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BEHAVIOURAL AND MECHANOSENSORY NEURONE RESPONSES TO SKIN STIMULATION IN LEECHES

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SUMMARY

1. Behavioural responses to electrical stimulation of mechanosensory neurones were characterized in two species of leeches, *Hirudo medicinalis* and *Macrobdella decora*.

2. Depending upon the site and intensity of stimulation, the stimuli elicited one or a combination of five different responses: local bending, curling, shortening, whole-body bending or swimming.

3. The electrical threshold for activating identified mechanosensory neurones, T (touch) cells and P (pressure) cells, was the same in all regions of the body.

4. The voltage range over which the electrical stimuli produced progressively more mechanosensory impulses was the same as the range that produced different behavioural responses.

5. These results suggest that the T and P mechanosensory neurones provide the entire sensory input for all the behavioural responses. The production of different behavioural responses to stimuli of different intensities at the same location are attributable to different firing rates of the same sensory cells, and different responses to the same stimulus at different locations suggest different interneuronal targets for the T and P cells in different body regions.

INTRODUCTION

Mechanical stimulation of a leech produces a variety of behavioural responses, depending, among other things, upon the intensity and location of the stimulus (Gee, 1913; Mann, 1962). For instance, touching the head end produces whole-body contraction, the shortening response, whereas the same stimulus applied to the tail end usually elicits swimming. More intense mechanical stimuli produce complex responses, usually starting with violent writhing movements followed by swimming or 'looping' – an inchworm type of locomotion using the head and tail suckers.

The neuronal basis of some of these responses is now partly understood. The motor neurones responsible for shortening have been described (Stuart, 1970), as have those for swimming (Ort, Kristan & Stent, 1974). The pre-motor neurones responsible for initiating and generating these two behavioural responses are also known (Bagnoli, Brunelli & Magni, 1972; Gardner-Medwin, Jansen & Taxt, 1973; Magni & Pellegrino, 1978; Friesen, Poon & Stent, 1978; Weeks & Kristan, 1978; Weeks, 1980). The

sensory neurones that are at least partially responsible for the shortening response have been identified (Nicholls & Purves, 1970, 1972; Muller & Nicholls, 1974) as the T, P and N cells, sensitive to light touch, pressure and nociception respectively and found in each of the midbody segmental ganglia (Nicholls & Baylor, 1968; Keyser & Lent, 1977). These cells were almost certainly activated by the mechanical stimuli used to produce all the above responses.

No differences have been observed between the homologous neurones in different body regions. One may wonder, then, what neuronal differences account for the different behavioural responses. Can differences in sensory cell responses explain why the leech swims when touched posteriorly and yet shortens when touched anteriorly, or will the explanation involve differences in the central connexions of the sensory cells? We began to investigate this question using mechanical stimulation, but observed great variability in the responses, seemingly because of difficulty in delivering a stimulus of a given intensity to a particular body location. Then we found that electrical stimulation of the skin produced the same behavioural responses as did mechanical stimulation, but with much less response variability. Furthermore, T, P and N cell impulses could be elicited one-to-one with repeated stimulus pulses, thus allowing greater control of the rate of sensory cell firing. This degree of control was used to assess the mechanosensory cell activity needed to produce particular behavioural responses.

MATERIALS AND METHODS

Medicinal leeches, *Hirudo medicinalis*, were obtained from French, Hungarian and Italian suppliers. American blood-sucking leeches, *Macrobdella decora*, were obtained from a supplier in Wisconsin. All were kept in a 5% saline solution (Nicholls & Baylor, 1968) at 15 °C and fed about every 90 days on bullfrogs.

Some behavioural experiments were performed on intact animals, but most involved animals whose anterior and posterior ganglionic masses, or 'brains', had been disconnected from the ventral nerve cord by cutting the appropriate interganglionic connectives. The semi-sterile techniques used to produce these 'brainless' animals have been described previously (Kristan & Guthrie, 1977). Electrical stimuli were delivered through electrodes composed of a pair of stainless-steel wires (0·15 mm diameter) mounted on a glass rod with their tips approximately 1 mm apart and insulated except for the terminal 1 mm. A Grass SD 9 or S 88 stimulator was used to produce 1 ms biphasic electric pulses, isolated from ground, at 10 Hz. To be sure that the observed behavioural responses were to electrical and not to mechanical stimulation, the electrodes were placed gently on the skin with the stimulator turned off. There was usually no response to the electrode placement, and the electrical stimulus was delivered about 1 s after the electrodes made contact. If there was a response to touch, the electrodes were left in place and the electrical stimulus delivered about 2 s after completion of the touch-elicited response. Stimuli were presented at 0·5 V gradations, ranging from intensities which did not elicit a response, usually about 2·0 V, to intensities above which no new behaviour could be produced, which was no higher than 12 V. Only at intensities above 20 V did the stimuli activate musculature directly, as tested by the ability of electrical stimuli to cause contractions in pieces of body wall that were disconnected from the central nervous systems. Initially both 5 and 10 Hz

stimuli were used. Since no differences were apparent in the response characteristics at these two frequencies, these data were combined in the tables, and all further experiments used 10 Hz as the stimulus rate. Both intensity and position of stimulation were varied randomly. Experiments were done blind, with one experimentor regulating stimulus intensity and another recording the behavioural responses. Temperature had a strong effect on the behaviour of the animals. Therefore, all behavioural experiments, as well as the physiological experiments, were conducted at $12 \pm 0{\cdot}5$ °C. Since cutting the cord is known to have behaviour effects on body segments after about a week (Kristan & Guthrie, 1977), all experiments were performed 1–4 days after surgery.

Physiological experiments were performed on preparations consisting of pieces of the body wall connected to a segmental ganglion by one or more of its nerves on one side. The body-wall pieces were generally at least three segments long and extended from ventral to dorsal midline on either the right or left side of the animal. The body wall and ganglion were pinned to wax on the bottom of a chamber filled with leech saline (Nicholls & Baylor, 1968), with the ganglion positioned over a glass wedge, permitting transmitted darkfield illumination. Mechanical stimulation of the skin was accomplished by a firepolished 1 mm glass rod which was made to vibrate on the skin at 10 Hz by means of a piezoelectric crystal (E. Licht Co., Denver). To stimulate the skin electrically, two types of electrodes were used. The first was the same wire electrodes used for the behavioural experiments. The second was a suction electrode with a tip diameter of about 0·5 mm, which is approximately half the width of an annulus in the pinned-out body wall. In order to maintain a constant site of stimulation during body wall movements, a bleb of skin was aspirated into the tip of the suction electrode. Since this arrangement gave significantly smaller stimulus artifacts than did the wire electrodes with no apparent differences in the responses elicited, suction electrodes were used routinely.

Intracellular recordings were made using 60–80 MΩ glass microelectrodes filled with 4 M potassium acetate. Recording and current passage were achieved by using either WPI model M 701 or Getting model 4 preamplifiers. Extracellular recording was achieved via 100×, 10–1000 Hz bandpass amplifiers fabricated from operational amplifiers. All electrical signals were amplified with a second set of amplifiers (d.c. to 1 kHz) to between 1 and 5 V and recorded on a Vetter–Crown, model A, tape-recorder with FM-5T plug-ins. All signals were also recorded on a Gould–Brush, model 260 chart recorder both on-line and from the tape-recorded signals played back at one-quarter speed for analysis and production of figures. With this recording system, signals from d.c. to 200 Hz were unattenuated.

RESULTS

(1) *Responses to electrical stimulation of the skin*

Initial behavioural experiments were performed by touching intact leeches on different regions of the skin with a blunt probe. As initially described using other leech species (Gee, 1913), touching the head region of either *Macrobdella* or *Hirudo* produced retraction, whereas touching the back produced locomotory movements, either

swimming or looping, away from the stimulus. However, the responses were variable. depending to a great extent upon the state of the animal at the time of stimulation. If, for instance, the leech was making searching movements. lightly touching any part of the body would usually cause shortening. On the other hand, animals that were curled up and motionless were unresponsive except to intense mechanical stimuli. This variability in responsiveness was significantly diminished after the head and tail brains were disconnected from the ventral nerve cord. A further reduction in response variability was achieved by the use of electrical rather than mechanical stimulation of the skin, presumably because a more reproducible stimulus intensity could be achieved with electrical stimulation. The removal of the brains eliminated both the looping behaviour and the 'spontaneous' searching movements, but did not cause any apparent change in the form of the other responses.

In both *Hirudo* and *Macrobdella* there were five different behavioural responses to electrical stimulation: local bending, shortening, curling, whole-body bending, and swimming (Fig. 1). Often two or three of these responses occurred in serial combination. The two most common such combinations were curling followed by swimming and whole-body bending followed by swimming.

Fig. 1 (A) illustrates the local bending reflex. In this example, a weak stimulus to the dorsal surface at the anterior end produced a localized contraction that involved only the segment stimulated and one or two segments on either side. This response resulted primarily from the contraction of the dorsal longitudinal muscles, producing a downward bending of the body which pulled the stimulated skin away from the site of stimulation. A similar response could be elicited from stimulation of any part of the body. Comparable stimulation of the ventral surface produced a similarly localized upward bending response at the site of stimulation.

At greater stimulus intensities to the front end there was usually a symmetric shortening of the whole body (Fig. 1 B) and often the animal also curled its anterior end downward, away from the stimulus (Fig. 1 C). Occasionally the animal would curl with no shortening. When applied to the posterior end, comparable stimuli often elicited a similar curling response in the posterior end.

Comparing Fig. 1 (B–E) shows that the same electrical stimulus, of moderate intensity, produced different responses when delivered to different body regions. Stimulation of the midbody region typically produced whole-body bending – a dorsoflexion of both ends of the animal – which was usually accompanied by body extension, as shown in Fig. 1 (D). Similar stimuli delivered to the posterior end often produced swimming (Fig. 1 E). At very high intensities at any point on the animal (not pictured) there was a violent twisting or writhing similar to that seen in an earthworm when stuck with a fishhook.

Many of the behavioural responses, such as shortening and swimming, appeared to be incompatible, because they involve different patterns of activation of the same muscles (Ort *et al.* 1974). However, some, like shortening and curling, were often performed simultaneously. In fact, local shortening seemed compatible with all responses, although it was often difficult to detect during strong whole body movements. Also, incompatible responses, such as curling and swimming, were often elicited in sequence by a single stimulus.

The behavioural responses produced by stimuli of different intensity and locati

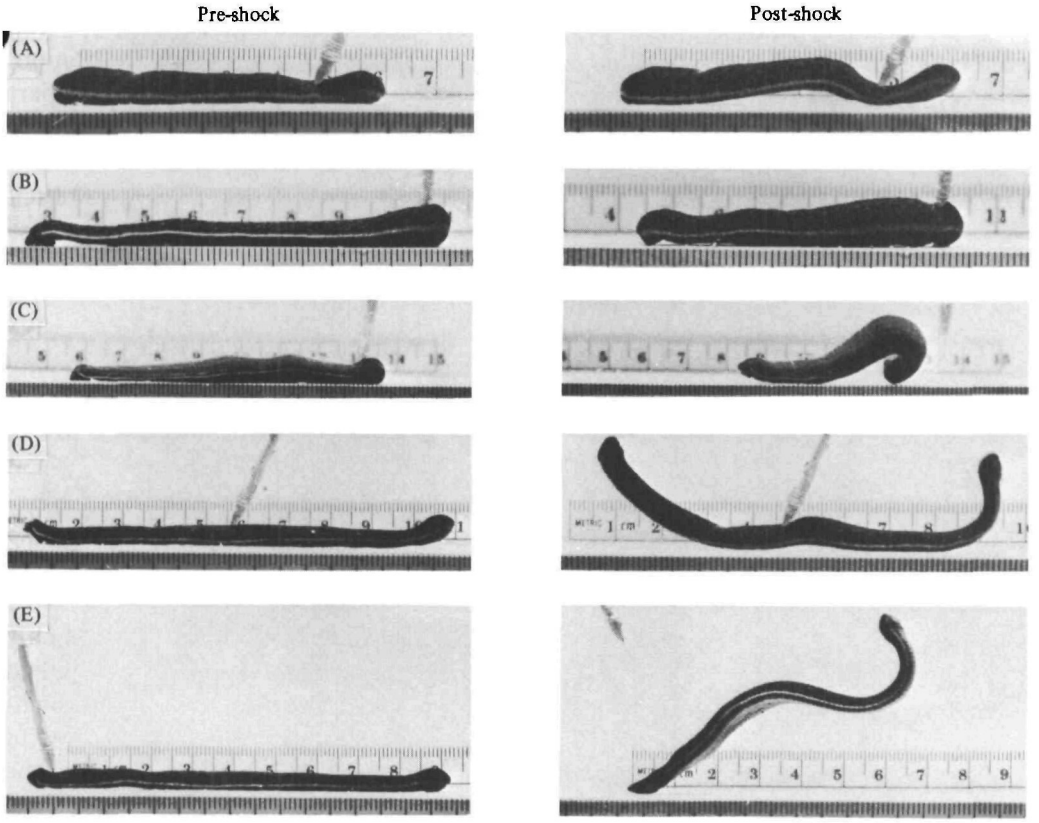

Fig. 1. Typical behavioural responses of leeches to electrical stimulation. All pictures are of a medicinal leech, *H. medicinalis*, lying on the bottom of a water-filled chamber with its anterior end to the right. In all cases, the pre-stimulation body form is shown on the left with the stimulating electrodes in place, and the response to stimulation (after 1–5 s) is shown on the right. Stimuli were 1 ms, biphasic pulses at 10 Hz. Five different responses are pictured: (A) local bending; (B) shortening; (C) curling, which consists of a ventroflexion of the stimulated end of the body, accompanied often by shortening of the rest of the body; (D) whole-body bending, which consists of a dorsoflexion, usually of both ends of the body; (E) swimming. The vertical lines are 1 mm marks on rulers attached to the front and back of the chamber; the leech is approximately half-way between the rulers.

are shown in Table 1 A for *Hirudo medicinalis* and Table 1 B for *Macrobdella decora*. During the course of an experiment, each leech received, in random order, 3 trials of stimulus trains at each half-volt step in the range from 2 to 12 V.

Tables 1 and 2 show four major features. First, both species showed a similar pattern of responses to the stimulus trains of different intensity at different locations. Secondly, low-intensity stimuli delivered to any region of the body caused primarily a local bending reflex. Thirdly, stimuli of moderate intensity elicited different responses depending upon the site of stimulation. For instance, stimuli between 4·5 and 7 V delivered to the anterior end elicited primarily shortening and curling, whereas the same stimuli were more likely to elicit whole-body bending when applied to the middle, and to elicit swimming or curling when applied to the posterior end. Fourthly, in any given region, as the stimulus intensity was increased, there was a progression in

Table 1 A. *Behavioural responses of* 8 Hirudo medicinalis, *stimulated* 3 *times at each* 0·5 *V interval from* 2·5 *to* 12·0 *V on the dorsal skin of segments* 4 (*anterior*), 10 (*middle*) *and* 17 (*posterior*)

Stimulus intensity	No response	Local bend	Shorten	Curl	Whole-body bend	Whole-body bend then swim	Curl then swim	Swim	Other
					Anterior				
2·5–3·0	16	19	—	5	—	—	—	—	2
3·5–4·0	5	22	5	13	1	—	—	—	1
4·5–5·0	—	20	3	21	—	—	4	—	—
5·5–6·0	—	5	5	18	—	—	9	1	—
6·5–7·0	—	4	—	17	2	—	2	1	1
7·5–12·0	—	4	—	34	1	—	5	—	—
					Middle				
2·5–3·0	16	22	—	—	—	—	—	—	5
3·5–4·0	5	27	—	—	13	—	—	—	2
4·5–5·0	—	20	—	—	9	12	—	1	—
5·5–6·0	—	13	—	—	7	16	—	—	1
6·5–7·0	—	6	—	—	8	8	—	—	—
7·5–12·0	—	1	—	1	2	39	—	—	1
					Posterior				
2·5–3·0	20	15	—	3	—	—	—	—	4
3·5–4·0	6	24	—	1	4	—	1	4	3
4·5–5·0	—	16	—	5	2	3	—	13	5
5·5–6·0	—	11	—	3	2	4	1	14	2
6·5–7·0	—	4	—	8	—	2	—	9	3
7·5–12·0	—	3	—	7	—	1	18	14	2

(Number of responses of each type described in the text and Figure 1 are indicated.)

Table 1 B. *Behavioural responses of* 4 Macrobdella decora, *stimulated* 3 *times at each* 0·5 *V interval from* 2·5 *to* 6·0 *V on the dorsal skin of segments* 4 (*anterior*), 10 (*middle*) *and* 17 (*posterior*)

Stimulus intensity	No response	Local bend	Shorten	Curl	Whole-body bend	Whole-body bend then swim	Curl then swim	Swim	Other
					Anterior				
2·5–3·0	12	11	6	—	—	—	—	—	—
3·5–4·0	—	5	10	13	1	—	1	—	—
4·5–5·0	—	—	2	8	1	—	14	—	—
5·5–6·0	—	—	—	1	—	—	15	1	—
					Middle				
2·5–3·0	12	15	—	—	—	—	—	—	—
3·5–4·0	—	11	—	—	12	8	—	—	—
4·5–5·0	—	2	—	—	3	18	—	—	—
5·5–6·0	—	—	—	—	—	23	—	—	—
					Posterior				
2·5–3·0	12	16	1	—	4	—	—	—	—
3·5–4·0	—	8	—	—	11	1	—	8	—
4·5–5·0	—	—	—	—	1	14	—	9	—
5·5–6·0	—	—	—	—	—	18	—	5	—

response type and complexity. For instance, when applied to the anterior end, the lowest effective stimulus intensity elicited only the local bending response; moderate intensities elicited local bending accompanied by shortening; high intensities caused shortening and curling, sometimes followed by swimming.

The vigour of the responses consistently increased with increased stimulus intensity (which is not indicated in Table 1). For instance, when a 5–6 V stimulus was applied to the posterior end of an animal, it tensed up as if startled, then started to swim. As the stimulus intensity was increased, the initial response and the swimming movements increased in vigour and duration until at 10–12 V, the animal typically writhed, sometimes for several seconds, then swam away at maximal speed.

It should be noted that all behavioural responses included in Fig. 1 and Table 1 were elicited by stimuli delivered on or near the dorsal midline. This procedure was used for convenience, since even brainless leeches keep their dorsal surface uppermost. A few experiments on leeches suspended by threads showed that the threshold and quality of response to electrical stimulation was the same on the ventral as on the dorsal body surface. This was somewhat unexpected because Gray, Lissman & Pumphrey (1938) reported that mechanical stimulation of the ventral surface produced a different response from that produced by dorsal stimulation, especially during swimming. We could not duplicate these results by presenting either mechanical or electrical stimuli. To deliver electrical stimuli, fine silver wires were sewn into the dorsal or ventral body wall, an arrangement which allowed the stimuli to be delivered reproducibly to moving animals. The responses to such stimuli were complex, with the animals initially speeding up then slowing down, but were the same whether the stimuli were delivered to the dorsal or ventral body surface.

(2) *Activation of mechanoreceptor neurones by stimulation of the skin*

The similarity of electrically evoked responses to those evoked by mechanical stimulation suggested that the electrical stimuli were activating mechanoreceptor neurones. To confirm this possibility, intracellular recordings were obtained from the identified mechanoreceptor neurones, the T, P and N cells, using ganglion/body-wall preparations, while stimulating the skin with mechanical or electrical stimuli capable of eliciting the various responses in intact or brainless animals. Although mechanical and electrical stimulation activated the same mechanoreceptors, mechanical stimulation produced a much more variable response (compare Fig. 2 with Figs. 3 and 4).

(A) *Mechanical stimulation.* The responses produced by mechanical stimulation were elicited using a piezoelectric crystal to push a small glass rod against the skin of a pinned-out piece of body wall while recording intracellularly from the T and P cells with appropriate receptive fields (Fig. 2A). As shown in Fig. 2(B), low-amplitude mechanical stimuli selectively stimulated the T cell without affecting the P cell. At slightly higher stimulus intensities, the P cell was also activated, and the response outlasted the stimulus by about 2 s (Fig. 2C). Both P and T cells produced a variable number and timing of impulses with each stimulus (compare Fig. 2C, D).

(B) *Electrical stimulation.* The responses to electrical stimulation were tested with electrical pulses delivered to the dorsal skin of a similar preparation via wire or suction electrodes, while recording intracellularly from the T, P and N cells with

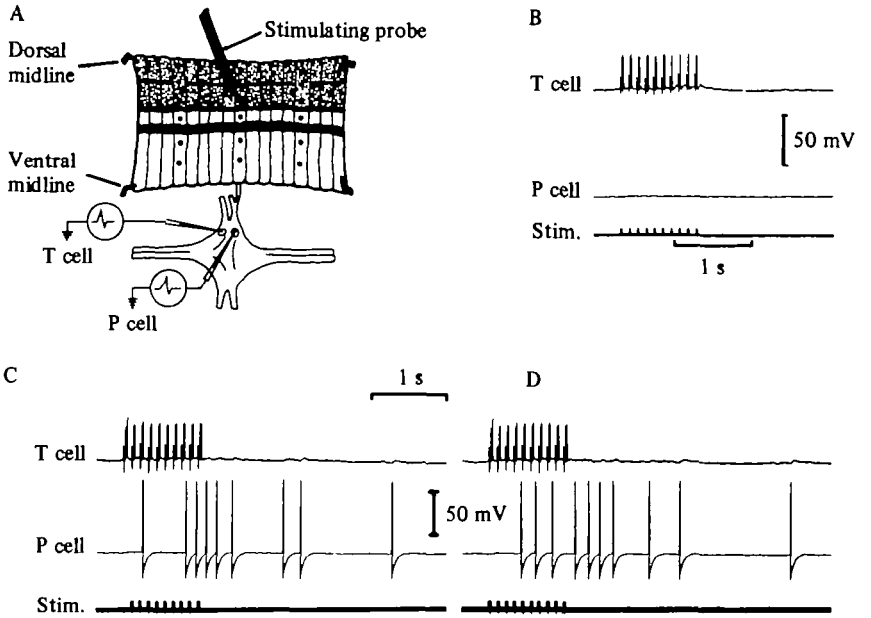

Fig. 2. Intracellular responses of T and P cells to mechanical stimulation of the skin. (A) The experimental arrangement consisted of a right or left half of the body wall, three segments in length, attached to a single ganglion by the dorsal branch of the posterior nerve (DP) nerve. Intracellular recordings were made from cells in the segmental ganglion. The scale for the ganglion is about ten times that used for the skin, so that details can be shown for both structures. The ganglion is about 0·5 mm across and an annulus is about 1 mm across. (B–D) The top two traces are intracellular recordings from the T and P cells respectively. The bottom trace shows the times of application of a small mechanical deformation to the skin, via a small glass rod pushed by a piezoelectric crystal driven by 1 ms square pulses at 10 Hz. (B) Responses of T and P cells to low-intensity mechanical stimuli. (C) Responses to more forceful stimuli. (D) A second set of responses to stimuli at the same intensity as in (C). Vertical lines in the T cell recordings just before the impulses are electrical artifacts produced by the mechanical stimulator.

dorsal receptive fields, and extracellularly from the one nerve attached to the dorsal skin. The central annulus, which contain fibres activated by water waves (Friesen, 1981), were avoided. Therefore, the three neurones being recorded were the only known mechanoreceptors being activated (Nicholls & Baylor, 1968). As the intensity of the stimulus was increased, the tactile sensory neurones were recruited in the order T, then P, then N (Fig. 3A). Expansions of the records (Fig. 3B–D) show that the latency to the action potentials recorded both in the cell body and in the DP nerve increased in the same order. Although conduction velocity cannot be determined accurately because the length of the axonal pathway can only be roughly estimated, these latencies correspond to conduction velocities of approximately 55, 30 and 7 cm/s.

The appearance of the mechanoreceptor action potentials recorded in the DP nerve is so reproducible in relative size, shape and latency that it is possible to identify the T and P cell impulses in every DP recording, although for all results in this report their identity was confirmed by intracellular recording. The N cell action potentials were small and of sufficiently long latency that they were often mixed with the motor neurone's action potentials reflexly activated by the stimuli. Hence, to identify N cell

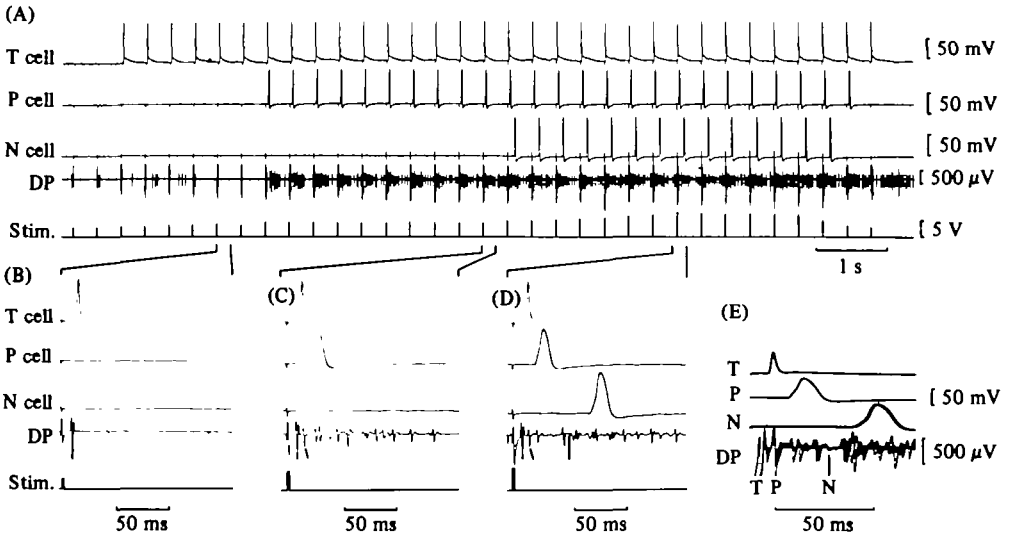

Fig. 3. Responses of mechanosensory cells to increasing intensities of electrical stimulation of the skin. The preparation used was that shown in Fig. 2(A), with the addition of an *en passant* recording from the DP nerve connecting the ganglion to the body wall. (A) The top three traces are intracellular recordings from the T, P and N mechanoreceptor cells, showing their responses to 1 ms electrical stimuli of different intensities delivered to the dorsal skin. The fourth trace shows the recording from the DP nerve. The lengths of the vertical lines in the bottom trace indicate the intensity of the electrical stimulus. (B–D) Tenfold time expansions of parts of the recording shown in (A). The extracellularly recorded T, P and N impulses are identified in the DP trace. (E) Four superimposed oscilloscope traces triggered by the stimulus to show the reproducibility of the mechanoreceptor responses and to identify the N cell impulse in the DP recording.

impulses in nerve recordings, it was usually necessary to examine multiple, superimposed traces triggered by successive stimuli, as shown in Fig. 3(E).

In anticipation of a discussion in the paper to follow (Kristan, 1982), it should be noted in Fig. 3(A) that reflex activation of motor neurones, represented by the relatively small impulses between the sensory cell impulses in the DP nerve recording, was minimal at stimulus intensities that activated only the T cell. At intensities that activated both the T and P cell, the motor neurone activity sharply increased, and did not increase noticeably as the N cell was recruited.

(C) *Electrical thresholds of mechanoreceptors.* In order to determine which tactile sensory neurones were activated by the electrical stimuli used to elicit behavioural responses, we attempted to determine the threshold for each sensory neurone over the extent of its receptive field. We did not measure the absolute threshold, i.e. the lowest voltage at which a single electrical shock produced a sensory cell impulse, because this was quite variable. Instead, we measured the minimum voltage at which every shock produced an impulse. We had to adopt a standard stimulation regime because the success of a particular shock was strongly dependent upon the frequency at which it was repeated. Since a delivery rate of 10 Hz was used in the behavioural experiments and a reflex response usually occurred within a second, we settled upon a 1 s train of 1 ms pulses at 10 Hz as the standard test regime.

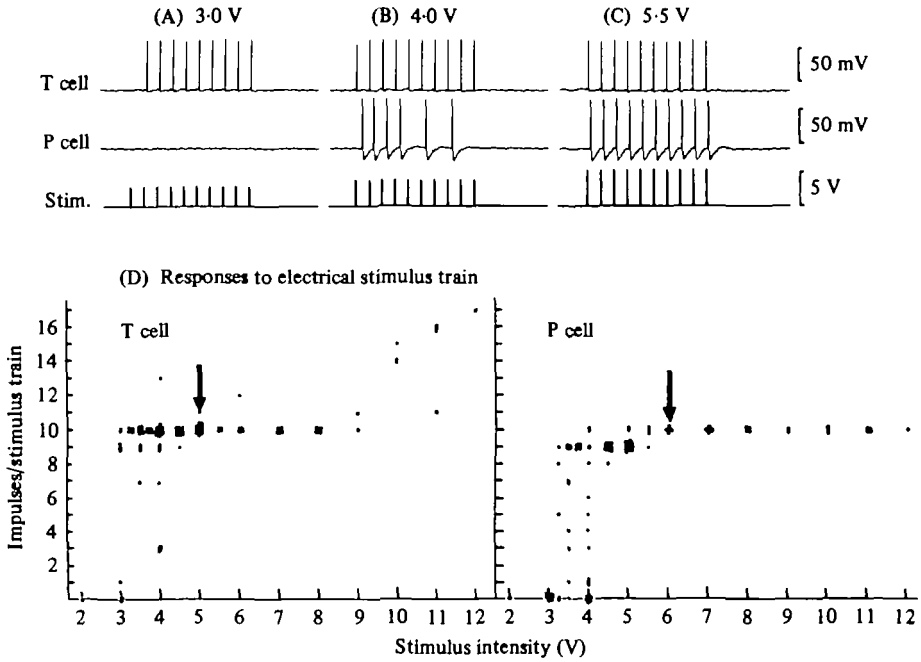

Fig. 4. Determination of electrical threshold of mechanosensory neurones. The preparation used was the same as that shown in Fig. 2(A). (A–C) Responses of T and P cells with dorsal receptive fields to electrical stimulation of the dorsal skin. The top trace in each case is an intracellular recording from the T cell, the middle trace is an intracellular recording from the P cell and the bottom traces mark the time of application of electrical pulses at 10 Hz for 1 s. The amplitude of the pulses was 3·0 V in (A), 4·0 V in (B) and 5·5 V in (C). (D) Graphs of the number of T and P cell impulses in response to ten pulse-stimulus trains at voltages between 2 and 12 V. Arrows mark the threshold, i.e. the minimum stimulus intensity at which every pulse produced an action potential. Points above ten impulses per train in the T cell graph were caused by doublets of impusles in response to some stimulus pulses, particularly those of high intensity. Such doublets were observed in about half the preparations used.

The stimulus intensity was raised from 2 V in 0·5 V steps with each successive standard regime. As the intensity was raised above 3 V, the T and P cells produced responses to more and more of the stimulus pulses (e.g. Fig. 4A–C). When an intensity was found at which each shock produced at least 1 impulse, the test stimulus at that intensity was repeated at least twice more. By these tests, the thresholds for T and P cell activation were 5 and 6 V, respectively (arrows, Fig. 4D), even though the minimum voltage at which any impulse was elicited in either neurone was lower by 2–3 V. Fig. 4(D) shows that, over a broad range of stimulus intensities, each stimulus train produced exactly the response pattern seen in Fig. 4(C), a much more uniform response than that to the mechanical stimuli shown in Fig. 3.

It was of some concern that we might be stimulating the secondary receptive fields of sensory cells whose cell bodies were located in adjacent ganglia (Yau, 1976). However, the threshold for activating these secondary fields via the large suction electrodes proved to be higher than that for the N cell primary receptive fields and, therefore, was well above the intensities used in this study.

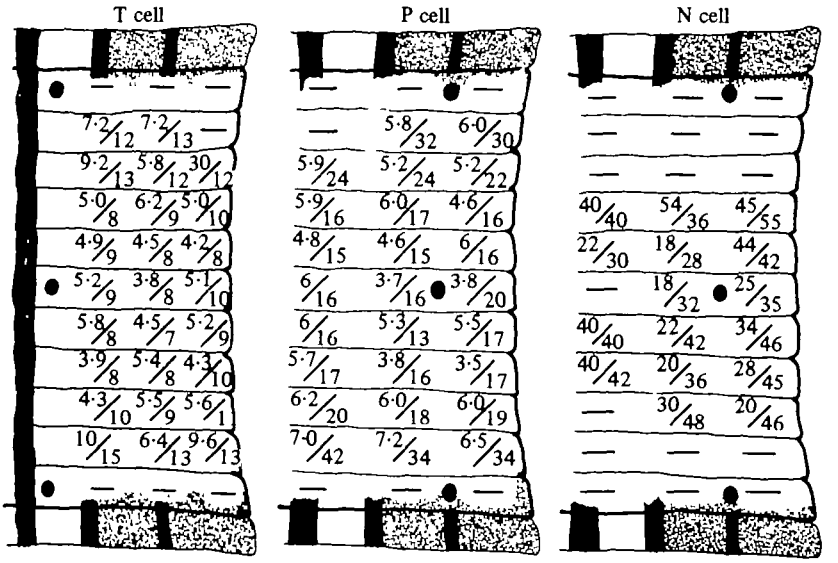

Fig. 5. Thresholds and latencies of mechanosensory cells to electrical stimulation of the skin. The preparation used was the same as that shown in Fig. 2(A). Thirteen annuli from the right side of the body wall are represented, with the anterior end uppermost, the dorsal midline on the right, and the longitudinal dark stripe just below the lateral midline shown on the left edge. Mechanoreceptors were recorded from a ganglion attached to the body wall at the middle annulus. Black circles indicate the location of several sensilla. The T, P and N cells with dorsal receptive fields in segment 4 were recorded intracellularly while determining the threshold to electrical stimulation using a suction electrode applied to the regions indicated on the drawing of the body wall. The numerator is the voltage required to elicit impulses reliably in the particular sensory cell at 10 Hz and the denominator is the latency, i.e. the number of milliseconds from the stimulus to the peak of the evoked intracellularly recorded action potential. As the stimulus intensity was increased, the latency decreased somewhat to a minimum value; the number shown is that minimum value. Dashes indicate that the sensory cell could not be activated at that site by electrical pulses up to 80 V in amplitude.

3. *Mechanoreceptor neurone receptive fields determined by electrical stimulation*

The sensory-cell primary receptive fields, as determined by touch or by electrical stimulation with suction electrodes, were roughly ovoid in shape and extended two or three annuli into adjoining segments on one side of the animal (Fig. 5). The voltage required to activate any mechanoreceptor (indicated by the numerator at each stimulus site) increased as the stimulating electrode was moved to the periphery of the receptive field. The thresholds for T and P activation were nearly equal at all overlapping responsive sites, but both had lower thresholds than did the N cell. The latencies for impulses recorded in a particular neurone (indicated by the denominator at each stimulus site) increased as the stimulus site was moved further from the middle of the central annulus. Also, there was a consistent pattern in the relative latencies among the mechanoreceptors: the P cell latency was about twice that of the T cell and the N cell latency was about twice the P cell latency. These general features were found in segments from the front, middle and back of both leech species.

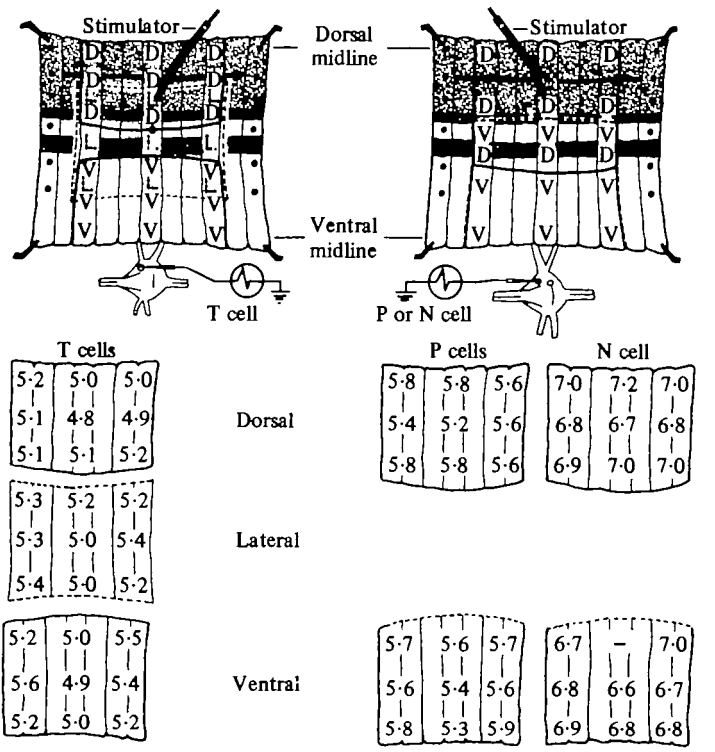

Fig. 6. Thresholds of the seven known mechanosensory cells innervating one side of the body wall. The numbers shown are the thresholds to electrical stimulation at nine locations within the sensory-cell receptive fields, represented schematically. The thresholds for the three T cells, with dorsal, lateral and ventral receptive fields, are shown on the left, and the thresholds for the two P and N cells are in the middle and to the right, respectively. The drawings at the top indicate the regions of the body wall represented in the rectangles below. The data shown were obtained from segment 10 in *H. medicinalis*.

(4) *Comparison of thresholds of mechanoreceptor neurones*

(A) *Within a segment.* The body wall was left attached to the ganglion by all its peripheral nerves on one side and dorsal, lateral, and ventral receptive field thresholds were determined. As indicated in Fig. 6, nine separate locations were tested in each receptive field. There appeared to be no significant difference in the thresholds of different cells of a particular type having different receptive fields. For instance, the thresholds for activating the dorsal, lateral and ventral T cells in the centre of their receptive fields were 4·8, 5·0 and 4·9, respectively. This similarity was seen over the whole receptive field for these cells, and also for the P and N cells.

(B) *Between segments.* The experiment illustrated in Fig. 6 was repeated using body-wall preparations obtained from the head and tail ends. Again, there was no apparent differences in the thresholds of corresponding sensory neurones (Fig. 7).

(C) *Statistical comparisons.* Thresholds were determined for all mechanorceptor neurones in segments 4, 10 and 17 in 4 different *Hirudo* and 4 different *Macrobdella*. The voltage threshold for the most sensitive, central section of the receptive field was averaged for each type of sensory cell in both species and compiled in Table 2. The

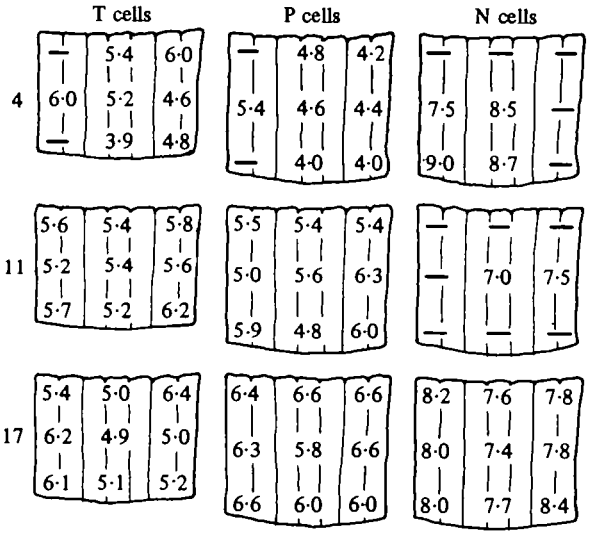

Fig. 7. Thresholds of the mechanosensory cells with dorsal receptive fields in segments 4, 10 and 17 in *H. medicinalis*. The preparations used were as per Fig. 2(A) and the techniques for determining threshold were as per Fig. 4. Dashes indicate that the thresholds were not determined at those locations.

Table 2. *Mean and standard deviation of threshold for dorsal (D) and ventral (V) T, P and N cells in preparations from anterior (segment 4), middle (segment 10) and posterior (segment 17) body regions for two species of leech*

Segment	Location	T	P	N
		(A) Data from 4 *Hirudo medicinalis*		
4	D	4·7 ± 0·41	4·9 ± 0·76	7·3 ± 0·41
	V	4·3 ± 0·41	4·5 ± 0·58	6·7 ± 0·76
10	D	4·3 ± 0·76	4·5 ± 0·9	7·9 ± 1·4
	V	3·9 ± 0·76	4·5 ± 0·46	7·4 ± 1·04
17	D	4·3 ± 0·41	4·2 ± 0·25	7·6 ± 1·29
	V	4·9 ± 0·91	5·0 ± 0·46	7·4 ± 1·37
		(B) Data from 4 *Macrobdella decora*		
4	D	4·8 ± 0·85	5·1 ± 0·74	6·6 ± 0·95
	V	4·4 ± 0·71	5·4 ± 0·51	6·6 ± 1·04
10	D	4·7 ± 0·68	5·2 ± 0·47	6·6 ± 0·60
	V	4·9 ± 0·21	5·4 ± 0·13	6·5 ± 0·23
17	D	4·6 ± 0·59	5·1 ± 0·23	6·5 ± 0·25
	V	4·7 ± 0·44	5·1 ± 0·31	6·4 ± 0·21

small standard deviations indicate that the stimulus thresholds are quite uniform between animals. Using these data, a *t*-test was performed testing the hypothesis that there was no difference in response threshold between:

(*a*) sensory neurones of a given type with different receptive fields in the same segment,

(*b*) sensory cells of a given type in different segments,

(*c*) sensory cells of a given type in the two different species,

(*d*) different types of sensory neurones.

Table 3. *Behavioural responses of a single* Hirudo medicinalis, *stimulated* 3 *times at ea* 0·5 *V interval from* 2·5 *to* 6·5 *V* (*these data are from the same leech as was used for th physiological data shown in Fig.* 7).

Stimulus intensity	No response	Local bend	Shorten	Curl	Whole-body bend	Whole-body bend then swim	Curl then swim	Swim	Other
					Anterior (4)				
2·5–3·0	3	3	—	—	—	—	—	—	—
3·5–4·0	—	3	1	3	—	—	—	—	—
4·5–5·0	—	—	1	5	—	—	—	—	—
5·5–6·0	—	—	—	4	—	—	2	—	—
6·5–7·0	—	—	—	1	—	—	2	—	—
					Middle (11)				
2·5–3·0	3	3	—	—	—	—	—	—	—
3·5–4·0	—	6	—	—	4	—	—	—	—
4·5–5·0	—	2	—	—	4	3	—	—	—
5·5–6·0	—	—	—	—	—	6	—	—	—
6·5–7·0	—	—	—	—	—	3	—	—	—
					Posterior (17)				
2·5–3·0	3	3	—	—	—	—	—	—	—
3·5–4·0	—	6	—	—	—	—	—	—	—
4·5–5·0	—	2	—	—	1	1	—	2	—
5·5–6·0	—	—	—	—	—	1	—	5	—
6·5–7·0	—	—	—	—	—	2	—	1	—

The null hypothesis could be rejected only in comparing either T or P cells to N cells; in these cases it could be rejected with great confidence ($P < 0{\cdot}001$). In all other cases, the null hypothesis could not be rejected even at the 10% level. Therefore, the T and P cell thresholds were indistinguishable from one another in all parts of all leeches, whether they were recorded in *Hirudo* or *Macrobdella*, but the thresholds for N cells were greater than the thresholds for the other mechanoreceptor neurones.

5. *Comparison of behavioural thresholds with mechanoreceptor neurone thresholds*

There is a good correlation between the behavioural data in Table 1 and physiological data in Figs. 5–7, as would be expected if the identified mechanoreceptor neurones were responsible for eliciting the behavioural responses. However, such comparison does not take into account the variation in behavioural threshold observed in the same animal on different days, variations in the behaviour of different animals, and variations in the responses of the same animal using different stimulating electrodes. To avoid these sources of variability, both behavioural and physiological experiments were performed on the same animal on the same day using the same stimulating electrodes.

Three days after having their brains disconnected from the ventral nerve cord, three leeches (*Hirudo medicinalis*) were first tested behaviourally to different intensities of electrical stimulation, and then were immediately dissected for determination of the physiological thresholds for dorsal T, P and N cells in segments 4, 11 and 17. All leeches showed similar results. Behavioural data from one leech are given in Table 3, and the corresponding physiological threshold data are those shown in Fig. 7.

The lowest stimulus intensity that produced behavioural responses was 3 V, whereas the minimal responses for T and P cell activation were at 5 V. Two reasons can be proposed. First, the behavioural thresholds were determined in a 5% saline solution whereas the physiological experiments were performed on preparations bathed in a full-strength saline solution. When behavioural experiments were performed in saline, the thresholds were found to increase by 0·5–1·0 V. Second, the thresholds recorded represent the voltages at which each stimulus pulse delivered at 10 Hz produced an action potential. The threshold for eliciting *any* impulses in these sensory neurones (Fig. 4) was 2–3 V lower than the values shown in Fig. 7.

This comparison shows that it is highly likely that T and P cells were activated by voltages in the range that produced the behavioural responses and indicate that the behavioural responses result from the activation of these mechanoreceptor neurones. This is shown conclusively for the local bending response in the following paper (Kristan, 1982).

DISCUSSION

(1) *The use of electrical stimulation*

Electrical stimulation of the skin provided a convenient means for delivering stimuli reliably and reproducibly to particular body locations. However, there are two ways in which eliciting behavioural responses with electrical stimulation might differ from eliciting them mechanically. First, the mechanoreceptor impulse activity pattern might be different. Whereas it is true that the normal transduction mechanism at the mechanoreceptor terminals is bypassed by electrical stimulation of the skin, the impulses reaching the ganglion, where all synaptic contacts are made, are identical in all respects to mechanically elicited impulses. The firing frequencies induced in the mechanoreceptors (up to 20 Hz in T cells and 10 Hz in P and N cells) are well within the response frequencies observed to natural stimuli, and are well below the frequencies at which failure of impulse propagation in peripheral branches has been observed (Van Essen, 1973). However, the firing frequency of the mechanoreceptors was constant throughout the electrical stimulus trains, whereas the firing frequency in response to mechanical stimulation decreased after an initially higher level. It is unlikely that this feature changes the responses qualitatively, however, because the responses obtained at electrical stimulation frequencies of both 5 and 10 Hz were similar to those obtained from mechanical stimuli.

A second potential problem that arises from the use of electrical stimulation is that other sensory neurones, either as yet unidentified mechanoreceptors or receptors for other sensory modalities, may have been activated by electrical stimulation of the skin. In the experiments reported here, the electrodes were not placed near the sensilla (specialized receptor organs found only on the centre annulus of each segment), and no time-locked extracellularly recorded impulses were seen in response to the levels of electrical stimulation used in this study. It is possible, however, that we did activate other sensory neurones with such small axons that they generated too little current to be detected in our extracellular recordings. Evidence that no such elusive sensory neurones contribute to the local bending response is presented in the following paper (Kristan, 1982).

The receptive fields defined by electrical stimulation are similar in size and shape to those defined by mechanical stimuli (Nicholls & Baylor, 1968). Using very fine-tipped mechanical probes or suction electrodes, Nicholls & Baylor found that the spots within the receptive field that would elicit mechanoreceptor impulses decreased in density but not in threshold at the margins of the receptive field. They proposed that these sensitive spots probably correspond to individual terminals of the mechanoreceptors. Using larger stimulating electrodes, we found that, rather than larger regions of inexcitability, the threshold of activation increased at the receptive field margins. Hence, assuming the Baylor & Nicholls interpretation, the density of sensory terminals was indicated in our studies by differences in the activation threshold. The increased latency that was seen as the stimulating electrode was moved to the edges of the receptive fields probably results both from a longer conduction pathway and a longer transduction time in areas of low terminal density.

(2) *Differences in behavioural responses to different stimulus intensities*

At any location along the animal, two distinct response thresholds were observed as the stimulus intensity was increased. At the lowest effective intensity (2·5–3 V) there was a local bending away from the stimulus and at higher intensities (around 4 V) there was a stronger movement away from the stimulus involving the whole body. Since the T and P cells have similar thresholds (Table 2), these two behavioural thresholds could not result from differential activation of the two types of mechanosensory neurone. It is more likely that the two types of behaviour resulted from different levels of activity in the T and P cells. The selection of a response might result from the activation of different interneurones, i.e. the 'pattern generators', each sensitive to different firing rates of T and P cells. These possibilities can be tested by stimulating the T cells and P cells individually and in combination, while observing the behavioural consequences, a strategy used for studying the bending response in the following paper (Kristan, 1982).

At higher stimulus intensities, the whole-body responses become stronger and more complex, with serial combinations of the individual responses predominating. At the highest intensities used, the response was clearly a violent writhing response similar to that seen when leeches are pinned out for dissection. It is possible that writhing is a distinct behaviour, with its own pattern generator. Such responses can sometimes be elicited by intracellular stimulation of individual N cells in semi-intact preparations (W. Kristan, unpublished observations), but they are not as strong as the responses elicited by high-intensity electrical or mechanical stimulation. Therefore, it is possible that activation of T and P cells also helps to elicite writhing responses.

(3) *Differences in behavioural responses to the same stimulus intensity in different body regions*

At moderate stimulus intensities (about 4–8 V) there is a clear distinction in the type of response elicited: anterior stimulation produced shortening or anterior curling; midbody stimulation produced whole body bending; and posterior stimulation produced posterior curling or swimming. Such a dichotomy has been found in other leeches using mechanical stimulation (Gee, 1913).

The very strong similarity in the properties of different mechanoreceptors of a given type within a segment, between segments and even between animals all but eliminates the possibility that differences in behavioural response are due to differences in the response characteristics of the mechanoreceptor neurones. It is much more likely that the behavioural differences are due to connexions of the mechanosensory neurones in different body regions on to different interneurones.

It should be noted that no stimulus intensity used in this study produced the type of inchworm locomotion termed 'looping' (Gee, 1913). This is probably because the animals in this study all had their head and tail ganglionic masses ('brains') surgically separated from the midbody region in which behavioural responses were tested. It has been noted previously (Gray *et al.* 1938) that the nerve cord must be intact between the two brains in order for the head and tail suckers to be used in coordination – a necessity for producing the looping behaviour.

(4) *Extensions of the behavioural studies*

It is hoped that the quantitative descriptions of the behavioural response will serve as a basis for further studies, such as those upon behavioural plasticity (Henderson & Strong, 1972), recovery of function after surgical damage (Kristan & Guthrie, 1977; Muller & Carbonetto, 1979) and the onset of different patterns of behaviour during development (Weisblat *et al.* 1980).

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