to be compared? Are the interneurons that respond to wave motion dedicated to this modality or do they process other sensory information as well? How does directional information obtained from the sensillar movement receptors become integrated with directional information from the photoreceptors?

In this report, as a first step towards answering some of these questions, we describe a small population of cells, located within the anterior medial packet of each segmental ganglion, that are excited by water movement. We refer to these cells as corner bilateral wave cells or CBW cells. Morphological and physiological techniques were used to identify the structure of the CBW cells and their relationship with the sensillar movement receptors, the mechanoreceptive neurons and the fast conducting system.

2. MATERIALS AND METHODS

(*a*) *Animals*

Adult medicinal leeches, *Hirudo medicinalis*, obtained from Biopharm (U.K.) Ltd, were maintained at 12 °C.

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They were fed on defibrinated sheep blood at three-monthly intervals.

(*b*) *Physiology*

We used a single ganglion-body wall preparation for physiological experiments. The ganglion remained attached, by roots on one side, to a flap of body wall which extended from the dorsal to the ventral midline. The section of body wall comprised one complete body segment together with two annuli from adjacent segments. The preparation was pinned out on Sylgard (Dow Corning) in a Petri dish and perfused with standard leech Ringer (Muller *et al.* 1981). In some experiments high Mg^{2+} (20 mM) Ringer was used to block chemical synaptic transmission (Nicholls & Purves 1970). Recording methods were as detailed in Muller *et al.* (1981). Water movement was created by a vertical pin positioned in the bath about 12 mm from the dorsalmost edge of the skin. The pin was connected to a Derriton (U.K.) electromagnetic driver and the form, velocity and amplitude of movement controlled by a function generator. Horizontal pin displacement varied between 50 and 350 μm. The single ramped movement of the pin generated a wave which was reflected from the side of the bath. Each reflection produced a response in the CBW cells. We measured the periodicity of waves in the bath by reflecting light from the surface of the bath water onto a photocell. The periodicity of the waves exactly matched the periodicity of response in the CBW cells. The diminishing amplitude of the reflections generated a series of diminishing responses in the CBW cells. The voltage controlling the wave generator, the voltage recorded from the cell(s) and the current injected into the cell were stored on a Racal Instrumentation recorder and subsequently transferred to a PC using a Cambridge Electronic Design interface and spike processing package. Records were printed direct from the PC onto a pen plotter (Hewlett Packard).

(*c*) *Cell morphology*

The morphology of the CBW cells was revealed using the fluorescent dye Lucifer Yellow (Muller *et al.* 1981). The dye-filled cells were viewed and photographed with a Zeiss (LSM 10) confocal laser scan microscope. The negatives were projected onto a screen and the structure of individual cells traced, using 3-D and colour-coded depth pictures to determine possible contact points between cells.

3. RESULTS

A large number of cells within the segmental ganglia of the leech have been mapped and given identification numbers (Nicholls & Van Essen 1974; Weeks & Kristan 1978; Muller *et al.* 1981). The cells that we refer to are located within the anterior medial glial packet of the body segmental ganglia. In the published maps (Muller *et al.* 1981) each cell in the anterior medial packet has a different number while those cells in the lateral packets which are paired, have been given the same number. For instance there is a left 162 and a right 162. The implication of the sequential numbering in the central packet is that these cells are unpaired. This is the case for the S cell. However, the cells that we describe below, like the lateral visual cells in the anterior medial packet of the first segmental ganglion (Peterson 1983), are paired cells (figure 2*c*). Therefore we have not used the published numbers to identify cells in the anterior medial packet. Instead, cells were identified by their physiology and shape. Where we use the terms ipsilateral and contralateral this is with reference to the cell soma.

(*a*) *Response of CBW cells to water motion*

We began by surveying all the cells on the ventral surface of the ganglion for their response to stimulation of the sensillar movement receptors. Several cells in the central glial packets were briefly excited or inhibited by sensillar movement receptor stimulation. The cells which showed the largest amplitude excitatory response, as recorded from the cell soma, are located in the posterior lateral corners of the anterior medial packet (figure 2*a*). It is these cells, the corner bilateral wave cells, which are described in this report.

Cells confirmed as CBW cells by dye injection exhibited some variation in spontaneous activity. The majority of corner cells were almost silent with occasional, irregular bursts of excitatory postsynaptic potentials (EPSPs), but a few fire small, high frequency, non-overshooting action potentials with an amplitude of 5–15 mV. These observed differences in activity could reflect variable degrees of damage caused by electrodes as they enter cells. All CBW cells responded to low amplitude (50–350 μm) pin movements with a prolonged depolarization (figure 1*a*). The response often starts with a rapid depolarization, followed by periodic depolarizations that correspond to wave reflections from the side of the bath (see §2). The excitatory response to wave motion is maintained in high (20 mM) Mg^{2+} Ringer (figure 1*a*). CBW cells continue to respond, without adaptation, for as long as wave stimuli are presented (figure 1*b*). The wave amplitude to which these cells respond is considerably less than that required to excite touch mechanosensory cells. All corner cells received excitatory input whether the wave was directed at the ipsilateral or contralateral body wall (figure 1*c*).

(*b*) *Morphology of CBW cells*

We examined the structure of 36 CBW cells from as far forwards as segmental ganglion 3 and as far back as segmental ganglion 20. CBW cells, as revealed with Lucifer Yellow have a characteristic shape (figure 2*b*) in which the monopolar cell body, located in one of the posterior lateral corners of the anterior medial packet, issues a single process which travels dorsally for a short distance before dividing. One larger diameter branch loops ventrally across the midline of the ganglion before reaching back up into the dorsal neuropile where it divides again to produce an anteriorly and posteriorly directed branch. At the margins of the ganglion one or both (figure 2*b*) of these branches turn

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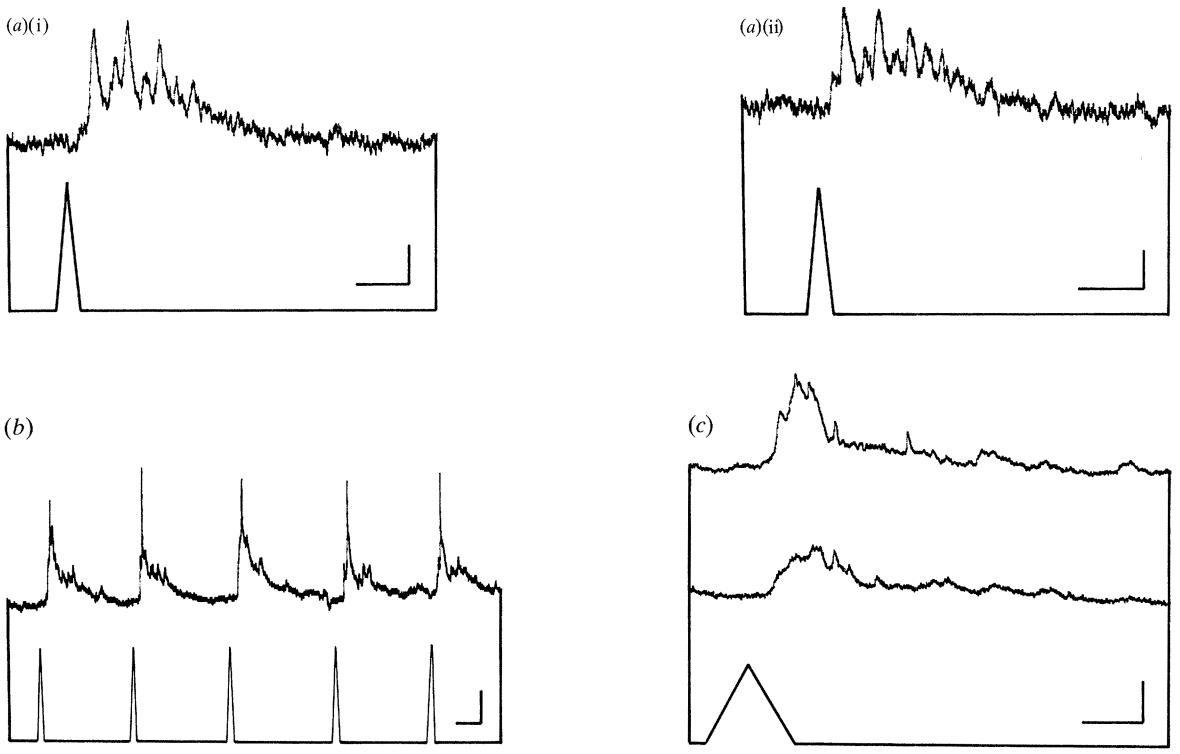

Figure 1. (*a*) Waves in the preparation bath induce a series of depolarizations in CBW cells at intervals which correspond to wave reflections from the side of the bath. (i) Normal Ringer; (ii) 20 mM Mg^{2+} Ringer. As the response is maintained in high Mg^{2+} Ringer the connections from the sensillar movement receptors to the CBW cells are probably via electrical synapses. Top trace, CBW cell voltage; bottom trace, movement of wave generating pin. Vertical scale bars; top trace 2 mV, bottom trace 62 μm. Horizontal bar, 200 ms. All vertical scale bars to be read, as here, from top trace to bottom trace. (*b*) Each wave in the bath is followed by a non-adapting depolarization in a CBW cell. Top trace CBW cell voltage; bottom trace, movement of wave generating pin. Scale bars, 2 mV, 62 μm, 300 ms. (*c*) CBW cells respond to water movements over ipsilateral and contralateral skin. In this preparation, as elsewhere, only the ganglion roots ipsilateral to the CBW cell are intact. Top trace, CBW cell voltage, ipsilateral to the stimulated sensillar movement receptors; middle trace, CBW cell voltage, contralateral to the sensillar movement receptors; bottom trace, movement of wave generating pin. (Scale bars, 10 mV, 10 mV, 230 μm, 100 ms.)

towards the midline to exit as axons from contralateral connectives. No CBW cell processes leave the ganglion on the ipsilateral side. In travelling through the neuropile, corner cell branches issue numerous secondary processes that mainly, but not exclusively, project towards the lateral margin of the ganglion. The main ipsilateral neurite radiates a large number of secondary processes which bear varicosities along their length. Varicosities are also present on secondary processes on the contralateral side of the ganglion. We assume that these varicosities indicate synaptic sites as shown to be the case in the mechanosensory cells where the varicosities proved to be both pre- and postsynaptic (Muller & McMahan 1976).

CBW cells show a degree of variability in the structure of their projections towards neighbouring ganglia (figure 2*b*). These variations might be related to the position of the cell within the nerve cord, as reported for some other leech neurons. Two examples of this are the anterior pagoda cells (Gillon & Wallace 1984) and annulus erector motor neurons (Gao & Macagno 1987). In both of these cell types the anteriorly located cells extend contralateral connections through the anterior connectives, the most posteriorly located cells send longitudinal projections caudally. Another explanation for the variety of CBW cell structure may be that all three types of CBW cell are present in every segmental ganglion. The maximum number of ipsilateral CBW cells filled so far in a single segmental ganglion is two (figure 2*b*). In this particular case both cells had the same structure. The CBW cells are bilaterally paired. Figure 2*c* shows a pair of CBW cells projecting to adjacent ganglia through the posterior contralateral connectives. Because the CBW cells are difficult to locate and fill we are not certain that we have succeeded in filling all the CBW cells on one side of a ganglion. Thus the exact number of CBW cells present in each segmental ganglion is not yet determined.

(*c*) *Interaction of the CBW cells with the mechanosensory cells and the rapid conduction system*

Corner bilateral wave cells process sensory information related to water motion. Within each segmental ganglion there are three classes of mechanosensory neurons which respond to touch, pressure and nociceptive stimuli. The properties of T, P and N cells were originally described by Nicholls & Baylor (1968). A wave sensitive cell, the S cell, receives excitatory T mechanosensory input through a coupling interneuron

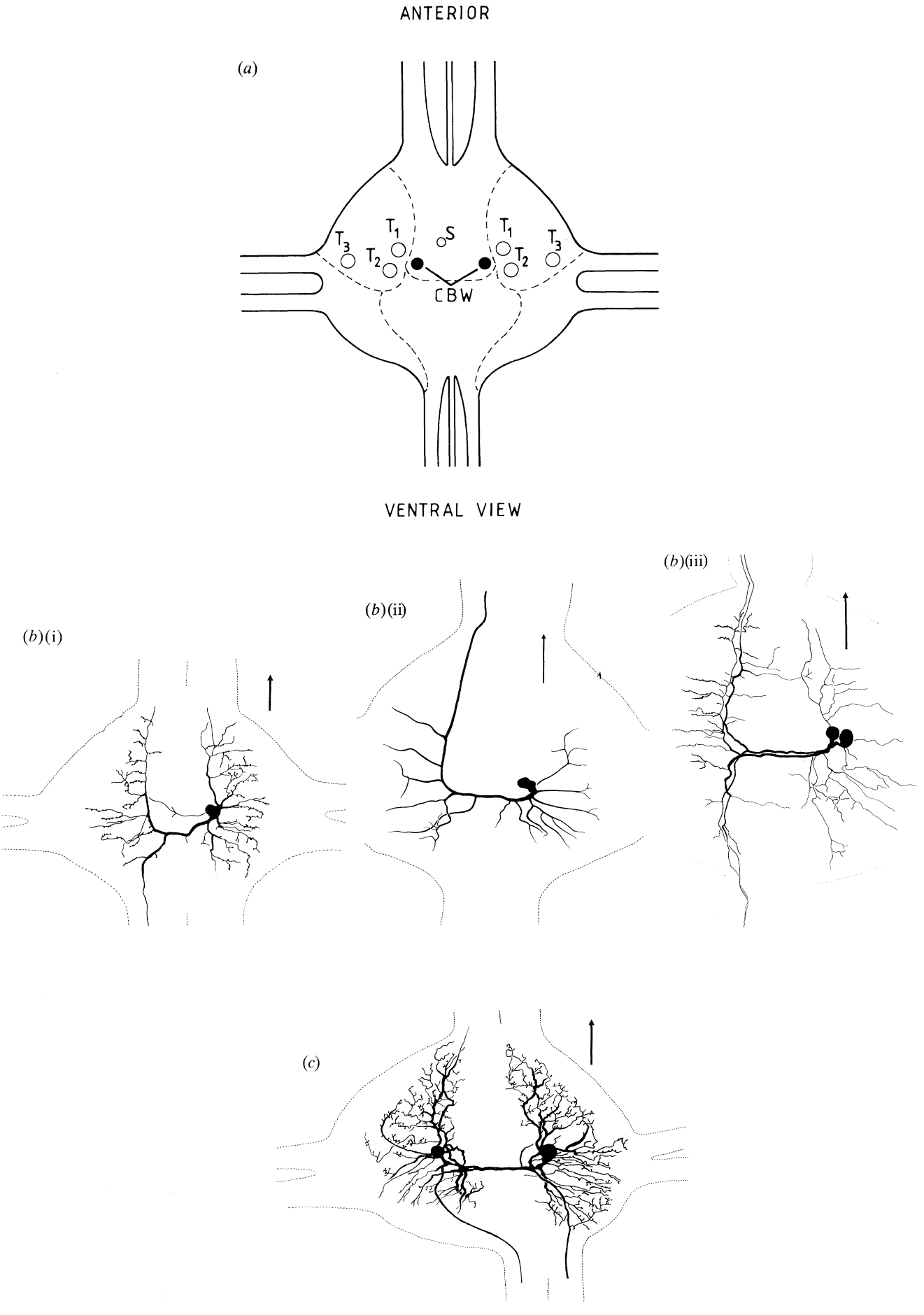

Figure 2. For description see opposite.

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(Muller & Scott 1981). We did dye injection and dual recording experiments to determine whether the CBW cells are synaptically coupled to the mechanosensory cells or to the rapid conduction network of S cells. One electrode was used to record from and with a balanced bridge, pass current into cells while the second electrode recorded the postsynaptic response.

(*d*) *Connections with T cells*

Intracellular staining revealed possible synaptic sites between two ipsilateral T cells and a CBW cell (figure 3). Each action potential in any one of the ipsilateral T cells was matched by an EPSP in the CBW cells (figure 4*a*). An action potential in a contralateral T cell produced an IPSP (2–3 mV) in CBW cells (figure 4*c*). As all known T-cell synapses are electrical and excitatory inhibition must be via an interneuron.

The delay between the beginning of an ipsilateral T-cell action potential and the start of an EPSP in the CBW cell was about 1 ms (figure 4*b*). The connection between the two cells was maintained in high (20 mM) Mg^{2+} Ringer (figure 4*a*). These results are consistent with the presence of a monosynaptic electrical synapse between the two cells since the disynaptic T cell to S cell connection has a longer delay of about 5 ms.

Hyperpolarization or subthreshold depolarization of ipsilateral T cells did not produce a corresponding response in the CBW cells. When the CBW cell is depolarized or hyperpolarized there is no response from either ipsilateral or contralateral T cells.

(*e*) *Connections with P cells*

A train of action potentials in ipsilateral P cells elicits EPSP's in CBW cells (figure 6*a*). The synaptic latency is about 14 ms (figure 6*b*) which is consistent with a polysynaptic pathway between these cells which includes chemical synapses. Ipsilateral P cell and CBW cell dendrites occupy similar areas of the neuropile without the clear positional disparity found between the T cells and the S cells (Muller & Scott 1981). Without examining the area where the processes from the two cells intermingle at higher resolution the possibility of a direct synapse between the P cells and the CBW cells cannot be ruled out (figure 5).

(*f*) *Connections with the N cells*

No physiological connection could be shown between ipsilateral or contralateral N mechanosensory neurons and the CBW cells.

(*g*) *Connections with the S cell*

The unpaired S interneuron (Frank *et al.* 1976) present in the anterior medial packet of all segmental ganglia is, excited by both touch and water movement (Friesen 1981). This raised the possibility that CBW cells are involved in the rapid conduction pathway. However, no physiological connection was uncovered, in either direction between the CBW cells and the S cell.

(*h*) *Wave pathway is independent of T cells*

Because the response of the CBW cells to action potentials in ipsilateral T cells was identical to that produced by sensillar movement receptor stimulation and because T cells can be depolarized by wave motion (Friesen 1981) we wanted to be certain that there is a parallel pathway from the sensillar movement receptors to the CBW cells that bypasses the T cells. We had been able to show that CBW cells are excited by wave stimuli too small to excite one T cell, but to be certain that there was a separate pathway we either had to monitor the activity of all three ipsilateral T cells while recording from a CBW cell or separate two T cells from their terminals in the skin so that they were no longer able to respond to water movements. We opted to cut the roots from the ganglion to the skin while keeping the dorsal posterior nerve intact. This isolates T1 and T2 from their terminals in the skin, while keeping T3 (Yau 1976) and the axons from sensilla six and seven intact (Kretz *et al.* 1976). After cutting the roots, dual recording from T3 and an ipsilateral CBW cell showed that the synapse from T3 onto the CBW cell was still functional but that small water movements depolarized the CBW cell without exciting T3 to threshold (figure 7). This experiment proved conclusively that the response of the CBW cells to water movement is independent of the pathway through the touch cells.

4. DISCUSSION

Hirudo medicinalis is abundantly supplied with water-movement-sensitive cilia (Derosa & Friesen 1981; Friesen 1981; Brodfuehrer & Friesen 1984) and photoreceptors (Hansen 1972; Rohlich & Torok 1964; Kretz *et al.* 1976). Both photoreceptors and sensillar movement receptors are distributed over the surface of the leech. Visual interneurons have been identified throughout the nervous system. These include anterior

Figure 2. (*a*) A schematic drawing shows the position of CBW cell somata in the segmental ganglia. The relative positions of the S cell and touch (T) cells are shown. The margins of glial packets are shown by dotted lines. Each T cell innervates a discrete field of ipsilateral skin. T3 terminals are located in dorsal skin, T1 and T2 innervate lateral and ventral skin, respectively. Touch cell identities were confirmed by their response to stroking dorsal, lateral or ventral skin with a fine hair. (*b*) CBW cells have a characteristic shape with dendritic trees on each side of the ganglion. An axon may leave the ganglion through: (i) the posterior connective, or (ii) the anterior connective. In (ii) only the major processes of the cell have been drawn., (iii) This preparation shows the presence of two CBW cells on one side of the ganglion. These two CBW cells have a similar structure and differ from (i) and (ii) because their axons leave the ganglion through both anterior and posterior contralateral connectives. The arrows point anteriorly and represent 100 μm. (*c*) Left and right CBW cells, located in the same central glial packet, form mirror images of each other. The arrow points anteriorly and represents 100 μm.

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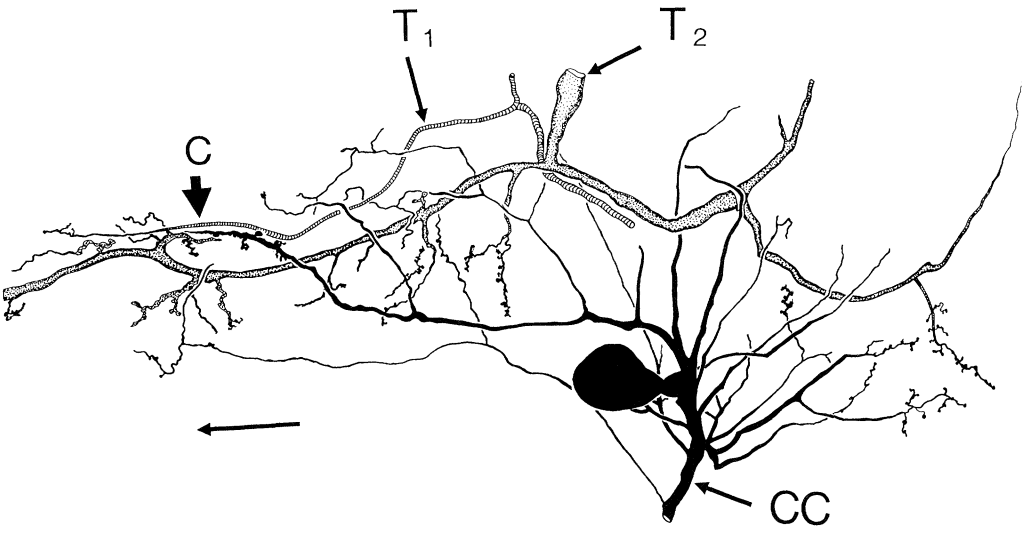

Figure 3. Both T1 and T2 touch cells have been filled with Lucifer Yellow, together with one CBW cell ipsilateral to the T cells. The dendrites of both T1, T2 and the ipsilateral dendrites of the CBW cell lie within the same region of neuropile and come into close contact with each other at the point shown by the arrow at c, where their processes lie at the same depth in the ganglion. T3 was not filled. The arrow points anteriorly and represented 50 μm.

visual cells in the supraoesophageal ganglion (Peterson 1985*b*) and lateral visual cells in the suboesophageal ganglion (Peterson 1985*a*) and first body segment ganglion (Peterson 1983 1985*b*). A small number of visual interneurons have been located in the segmental ganglia (Kretz *et al.* 1976), in addition to the well-documented S cell which responds to both touch and changes in illumination falling on the annular photoreceptors (Laverack 1969; Frank *et al.* 1975; Gardner-Medwin *et al.* 1973; Muller & Carbonetto 1979; Bagnoli *et al.* 1972, 1974, 1975). The S cell is found in all body segments from the suboesophageal ganglion to the tailbrain (Peterson 1984*a*).

In contrast very little is known about interneurons that respond to water moving over the surface of the leech. Friesen (1981) showed that the rapidly conducting S cells were synaptically excited by the sensillar movement receptors and identified two further interneurons, cells 201 and 202 (Nicholls & Van Essen 1974) whose activity was perturbed by water currents. The properties of these cells have not been investigated further.

In this study we have identified a small population of cells which are excited by the sensillar movement receptors. These cells lie in the posterior lateral corners of the anterior medial glial packet and have been found in every body segment examined, that is from segmental ganglion 3–20. These cells are depolarized by stimulation of the sensilla movement receptors. The response does not adapt to repeated stimulation. Phillips & Friesen (1982) were unable to find synapses beneath the ciliated soma and concluded that each ciliated cell projects an axon to the ganglion. The observed response of the CBW cells to sensillar movement receptor activity is similar to the response of another first-order sensory interneuron, the lateral visual cells, onto which photoreceptor axons are known to synapse directly (Peterson 1983). If the sensillar movement receptors synapse directly onto the CBW cells we would expect to find that the large numbers of sensillar movement receptor axons would produce a depolarization in the CBW cells consisting of summating EPSP's. This is apparently the case since surface waves induce depolarizations of the CBW cells which occasionally give rise to spike-like events (figure 1*b*). The Mg^{2+} resistance of the CBW cell response is consistent with the presence of direct electrical synapses from sensillar movement receptor axons onto the CBW cells. However, we cannot be certain that there is not an interneuron interposed between the sensillar movement receptor axons and the CBW cells.

When the structure of the CBW cells is revealed by Lucifer Yellow they are found to have a bilaterally organized dendritic tree, linked by a neurite across the ganglion. The axon always leaves the ganglion from a connective contralateral to the cell body. CBW cells are found with a variety of axonal projections. We found CBW cells with axons projecting to the next anterior ganglion, the next posterior ganglion and in some cases a bifurcating axon projecting both anteriorly and posteriorly.

We wanted to find out whether the CBW cells responded to one sensory modality, that is whether they were dedicated to processing water movement stimuli or whether they had a more general role of collating information from a number of sensory systems. Dual recording experiments failed to reveal the presence of synapse between CBW cells and ipsilateral or contralateral N cells. However, there is a Mg^{2+} susceptible excitatory synapse or synapses between ipsilateral P cells and CBW cells. Action potentials in ipsilateral P cells induced small, summating EPSPs in CBW cells with a 14 ms delay. Action potentials in ipsilateral T cells produced large, Mg^{2+} resistant, summating EPSPs in CBW cells, with a delay of 1 ms, evidence consistent with, but not absolute proof of, the presence of a direct electrical synapse. However, current could not be passed between ipsilateral T cells and CBW cells, suggesting that the synaptic sites between these two cells are electronically

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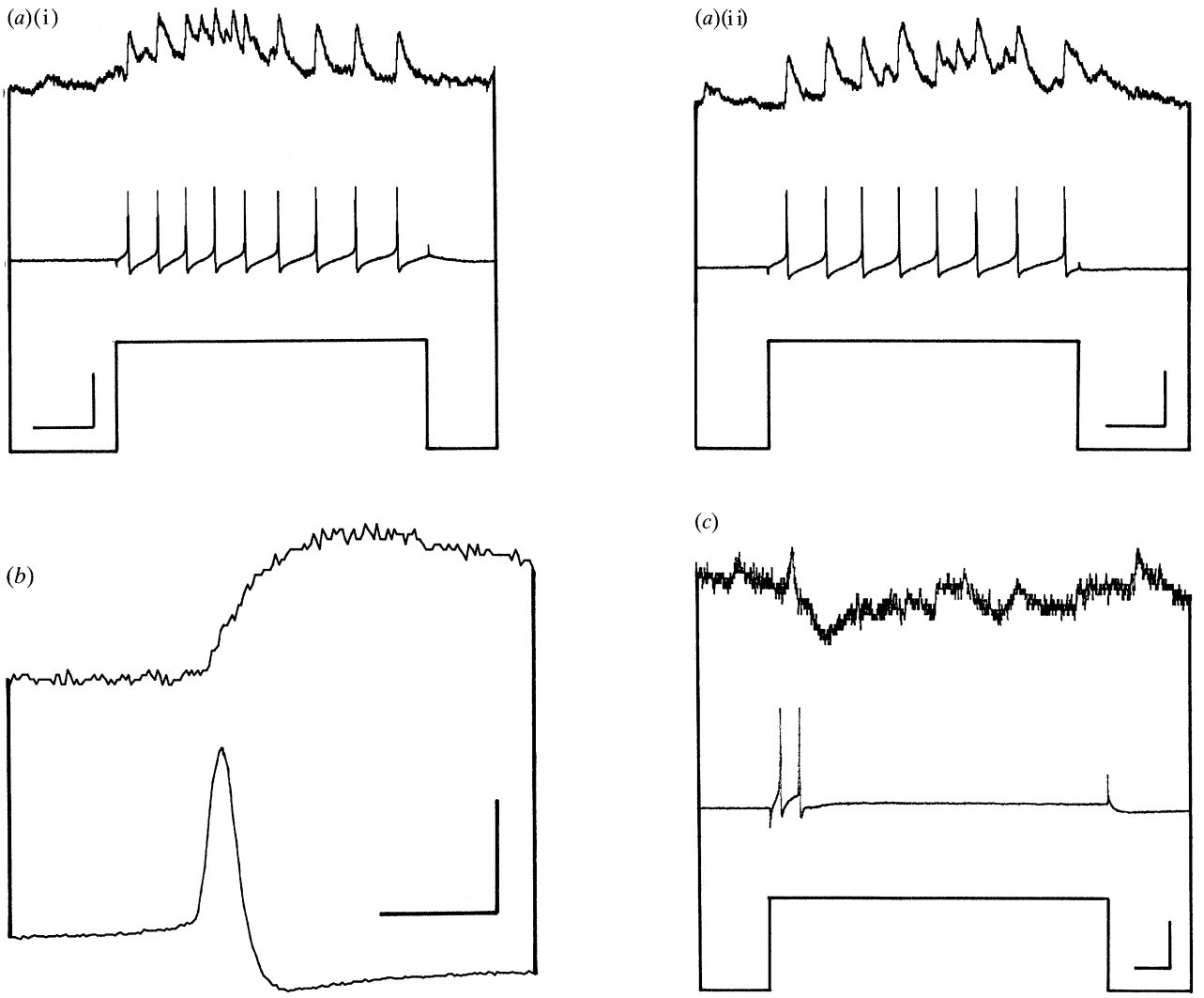

Figure 4. (*a*) Every action potential in T2 is matched by an EPSP in the corner cell, though there are EPSPs in the corner cell which do not correspond to action potentials in T2. (i) Normal Ringer and (ii) with the preparation bathed in 20 mM Mg^{2+} EPSP amplitude is unaffected, implying the presence of electrical synapses between T2 and CBW cells. Top trace, CBW cell voltage; middle trace, T2 cell voltage ipsilateral to the CBW cell; bottom trace, current injected into T2. Scale bars, 6 mV, 32 mV, 0.05 nA, 100 ms. (*b*) An action potential in T2 is followed, 1 ms later, by an EPSP in an ipsilateral CBW cell. Top trace, CBW cell voltage; bottom trace, T2 cell voltage. Scale bars. 5 mV, 25 mV, 5 ms. (*c*) Action potentials in a contralateral touch cell induce an IPSP in the CBW cell. Top trace, CBW cell voltage; middle trace, contralateral T3 cell voltage; bottom trace, current injected into T3. Scale bars, 2 mV, 34 mV, 0.4 nA, 50 ms.

remote from the T cell soma. In contrast, contralateral T cells induced IPSPs in CBW cells. No connection was established between contralateral P cells and CBW cells.

CBW cells are thus depolarized by ipsilateral touch cells, ipsilateral pressure cells and bilateral sensillar movement receptors. The response of CBW cells to touch is indistinguishable from the response to water movement. Since wave activity can produce EPSPs on T cells (Friesen 1981) there is apparently a parallel pathway from the sensillar movement receptors to the T cells.

S cells, like CBW cells, respond to water movement (Friesen 1981). This raised the possibility that CBW cells are either post- or presynaptic to the S cells and are involved in the rapid conduction pathway through the nervous system (Gardner-Medwin *et al.* 1973). However, CBW cells were not depolarized by action potentials in S cells neither were we able to pass current between them. Unlike the S cells, the CBW cells do not respond to changes in illumination falling on the skin.

Lockery & Kristan (1990*a*, *b*) have identified a number of interneurons within the segmental ganglia which are involved in the control of local bending reflexes. Depolarization of P cells leads to EPSPs in a discrete set of local bend interneurons. The pattern of activity distributed across the local bend interneurons determines which group of motorneurons are excited. One of the interneurons involved in local bending is cell 212 (Lockery & Kristan 1990*b*) located in the same region of the segmental ganglia as the CBW cells and whose structure is indistinguishable from a CBW cell with an anterior projection. There, are, however, some differences between these two cells. Lockery & Kristan (1990*a*) found that P cells were more effective in promoting the local bending reflex than were T cells

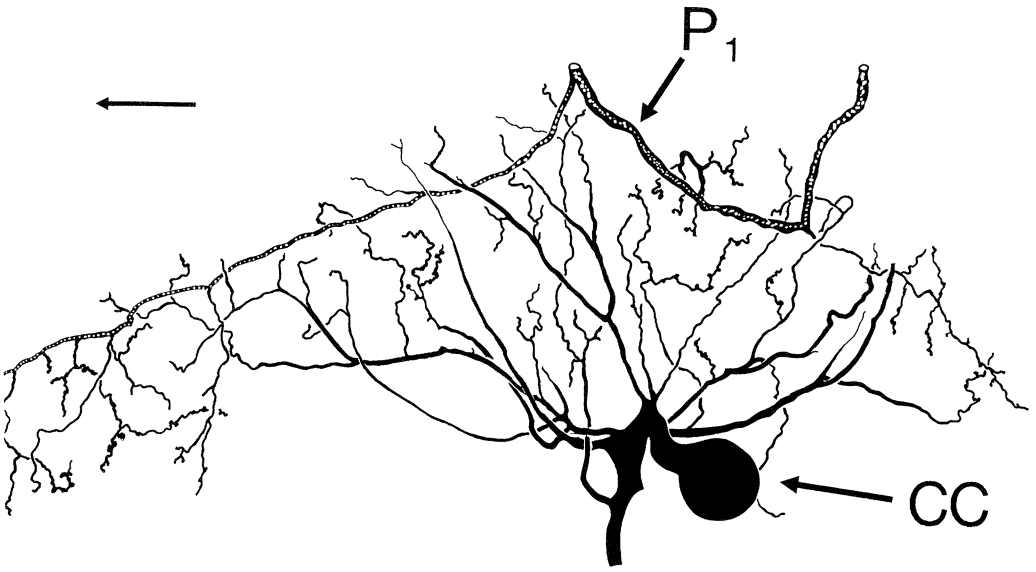

Figure 5. The ipsilateral pressure mechanoreceptive cell serving dorsal skin has been filled with Lucifer Yellow, as has one CBW cell. The processes of these cells intermingle in the same region of neuropile. The arrow points anteriorly and represents 50 μm.

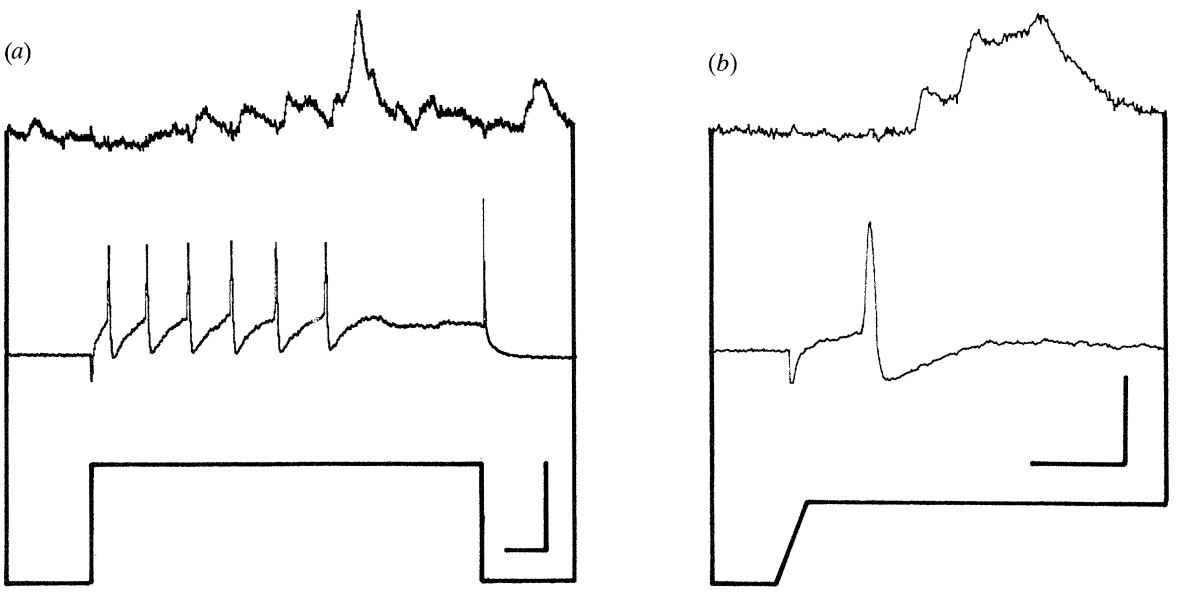

Figure 6. (*a*) Repetitive firing of pressure mechanoreceptive cells, represented by P2 innervating ventral skin, induces a series of EPSPs in a CBW cell. Top trace, CBW cell voltage; middle trace, ipsilateral P2 cell voltage; bottom trace, current injected into P2. (*b*) An action potential in P2 is followed, after a delay of 14 ms, by an EPSP in a CBW cell. The delay, measured from the peak of the action potential to the onset of the EPSP, is consistent with a polysynaptic pathway through chemical synapses. The first EPSP in the CBW cell is followed by further depolarizations which do not correlate clearly with the P cell action potential. Top trace, CBW cell voltage; middle trace, P2 cell voltage; bottom trace, current injected into P2. Scale bars for (*a*) and (*b*), 10 mV, 44 mV, 0.6 nA, 30 ms.

but we found that T cells had a stronger effect on CBW cells than do P cells. Cell 212 is depolarized by all four P cells in the ganglion whereas we found that only ipsilateral P cells were effective. Lockery & Kristan (1990*b*) found only one cell with a projection similar to cell 212 on each side of the ganglion. While we have found only one CBW cell on each side with a projection that is exactly the same as cell 212 we believe that this cell forms part of a small population of cells in the ganglion with similar physiological properties but different axonal projections. The latency between the peak of action potentials in P cells and the onset of the CBW response in our preparations is about 14 ms, whereas Lockery & Kristan (1990*b*) report a latency of about 3 ms. Despite these differences the structural similarities between their cell 212 and the CBW cells are striking. The response of CBW cells to ipsilateral T and P cells is consistent with their proposed role of a local bending interneuron. However, the marked response of CBW cells to sensillar movement receptors on either side of the body suggests that the CBW cells may have additional roles, if as seems likely CBW cells prove to be involved in local bending reflexes.

The bilateral structure of the CBW cells is reminiscent of the bilateral structure of the omega neurons in crickets (Wholers & Huber 1978) which are first-

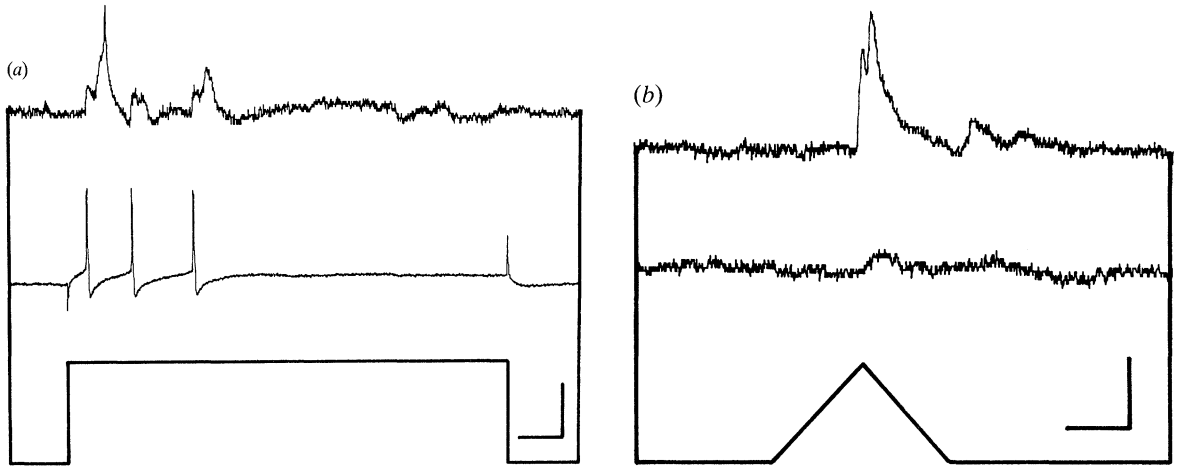

Figure 7. (*a*) T3 is the only touch mechanoreceptor in which the cell soma is still connected to its peripheral terminals in the skin. Current injected into T3 induces EPSPs in an ipsilateral CBW cell, showing that the synapse between T3 and the CBW cell is still intact and effective. Top trace, CBW cell voltage; middle trace, ipsilateral T3 cell voltage; bottom trace, current injected into T3. Scale bars, 5 mV, 20 mV, 0.1 nA, 50 ms. (*b*) Water movement depolarizes the CBW cell, accompanied by a small, subthreshold depolarization in T3. Top trace, CBW cell voltage; middle trace, T3 cell voltage; bottom trace, movement of wave-generating pin. Scale bars, 5 mV, 4 mV, 250 μm, 50 ms.

order auditory interneurons. We anticipated that like the omega neurons, the CBW cells would be excited by ipsilateral stimuli and inhibited by contralateral stimuli, thus accentuating spatial discrimination. While the inhibitory response to the contralateral T cells appeared to support this idea we were disappointed to find that the CBW cells were excited by water motion on either side of the leech (figure 1*c*).

CBW cells have similar properties to the lateral visual cells in the suboesophageal ganglion and segmental ganglion 1. Like the CBW cells, the lateral visual cells are excited by their receptors through electrical synapses. There is also structural similarity between lateral visual cells and CBW cells. Both have ipsilateral and contralateral dendritic trees and both project an axon into neighbouring ganglia through a contralateral connective. All the lateral visual cell axons project anteriorly, reaching forwards to the supraoesophageal ganglion, while the CBW cells may project forwards or backwards or in both directions. The degree of spatial discrimination exhibited by the lateral visual cells depends upon the ganglion in which they are located (Peterson 1985*a*).

Homologues of T, P and N cells (Yau 1976), S cells and coupling interneurons (Peterson 1984*a*) have been found in all segmental ganglia, including the suboesophageal ganglion. We presume that the CBW cells will be found in ganglion 1 and the suboesophageal ganglion. If so, we shall want to know if their structure enables them to make spatial discriminations when placed in the context of the anterior ganglia. We shall also want to know if the lateral visual cells and the CBW cells are examples of a common type of interneuron that carries out sensory processing.

In intact leeches, swimming can be started by wave motion (Young *et al.* 1981). Swimming can also be induced in semi-intact preparations by water waves (Willard 1981; Brodfuehrer & Friesen 1984) if serotonin is present in the saline bathing the preparation. Previous reports have failed to identify interneurons which trigger swimming on the basis of sensillar movement receptor activity. Depolarization of T, P or N cells in midbody ganglia is capable of generating the swimming rhythm (Debski & Friesen 1987) probably by exciting cell 204, a neuron that gates swimming activity (Weeks & Kristan 1978). Depolarization of T, P and N cells produce EPSPs in the contralateral swim trigger cells in the suboesophageal ganglion (Brodfuehrer & Friesen 1986*b*).

One route by which sensillar movement receptors could prime or trigger swimming would be for the CBW cells to synapse onto the mechanoreceptor cells, thus providing them with a route to cell 204 and in the anterior ganglia, the trigger cells (Brodfuehrer & Friesen 1986*a*). However, we failed to find any connections from CBW cells onto the mechanoreceptor cells; the connections between the CBW cells and T and P cells are in the opposite direction. If the CBW cells are involved in triggering swimming then their contact with the swim pattern generator must be through a pathway which is separate from or parallel to the mechanoreceptor pathway.

Can leeches distinguish wave motion from touch? Water motion strongly excites the CBW cells but only weakly excites the T cells. Conversely, T cell activity excites the ipsilateral CBW cells. Thus depolarization of CBW cells is not sufficient to distinguish between touch and water movement. However, the pattern of activity through the central nervous system will probably be different for each of these two modalities since water movement will excite a large number of CBW cells throughout the central nervous system whereas local touch stimuli will excite a limited number of touch and CBW cells in or adjacent to the segment stimulated.

More work is required before we can understand how leeches distinguish between water movement and touch and how they can use information inherent in wave motion to deduce the direction from which the waves are coming.

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REFERENCES

Bagnoli, P., Brunelli, M. & Magni, F. 1972 A fast-conducting pathway in the central nervous system of the leech *Hirudo medicinalis*. *Archs ital. Biol.* **110**, 32–51.

Bagnoli, P., Brunelli, M., Magni, F. & Pellegrino, M. 1974 Suprasegmental inputs to the fast conducting system in the central nervous system of *Hirudo medicinalis*. *Archs ital. Biol.* **112**, 307–329.

Bagnoli, P., Brunelli, M., Magni, F. & Pellegrino, M. 1975 The neurons of the fast conducting system in *Hirudo medicinalis*: identification and synaptic connections with primary afferent neurons. *Archs ital. Biol.* **113**, 21–43.

Brodfuehrer, P. D. & Friesen, W. O. 1984 A sensory system initiating swimming activity in the medicinal leech. *J. exp. Biol.* **108**, 341–355.

Brodfuehrer, P. D. & Friesen, W. O. 1986*a* Initiation of swimming activity by trigger neurons in the leech suboesophageal ganglion. I. Output connections of Tr1 and Tr2. *J. comp. Physiol.* A **159**, 489–502.

Brodfuehrer, P. D. & Friesen, W. O. 1986*b* Initiation of swimming activity by trigger neurons in the leech subesophageal ganglion. III. Sensory inputs to Tr1 and Tr2. *J. comp. Physiol.* A **159**, 511–519.

Debski, E. A. & Friesen, W. O. 1987 Intracellular stimulation of sensory cells elicits swimming activity in the medicinal leech. *J. comp. Physiol.* A **160**, 447–457.

Derosa, S. Y. & Friesen, W. O. 1981 Morphology of leech sensilla: observations with a scanning electron microscope. *Biol. Bull.* **160**, 383–393.

Dickinson, M. H. & Lent, C. M. 1984 Feeding behaviour of the medicinal leech, *Hirudo medicinalis* L. *J. comp. Physiol.* A **154**, 449–455.

Frank, E., Jansen, J. K. S. & Rinvik, E. 1975 A multisomatic axon in the central nervous system of the leech. *J. comp. Neurol.* **159**, 1–14.

Friesen, W. O. 1981 Physiology of water motion detection in the medicinal leech. *J. exp. Biol.* **92**, 255–275.

Gao, W.-Q. & Macagno, E. R. 1987 Extension and retraction of axonal projections by some developing neurons in the leech depend upon the existence of neighbouring homologues. I. The AP and AE neurons. *J. Neurobiol.* **18**, 295–313.

Gardner-Medwin, A. R., Jansen, J. K. S. & Taxt, T. 1973 The 'giant' axon of the leech. *Acta Physiol. Scand.* **87**, 30A–31A.

Gillon, J. W. & Wallace, B. G. 1984 Segmental variation in the arborization of identified neurons in the leech central nervous system. *J. comp. Neurol.* **228**, 142–148.

Hansen, J. 1962 Elektronenmikroskopische Untersuchung der Hirudineen-Augen. *Zool. Beitr. Neue Folge* **7**, 83–128.

Kretz, J. R., Stent, G. S. & Kristan, W. B. 1976 Photosensory input pathways in the medicinal leech. *J. comp. Physiol.* **106**, 1–37.

Laverack, M. S. 1969 Mechanoreceptors, photoreceptors and rapid conduction pathways in the leech, *Hirudo medicinalis*. *J. exp. Biol.* **50**, 129–140.

Lockery, S. R. & Kristan, W. B. 1990*a* Distributed processing of sensory information in the leech. I. Input-output relations of the local bending reflex. *J. Neurosci.* **10**, 1811–1815.

Lockery, S. R. & Kristan, W. B. 1990*b* Distributed processing of sensory information in the leech. II. Identification of interneurons contributing to the local bending reflex. *J. Neurosci.* **10**, 1818–1829.

Mistick, D. C. 1978 Neurones in the leech that facilitate an avoidance behaviour following nearfield water disturbances. *J. exp. Biol.* **75**, 1–23.

Muller, K. J. & McMahan, U. J. 1976 The shapes of sensory and motor neurones and the distribution of their synapses in the ganglia of the leech: a study using intracellular injection of horseradish peroxidase. *Proc. R. Soc. Lond.* B **194**, 481–499.

Muller, K. J. & Carbonetto, S. T. 1979 The morphological and physiological properties of a regenerating synapse in the C.N.S. of the leech. *J. comp. Neurol.* **185**, 485–516.

Muller, K. J., Nicholls, J. G. & Stent, G. S. 1981 *Neurobiology of the leech*. Cold Spring Harbour Laboratory.

Muller, K. J. & Scott, S. A. 1981 Transmission of a 'direct' electric connexion mediated by an interneurone in the leech. *J. Physiol.* **311**, 565–583.

Nicholls, J. G. & Baylor, D. A. 1968 Specific modalities and receptive fields of sensory neurons in the C.N.S. of the leech. *J. Neurophysiol.* **311**, 565–584.

Nicholls, J. G. & Purves, D. 1970 Monosynaptic chemical and electrical connections between sensory and motor cells in the central nervous system of the leech. *J. Physiol.* **209**, 647–667.

Nicholls, J. G. & Van Essen, D. 1974 The nervous system of the leech. *Sci. Am.* **230**, 38–48.

Peterson, E. L. 1983 Visual processing in the leech central nervous system. *Nature, Lond.* **303**, 240–242.

Peterson, E. L. 1984*a* The fast conducting system of the leech: a network of 93 dye-coupled interneurons. *J. comp. Physiol.* A **154**, 781–788.

Peterson, E. L. 1984*b* Two stages of integration in a leech visual interneuron. *J. comp. Physiol.* A **155**, 543–557.

Peterson, E. L. 1985*a* Visual interneurons in the leech brain. I. Lateral visual cells in the suboesophageal ganglion. *J. comp. Physiol.* A **156**, 697–706.

Peterson, E. L. 1985*b* Visual interneurons in the leech brain. II. The anterior visual cells of the supraesophageal ganglion. *J. comp. Physiol.* A **156**, 707–717.

Phillips, C. E. & Friesen, W. O. 1982 Ultrastructure of the water-movement-sensitive sensilla in the medicinal leech (*Hirudo medicinalis*). *J. Neurobiol.* **13**, 473–486.

Rohlich, P. & Torok, L. J. 1964 Elektronenmikroskopische Beobachtungen an den Sehzellen des Blutegels, *Hirudo medicinalis* L. *Z. Zellforsch. mikrosk. Anat.* **63**, 618–635.

Weeks, J. C. & Kristan, W. B. 1978 Initiation, maintenance and modulation of swimming in the medicinal leech by the activity of a single neurone. *J. exp. Biol.* **77**, 71–88.

Wohlers, D. W. & Huber, F. 1978 Intracellular recording and staining of cricket auditory interneurons (*Gryllus campestris* L., *Gryllus bimaculatus* DeGeer). *J. comp. Physiol.* A **127**, 11–28.

Willard, A. L. 1981 Effects of serotonin on generation of the motor program for swimming by the medicinal leech. *J. Neurisci.* **1**, 936–944.

Yau, K.-W. 1976 Receptive fields, geometry and conduction block of sensory neurones in the central nervous system of the leech. *J. Physiol., Lond.* **263**, 513–538.

Young, S. R., Dedwylder, R. D. & Friesen, W. O. 1981 Responses of the medicinal leech to water waves. *J. comp. Physiol.* A **144**, 111–116.

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