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NEURONES IN THE LEECH THAT FACILITATE AN AVOIDANCE BEHAVIOUR FOLLOWING NEARFIELD WATER DISTURBANCES

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SUMMARY

1. A multimodal, multisegmental interneurone (Röhde's fibre, RF) and previously identified mechanoreceptors (T-cells) are shown to respond to nearfield disturbances. Both the T-cells and RF can fire for hundreds of milliseconds following a brief stimulus, and both have subthreshold excitatory synapses onto motor neurones that cause longitudinal contraction of the body wall, an avoidance response.

2. Natural stimulation or electrical stimulation of T-cells in one hemiganglion causes synaptic excitation of T-cells in adjacent ipsilateral hemiganglia and re-excitation of T-cells in the hemiganglion stimulated. A model of repetitive T-cell activity that incorporates previously described synapses among T-cells is presented: the T-cells in adjacent ipsilateral hemiganglia form a reverberatory circuit, re-exciting one another via electrical synapses; repetitive firing is terminated by synaptic inhibition onto T-cells provided by an interneurone excited by the T-cells. With repeated stimulation (0·1–0·2 Hz, 0·2 ms pulses) of a segmental root (directly exciting all the T-cells of a hemiganglion), the number of T-cell impulses per stimulus decreases. Facilitation of inhibition may contribute to the response decrement.

3. The T-cell–RF pathway is investigated. T-cell stimulation can elicit RF impulses in the same and in adjacent ganglia. The long delay between mechanoreceptor stimulation and a response in the interneurone suggests that spatial and temporal summation of T-cell inputs may be required to reach firing threshold in the interneurone.

4. The impulse frequency of the RF response was compared for a travelling surface wave that is approaching a segment *v.* one that is moving away from the segment. It was found that the frequency was greater as the stimulus approaches; this should allow more effective temporal summation of the subthreshold synaptic potentials which RF evokes in motor neurones that cause longitudinal contraction of the body wall. Therefore, the probability of contraction is greater in segments toward which a stimulus is moving.

INTRODUCTION

It is well known that leeches respond behaviourally to nearfield water disturbances. A *Glossiphonia* resting in a shallow dish of water responds to a disturbance of the water

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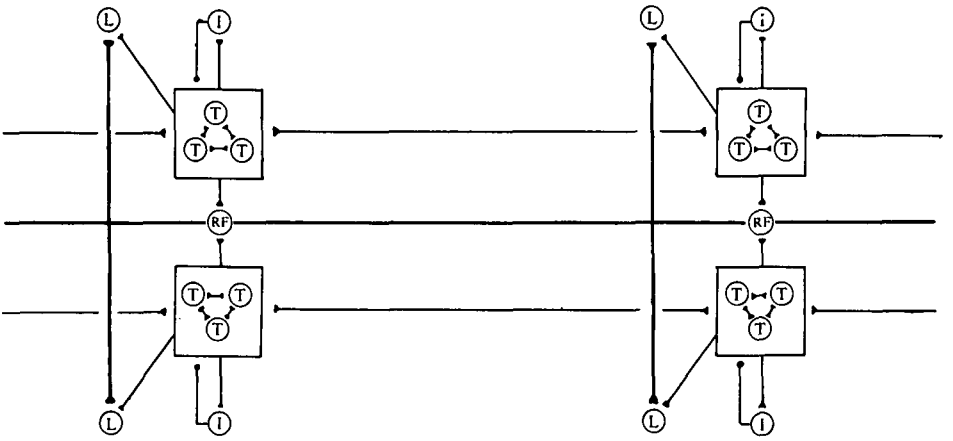


Fig. 1. Connexions among T-cells, RF, and longitudinal motor neurones are shown for two segmental ganglia. These connexions are repeated throughout the abdominal nerve cord, which consists of serially homologous ganglia. The T-cells (T) in each hemiganglion are connected by doubly rectifying electrical synapses, which allow only depolarizing current to pass (Baylor & Nicholls, 1969*b*). (The T-cells of a hemiganglion make parallel connexions with other cells; for simplicity in the diagram, the T-cells are enclosed in a square and only one connexion is drawn.) The T-cells of each hemiganglion are also connected to their contralateral homologues in the same ganglion and to ipsilateral homologues in adjacent ganglia via doubly rectifying electrical synapses (Baylor & Nicholls, 1969*b*). The presence in each hemiganglion of an inhibitory interneurone, as yet unidentified, that is excited by the T-cells and in turn inhibits them, was deduced by Baylor & Nicholls (1969*b*). The T-cells make subthreshold excitatory electrical synaptic connexions onto the single RF cell in each ganglion and onto the pair of motor neurones which cause longitudinal contraction of the body wall (L) (Gardner-Medwin *et al.* 1973; Nicholls & Purves, 1970). RF has a soma in each segmental ganglion and a functionally continuous axon that runs the length of the nerve cord (Gardner-Medwin *et al.* 1973; Mistick, 1974). It has subthreshold excitatory synapses onto L motor neurones (Gardner-Medwin *et al.* 1973). Triangles represent electrical synapses; circles represent inhibitory chemical synapses.

surface by ceasing undulatory movements or by pressing its body closer to the substrate (Gee, 1912). Predatory leeches, if hungry, swim toward the centre of a disturbance, even if the disturbance is caused by a stick rather than an animal (Mann, 1962).

Röhde's fibre (RF), the largest axon in the ventral nerve cord of the leech, has been shown previously to respond to several modalities of sensory stimulation (Laverack, 1969; Bagnoli, Brunelli & Magni, 1973; Smith & Page, 1974). Primary mechanoreceptors (touch or T-cells) synapse onto RF (Gardner-Medwin *et al.* 1973). Both the mechanoreceptors (Nicholls & Purves, 1970) and RF (Gardner-Medwin, Jansen & Taxt, 1973) have subthreshold excitatory inputs onto motor neurones that cause longitudinal contraction of the body wall, an avoidance response (Fig. 1). This study examines the role of the T-cells and RF in mediating the avoidance response of *Hirudo medicinalis* to a nearfield water disturbance.

MATERIALS AND METHODS

Medicinal leeches, *Hirudo medicinalis*, were maintained in a solution of 95% distilled water and 5% leech saline (Nicholls & Baylor, 1968) at 15 °C and fed regularly with frogs. Prior to dissection, a leech was cooled on ice and during most experiments was maintained at 15 °C by a Peltier cooling apparatus.

In some experiments, the whole animal was pinned ventral side up in a Plexiglas dish lined with Sylgard and filled with saline, and the body wall was slit along the midline to expose the ventral blood sinus. The blood sinus was cut, exposing the ventral nerve cord which lies within it. For intracellular recording, the animal was also slit along the dorsal midline above the ganglion of interest and the gut in that segment was removed. This allowed trans-illumination of the ganglion, providing clear visibility of neuronal somata. In other experiments, the preparation consisted of several ganglia attached to a flap of body wall by the roots on one side. Preparations consisting of one to four segmental ganglia isolated from the periphery were used to investigate the response of RF and the T-cells to electrical stimulation of the peripheral roots. For experiments in which the RF response to travelling surface waves originating at various distances from the leech was examined, a leech was pinned ventral side up parallel to the shorter side of a rectangular dish (12 × 14 cm) and covered with saline to a depth of 1·5 cm. A small slit along the ventral midline allowed *en passant* recording from the connective (Fig. 2).

Glass suction electrodes were used for extracellular recording from and stimulation of the roots and connective. In all experiments, extracellular recording from the connective was used to monitor the response of RF. Identification of the RF action potential was made from its amplitude, the largest recorded, and its duration (see Mistick, 1974). Glass capillary microelectrodes filled with 4 M potassium acetate (resistances of 25–100 MΩ) were used for intracellular recording. Transmembrane potentials were measured using standard electrophysiological apparatus and recorded on an FM tape recorder. The input voltage to the tape recorder was limited to prevent overload of the amplifier, resulting in truncation of intracellularly recorded action potentials.

A nearfield disturbance was created by a travelling surface wave produced by dropping 0·1 ml drops of saline onto the surface of the bathing medium. The amplitude of the surface wave could be varied by changing the height from which the drop was released. Drops were monitored with a photocell.

Rudolph (1967) has shown that most of the energy of a surface wave is concentrated in a frequency band (10 Hz) for which the conduction velocity is almost constant. For latency comparisons, waves reflected from the sides of the dish can be ignored since they will arrive with a greater delay than the wavefront caused directly by the drop. The conduction velocity of the travelling surface wave was measured by placing the tip of a microelectrode at the surface of the saline. As the wave passed the microelectrode, it was recorded as a change in DC potential. The latency between the output of the drop monitor and both the arrival of the drop at the surface of the saline and the arrival of the travelling wave at various distances were then measured. Given these data, the conduction velocity of the travelling wave was calculated to be 0·18 m/s.

Impulse distribution plots were constructed by amplifying the output of the tape recorder so that only the RF action potential (and the stimulus artifact) were of adequate voltage to trigger a Tektronix type 162 waveform generator. The output of the waveform generator triggered a Tektronix type 161 pulse generator, which was used to produce z-axis modulation of a Tektronix 564 storage oscilloscope. Each plot was carefully checked against chart records to ascertain that neither artifacts nor impulses originating in other cells had been recorded.

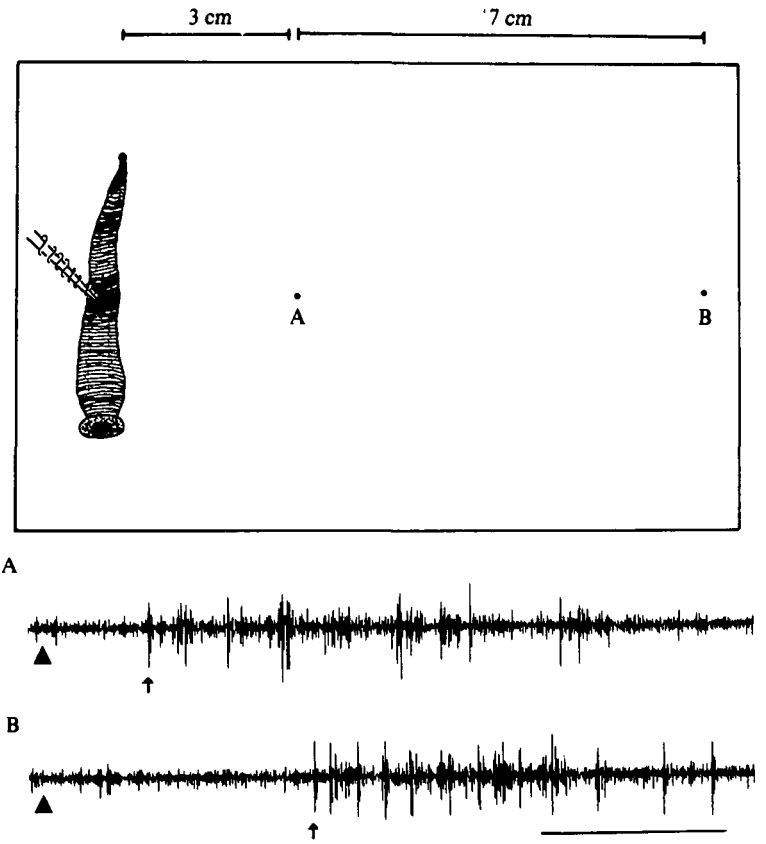


Fig. 2. Response latency of RF to pressure waves initiated at two distances from the leech. Carets indicate the time of initiation of the travelling pressure wave. Arrows indicate the first RF response. The difference in response latency to the two stimuli corresponds to the conduction time of the travelling surface wave (see text). Cal.: 405 ms.

RESULTS

(1) *RF responds to nearfield water disturbances*

(A) *The effective stimulus.* In its natural habitat of ponds and streams, a leech encounters disturbances in the water. These result in two sorts of pressure waves: the first travels at the speed of sound (1437 m/s at 15 °C), while the other is much slower and is caused by a travelling surface wave. The latencies of response of RF to drops at different short distances from the animal were not similar, as would be expected if the response were to the almost instantaneous pressure wave. In the experiment shown in Fig. 2, the response latency of RF to a pressure wave originating 10 cm from the leech was 595 ms, while the response latency to a drop 3 cm from the leech was 235 ms. The latency difference (360 ms) is very close to the conduction time of the travelling wave over the same distance (7 cm in 371 ms) and provides strong evidence that RF responds to the nearfield disturbance created by the travelling surface wave and not to the pressure wave.

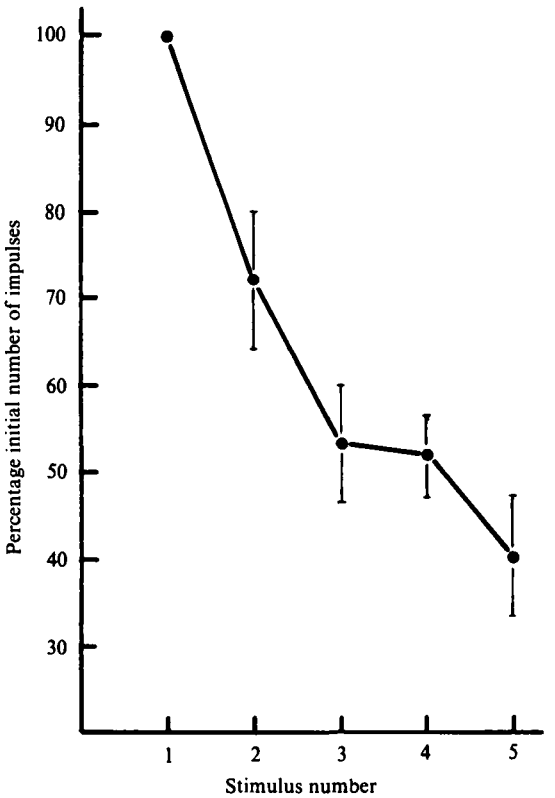


Fig. 3. Decrement in the number of RF impulses to repeated travelling wave stimuli. Experimental conditions were like those shown in Fig. 1 except that the recording was taken from a chain of five segmental ganglia isolated from the rest of the nervous system and attached to the periphery by the roots of the three middle ganglia. The travelling wave stimuli were presented at approximately 2 s intervals; three blocks of five stimuli each were separated by 10 min recovery periods. The means and standard deviations for the combined results of six preparations are shown.

The wave originating 10 cm from the leech was initiated by a drop released 11 cm above the saline surface while the wave originating 3 cm from the leech was initiated by a drop released 6·5 cm above the saline surface. The neural response to the more distant drop is more vigorous than that to the closer drop. It is therefore most unlikely that the longer response latency to the more distant drop stimulus is due to a weaker intensity of stimulation.

(B) *Response latency*. For a drop 3 cm from the leech, the latency between arrival of the wave at the leech and the RF response was calculated to be 235 ms. The latency between the change in pressure at the skin and the RF response was 72 ms. Interestingly, this figure is close to the RF response latency to a punctate mechanostimulus to the skin, about 70 ms, reported by Bagnoli *et al.* (1973).

(C) *Response decrement*. The response of RF to repeated travelling wave stimuli was measured in a chain of five ganglia (the centre three of which were attached to the periphery) by recording from the cut end of the connective. Blocks of five drop stimuli were presented at a rate of one per second and blocks of trials were separated by 10 min recovery periods. The rate of response decrement is shown in Fig. 3,

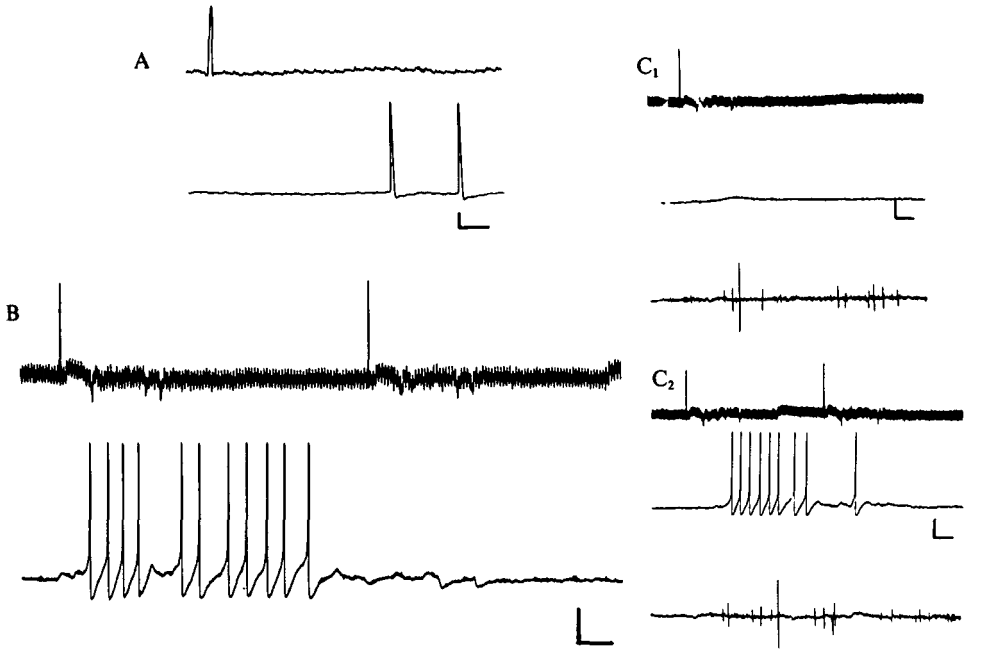


Fig. 4. Intracellular response of touch (T-) cells to travelling surface waves. Upper trace: drop monitor response. Lower trace: intracellular recording from a T-cell. (Impulse heights were attenuated, see *Methods*.) A drop of saline hitting the saline surface several centimetres from the leech produces a travelling surface wave which initiates impulse activity in the T-cells. (A) Record from 'whole animal' preparation with intact nervous system. Impulses rise directly from the baseline, indicating that they are initiated by sensory stimulation at the periphery (Nicholls & Baylor, 1968). Cal.: 10 mV, 50 ms. (B) Record from T-cell not attached directly to the periphery via its main axon. Preparation consists of three segmental ganglia; the middle ganglion is attached to a flap of body wall by the roots on one side, and the response of a T-cell in an adjacent ganglion is recorded. The T-cell is driven by depolarizing synaptic potentials in response to the first travelling wave. Occasionally, inhibitory synaptic potentials are also observed. Cal.: 10 mV, 200 ms. (C) Hyperpolarization of the T-cell blocks the synaptically driven response to travelling pressure waves. Preparation is same as that of B. (1) Injection of hyperpolarizing current blocks synaptically driven spikes and reveals a small excitatory synaptic potential. (2) The first travelling wave after release from hyperpolarization causes multiple spiking. Lower trace: extracellular recording from connective. The largest spike is that of Röhde's fibre. Cal.: 10 mV, 200 ms.

which summarizes results from six preparations, each of which received three blocks of drop stimuli. These results show that the number of RF impulses in response to a nearfield disturbance decrements with repetitive stimulation.

(2) *Evidence that the T-cells mediate the RF response to nearfield water disturbances*

(A) *T-cells respond to nearfield water disturbances.* Intracellular recordings from the central somata of the three classes of body wall mechanoreceptors, T-, P-, and N-cells (Nicholls & Baylor, 1968) were made in semi-intact preparations and preparations consisting of several segmental ganglia, some of which were still attached to a flap of body wall by their roots. Touch cells were excited by a travelling surface wave (Fig. 4A) but P- and N-cells were not. Furthermore, T-cells not connected to the periphery in their segment of origin were driven synaptically (Fig. 4B), possibly by the direct excitatory connexions from T-cells in adjacent ganglia (Baylor & Nicholls, 1969).

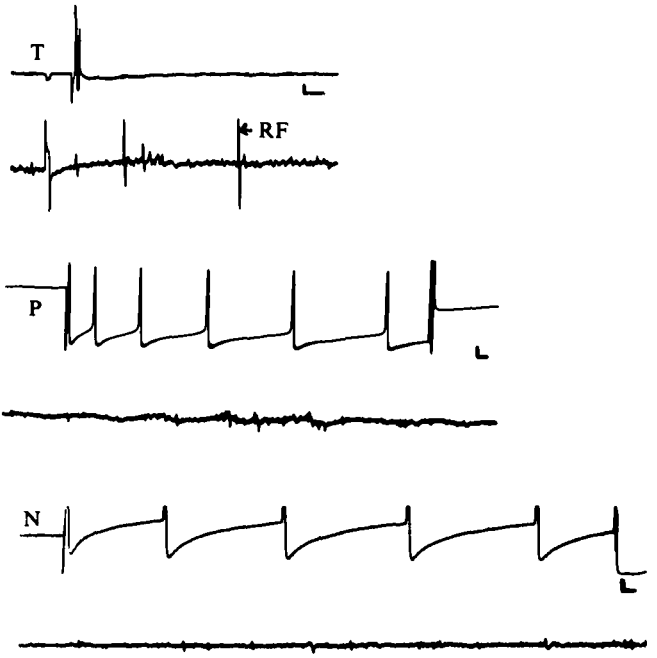


Fig. 5. Intracellular stimulation of mechanoreceptors and the response in RF. Stimulation of P- or N-cells was never observed to cause a response in RF, while stimulation of T-cells often did. (N-cell impulses in this record were clipped (see *Methods*) before reaching their full amplitude of 90 mV.) Cal.: 10 mV, 50 ms.

To examine the source of the T-cells' synaptic drive following the travelling wave stimulus, the level of T-cell membrane polarization was altered by injected current. Hyperpolarization is expected to augment chemically-mediated synaptic excitation and to decrease the effect of the doubly rectifying synapses among T-cells (Baylor & Nicholls, 1969*b*). The greatly *decreased* depolarization recorded in a hyperpolarized cell (Fig. 4C) suggests that much of the excitation is mediated by the electrical synapses from other T-cells.

(B) *T-cells can excite threshold activity in RF.* The response of RF to intracellular stimulation of single mechanoreceptors of each class was examined. Stimulation of a single P- or N-cell did not cause a response in RF (Fig. 5), in agreement with the findings of Smith & Page (1974) and Bagnoli *et al.* (1975). Stimulation of a single T-cell, on some occasions a single T-cell impulse, caused a response in RF. The latency of the RF response was variable and long (more than 30 ms with multiple T-cell impulses and more than 300 ms in the example shown in Fig. 5, where a single T-cell impulse initiates an RF impulse). An RF impulse may be initiated in the same ganglion or in adjacent ganglia (Fig. 6); the response is blocked in 20 mM-Mg^{2+} saline (Fig. 7), suggesting either that there is a necessary chemical component in the circuit or that raising the divalent ion concentration increases the threshold of the cells and blocks the response to depolarization caused by electrical synapses.

(C) *The RF response to nearfield disturbances is not mediated by nerve cord sheath stretch receptors.* The response of RF to nearfield disturbances was blocked when the preparation is bathed in saline containing 20 mM-Mg^{2+} and when all the roots are cut.

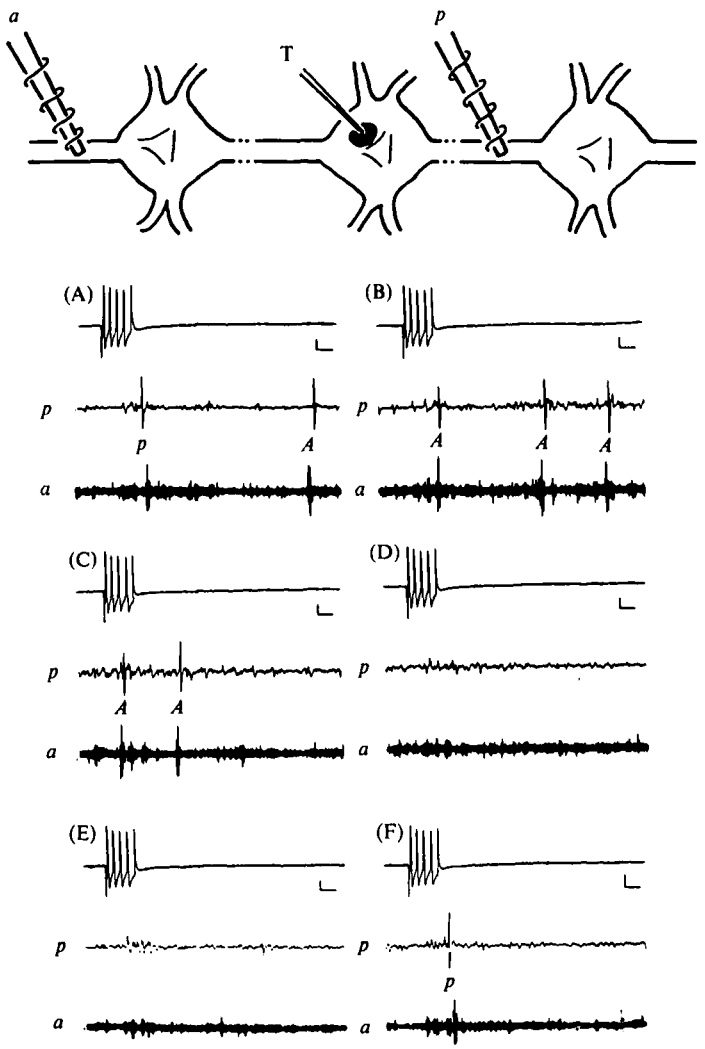


Fig. 6. Response of RF to intracellular T-cell stimulation: ganglion of origin of action potential. A–F are six consecutive stimulations of a T-cell. By comparing the arrival time of the RF impulse at the two recording sites *a* and *p*, the ganglion of origin was determined. *P*, impulse initiated in the posterior ganglion. *A*, impulse initiated in the anterior ganglion. Cal.: 10 mV, 50 ms.

The RF response to deformation of the ganglion sheath persisted under both these conditions. These findings suggest that the cord stretch receptors are not responsible for the RF response to nearfield water disturbances.

(3) *RF response to electrical stimulation of a single segmental root*

The organization of the T-cell and RF network was investigated further in preparations consisting of three segmental ganglia isolated from the periphery. A single 0·2 ms stimulus to either an anterior or posterior root of a segmental ganglion caused a multiple-impulse RF response (Fig. 8A). The repetitive RF response was not caused

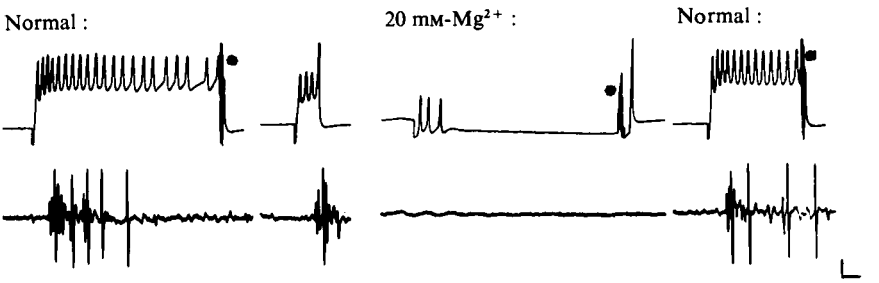
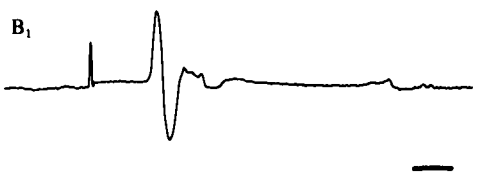


Fig. 7. RF response to intracellular T-cell stimulation is blocked by high Mg^{2+} concentrations. After changing the bathing medium from normal saline to 20 mM-Mg^{2+} saline, the same current which caused multiple T-cell firing in normal saline did not cause threshold T-cell activity. An increase in current produced the three impulses shown in the figure. These impulses did not drive RF, even though three T-cell impulses in normal saline were effective. These results show a significant increase in impulse threshold to electrical stimulation in 20 mM-Mg^{2+} saline. They do not prove that a chemical component is present in the T-cell–RF circuit (see text). Cal.: 20 mV, 50 ms.



B_2

ISI (ms)	Stimulus number 1	2	3	4	5	6	7	8	9	10
50	1	1	1	1	1	1	1	1	1	1
40	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	1
25	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	0	1	1	1	0	1
16	1	0	1	0	1	0	1	0	1	0
13	1	0	1	0	1	0	1	0	1	0
10	1	0	1	0	1	0	1	0	1	0

Fig. 8. (A) Response of RF to a single 0·2 ms suprathreshold stimulus to a segmental root. Preparation consisted of three isolated segmental ganglia. Cal. 100 ms. (B_1) Response of RF to a threshold electrical stimulus to the connective. A suction electrode was placed on the connective four ganglia from the recording site. The first upward deflexion is the stimulus artifact. Cal.: 10 ms. (B_2) Response of RF to stimulation of the connective just suprathreshold to RF at various frequencies. Same preparation as B_1. No more than one impulse per stimulus is observed. At interstimulus intervals of 10–16 ms, RF responds to alternate stimuli. Therefore, for this preparation, the refractory period lasts between 16 and 25 ms, depending upon the number of previous stimuli.

by feedback excitation of the fibre onto itself: stimulation of the connective at RF threshold caused only a single, fixed latency impulse (Fig. $8B_1$), even if RF was driven in high frequency bursts (Fig. $8B_2$).

The RF response to root stimulation can be divided into two components. The first

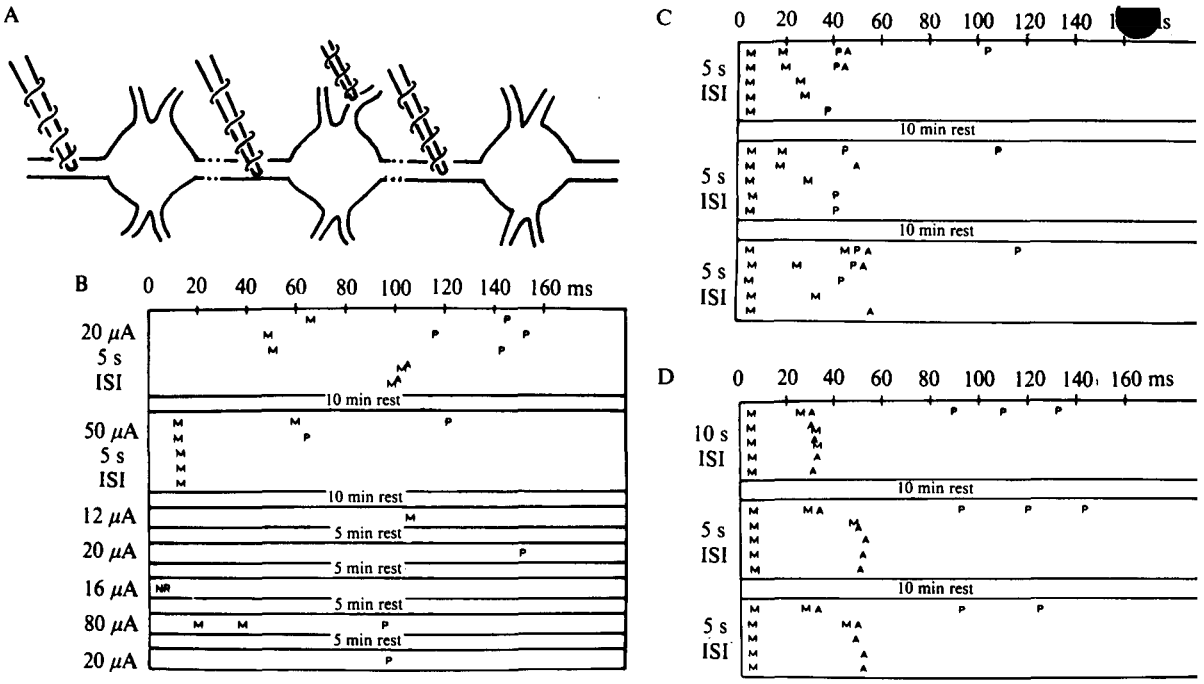


Fig. 9. Ganglion of origin of RF response to root stimulation. (A) By comparing the time of arrival of a RF impulse at three recording sites on the connective, the ganglion of origin was determined. B: Root stimulation at subthreshold, threshold, and suprathreshold current intensities. Note that threshold stimulation evokes a long latency response *only*, while suprathreshold stimulation evokes both short and long latency responses. (C, D) Response to suprathreshold stimulation, data from two different preparations. (B–D) Response latency is plotted on the abscissa. *M*, impulse initiated in middle ganglion; *P*, impulse initiated in posterior ganglion. *A*, impulse initiated in anterior ganglion.

component, which is observed only with stimulus intensities 2–3 × suprathreshold to RF, is of short latency, originates in the ganglion of which the root is stimulated, and is resistant to decrement. The fibres in the root responsible for this component have not been identified. The second component is low threshold, of long and variable latency, may originate in the same or in adjacent ganglia, and decrements rapidly. It will be argued below that this component is caused by stimulation of the large axons of T-cells whose somata are located in the ipsilateral hemiganglion.

(A) *Ganglion of origin of RF impulses.* The long latency RF impulse, along with several unidentified impulses, was the lowest threshold response observed in the connective following root stimulation. Because the T-cell axons are the largest in the segmental roots and the T-cells are known to synapse onto RF, it was hypothesized that the RF response was caused by stimulation of the T-cell axons in the segmental roots. The T-cells have processes that extend to neighbouring ganglia, with the possibility of synapses onto RF in those ganglia. To see whether root stimulation causes initiation of RF impulses in adjacent ganglia, as well as in the ganglion stimulated, the following experimental paradigm was used. In a preparation consisting of three segmental ganglia, recording electrodes were placed on both ends of the con-

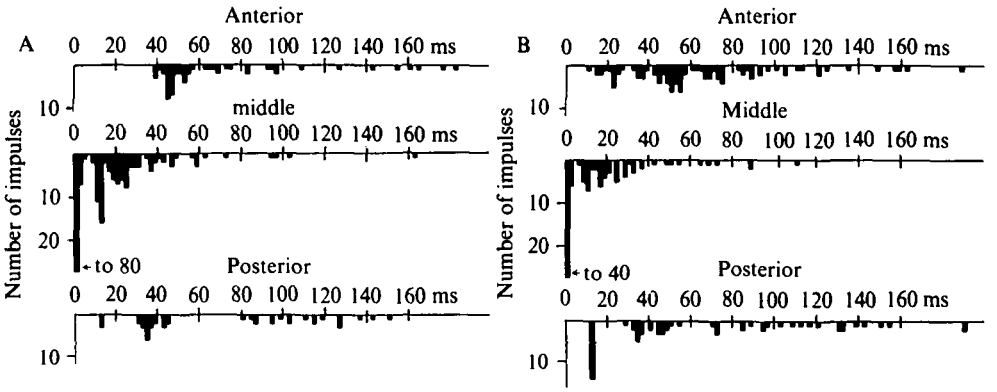


Fig. 10. Site and time of origin of RF impulses in response to suprathreshold stimulation of the posterior (A) or anterior root (B) of a segmental ganglion. A and B each combine data from six preparations like those shown in Fig. 9C, D. Stimulation was given at interstimulus intervals of 5 or 10 s, with 10 min rests between blocks of five stimuli.

nective and between two of the ganglia (Fig. 9). By comparing the time of arrival of RF impulses at the three electrodes, the ganglion of origin of the impulse was determined (cf. Kennedy & Mellon, 1964*a*, *b*). Furthermore, the true latency to impulse initiation following the stimulus was calculated by measuring the conduction time of an impulse from the ganglion of origin to one of the recording electrodes and subtracting that value from the latency between the root stimulus and recorded response.

The results from two such experiments are shown in Fig. 9 C, D. Impulses are initiated in RF in all three ganglia, with the short latency impulses initiated in the stimulated ganglion and longer latency impulses initiated in the adjacent ganglia. The pattern of impulse initiation was rather constant from trial to trial within a single preparation but varied between preparations (compare Fig. 9C and D).

One striking feature of the RF response to root stimulation is the rather regular intervals between impulses. This interval corresponds to the refractory period of RF, as shown in Fig. $8B_2$. The results presented in Fig. 9 suggest that the refractory period caused by impulses initiated in other ganglia can block impulse initiation even though synaptic excitation is present. For example, in the first stimulus series shown in Fig. 9C, impulses are initiated in the same ganglion no more frequently than once every 13 ms. In response to the first two stimuli, impulses are initiated in the posterior ganglion with a latency of 42 ms and in the anterior ganglion with a latency of 46 ms. (These two impulses collide.) In response to the third stimulus, an impulse in the middle ganglion occurs at 26 ms. Conduction time in the connective is approximately 5 ms between ganglia, so the RF cells in the adjacent ganglia are in the refractory state for approximately 18 ms following the stimulus. This may account for the absence of impulses initiated in the anterior and posterior ganglia in response to the third stimulus. Further, if the RF cells in all three ganglia are receiving threshold excitatory inputs, the one that fires first should come out of refractoriness first and fire again. Thus the locus of impulse initiation in RF shifts to a different ganglion only when excitation becomes subthreshold in the cell that has been firing first.

To combine the results from several preparations, the first response of RF initiated in the middle ganglion was taken as time 0. This was considered a better reference point than the stimulus artifact, since the latency to the first impulse varied among

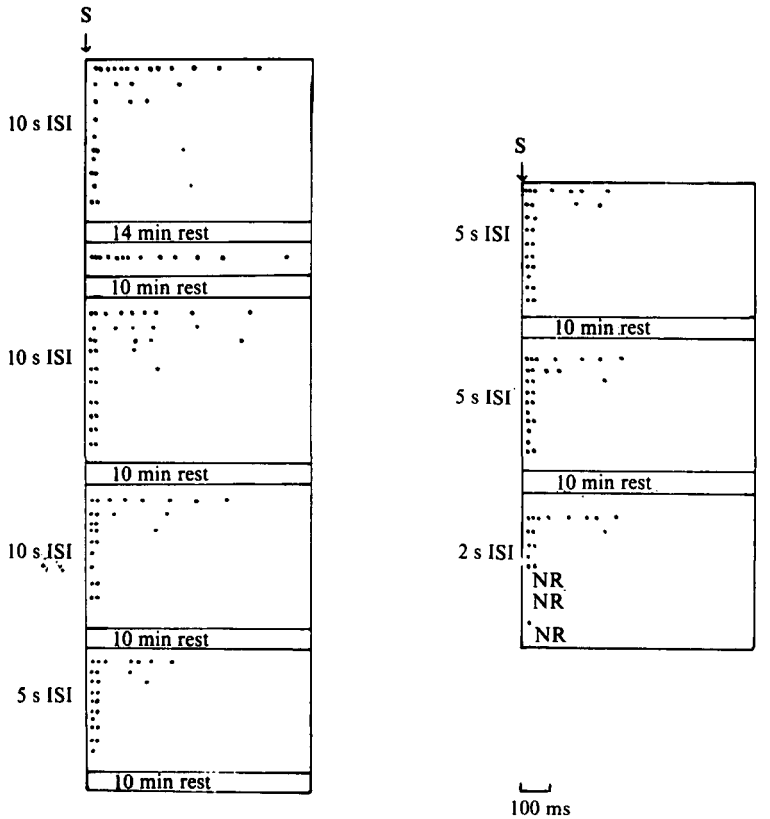


Fig. 11. RF response to repetitive suprathreshold stimulation of a segmental root: decrement and recovery. Note that long latency impulses disappear after several stimuli at interstimulus intervals of 5 and 10 s while short latency impulses persist for at least 10 successive stimuli. Short latency impulses fail at interstimulus intervals of 2 s.

preparations. Response latency histograms of impulses originating in the middle, anterior, and posterior ganglia when a posterior root of the middle ganglion was stimulated are shown in Fig. 10 A (N = 6 preparations; 87 stimuli). Figure 10 B shows the results for stimulation of the anterior root (N = 6 preparations; 90 stimuli).

The probability of impulse initiation in the middle ganglion falls rapidly following the first impulse in this ganglion. The most probable time for impulse initiation in the posterior ganglion is tens of milliseconds later, slightly earlier than for the anterior ganglion. In all six preparations in which the posterior root was stimulated, there were more impulses initiated in the anterior ganglion than in the posterior ganglion. This was also the case in three out of five preparations in which the anterior root was stimulated. (One preparation, in which there was no impulse initiation in the anterior ganglion, was not considered.)

(B) *Threshold to electrical stimulation.* Root, stimuli suprathreshold to RF caused both a short latency impulse originating in the middle ganglion and long latency impulses which could originate in any of the three ganglia (Fig. 9 B, 50 μA stimulus series). Threshold stimulus values (see Fig. 9 B, 20 μA stimulus series) caused only a long latency RF response (49–151 ms in the preparation shown in Fig. 9 B). This com-

ponent most probably results from stimulation of the T-cell axons, which are the largest in the segmental roots: intracellular recording from a T-cell soma during root stimulation just threshold for a RF response showed that the T-cell axon was excited by the root stimulus. (See Fig. 12: the stimuli used to excite the T-cell were just suprathreshold to a RF response recorded in the connective.) The finding that intracellular stimulation of a T-cell also causes a long and variable latency RF response (Fig. 6) adds further support to this notion.

(C) *Response decrement with repetitive stimulation.* Repetitive stimulation of a root results in a decrement in the number of impulses initiated in RF (Fig. 11). Two components of the response can be distinguished on the basis of rates of decrement upon repeated stimulation. The early component fails only after tens of stimuli at an interstimulus interval of 10 s, and it corresponds to the initial impulses(s) initiated in the middle ganglion (see preceding section). The later component, caused by T-cell excitation, fails after only a few stimuli at interstimulus intervals of 10 s.

(4) *T-cell response to electrical stimulation of a single segmental root*

A brief stimulus to the peripheral axons of two (anterior root) or three (posterior root) T-cells was shown to cause long-lasting, repetitive activity in RF. The repetitive firing behaviour of RF did not occur when its axon was stimulated directly. It is therefore most probable that the repetitive activity in RF results from long-lasting synaptic activation. The T-cells are known to synapse onto RF (Gardner-Medwin *et al.* 1973), and there were reasons to suspect that the T-cells themselves might fire repetitively. Their activity is affected not only by sensory stimulation but also by synaptic interactions in the ganglionic neuropil. Known inputs include electrical coupling to other T-cells and polysynaptic inhibitory inputs observed following multiple T-cell impulses (Baylor & Nicholls, 1969*b*). Furthermore, it was observed here that T-cells not connected directly to the periphery were driven synaptically when the receptive fields of T-cells in an adjacent ganglion were excited by a travelling surface wave (Fig. 4B). The synaptic potentials thus observed were abolished by hyperpolarization of the postsynaptic cell, as are the synaptic potentials caused by activity in other T-cells.

The behaviour of T-cells following single, brief shocks to a segmental root was examined in preparations consisting of three segmental ganglia. (Each T-cell sends a large process out of one or both ipsilateral roots of the ganglion that contains its soma (Nicholls & Baylor, 1968) and much smaller processes out of the ipsilateral roots of adjacent ganglia (Yau, 1976). The latter were not excited by the stimuli used in this study; they are fine processes with a high threshold to electrical stimulation (see Yau, 1976).) The posterior root, containing the axons of all three ipsilateral T-cells, of the middle ganglion was stimulated, and the responses of T-cells ipsilateral and contralateral to the stimulated root in both the middle and anterior ganglia were examined. Intracellular recordings from T-cells (Figs. 12–14) showed that these primary afferent neurones are driven synaptically for hundreds of milliseconds following a single 0·2 ms stimulus to the root. T-cells ipsilateral to the stimulated root in both the middle and in the anterior ganglia fired multiple impulses in response to the stimulus; cells contralateral to the stimulated root, however, seldom reached threshold.

The response of a T-cell in the middle ganglion ipsilateral to the root stimulated is

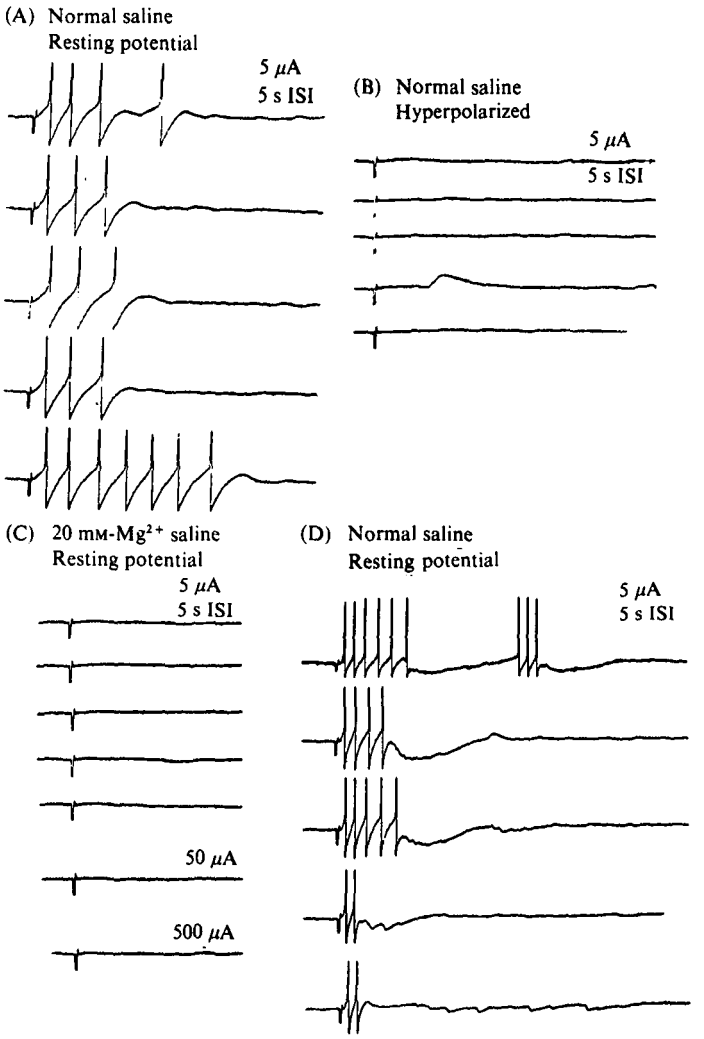


Fig. 13. Response of a T-cell to stimulation of the ipsilateral posterior root of an adjacent ganglion. The preparation consisted of three segmental ganglia. A posterior root of the middle ganglion was stimulated, and the response of an ipsilateral T-cell of the anterior ganglion was recorded. Cal. pulse: 5 mV, 10 ms.

T-cell process (Baylor & Nicholls, 1969*b*). Again, multiple spiking was blocked by 20 mM-Mg^{2+} (Fig. 13C) and by the injection of hyperpolarizing current (Fig. 13B). Inhibitory synaptic potentials were sometimes observed at the end of impulse trains: the repetitive activity shown in Fig. 13A is not terminated by hyperpolarization while the activity shown in Fig. 13D is terminated by a massive hyperpolarization.

Root stimulation rarely caused contralateral T-cells, either in the same or in the anterior ganglion, to reach firing threshold. The response of a contralateral T-cell of the middle ganglion is shown in Fig. 14. A depolarizing potential begins with a latency of about 10 ms. This is followed by a hyperpolarizing potential of variable duration. In 20 mM-Mg^{2+}, the duration of the depolarizing potential is increased and the hyper-

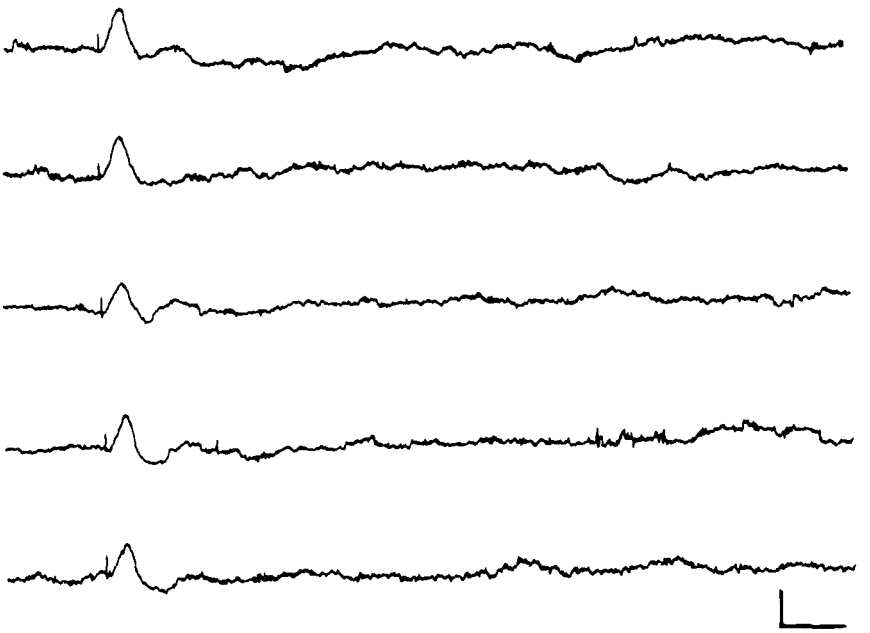


Fig. 14. Response of a T-cell to stimulation of the contralateral root of the same ganglion. Preparation consisted of three segmental ganglia. This T-cell was located in the middle ganglion, and the posterior root on the opposite side of the ganglion was stimulated. Cal.: 5 mV, 100 ms.

polarizing potential is abolished. Root stimulation causes only a very small depolarization of T-cells on the contralateral side of the anterior ganglion. Presumably, this potential is caused by the activity of T-cells located in the anterior ganglion ipsilateral to the root stimulus. (There are no known direct connexions between contralateral T-cells of adjacent ganglia).

(5) *The effect of direction of travelling wave on output frequency of RF*

Kennedy & Mellon (1964*a*, *b*) have suggested that the spike frequency of multisegmental interneurones with spike initiating zones in each ganglion could reflect the direction of movement of a stimulus. A stimulus moving toward a ganglion will result in a shorter interspike interval than will a stimulus moving away from that ganglion. This hypothesis, never examined using natural stimulation, was tested by monitoring the RF response at anterior and posterior recording sites as a surface wave moved along the longitudinal axis of the body.

The preparation consisted of a leech, intact except that the nerve cord was sectioned one ganglion below the anterior brain and one ganglion above the anal brain. RF activity was monitored at each end of the connective (see Fig. 15). Travelling waves were initiated at the posterior and anterior ends of the animal, and the response of RF was monitored. A microelectrode was used to measure latency from the drop monitor response to the arrival of the wave at the anterior and posterior electrodes.

The results illustrated in Fig. 15 show that when the wave is initiated at the posterior recording site, the RF interspike interval is shorter at the anterior one; when the

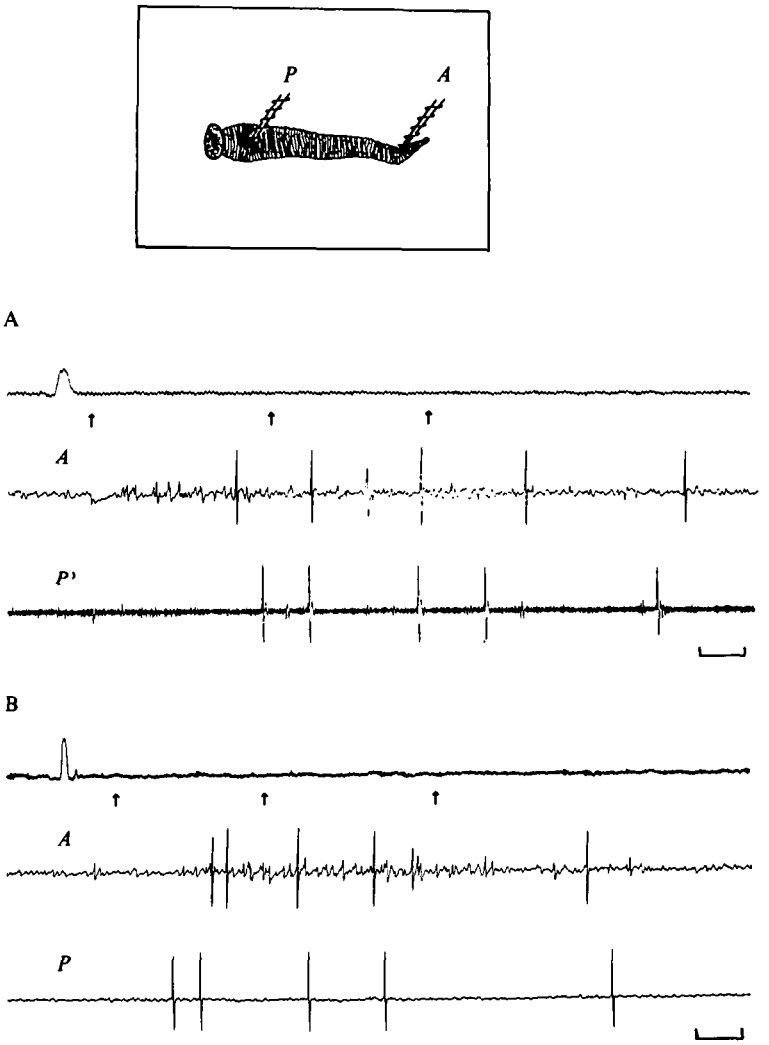


Fig. 15. Response of RF to travelling surface waves initiated at the anterior (A) and posterior (B) of the leech. In this preparation, the ventral nerve cord was cut one ganglion below the anterior brain and one ganglion above the anal brain. Upper trace: drop monitor response; arrows point to the time of arrival of the travelling wave at each electrode and at the midpoint between the two electrodes. Middle trace: response of connective as recorded at the anterior end. In both the middle and lower traces, the largest impulses are the extracellularly recorded RF response. When a travelling surface wave is initiated at the anterior, the interspike interval recorded at the posterior is shorter than that recorded at the anterior. When the travelling surface wave is initiated at the posterior, the converse is true. Cal.: 100 ms.

travelling wave is initiated at the anterior, the RF interspike interval is shorter at the posterior.

The shorter interspike interval should facilitate the temporal summation of the electrical PSPs that RF causes in motor neurones which cause longitudinal contraction of the body wall. This may explain a behavioural observation (Mistick, unpublished observation) that the leech contracts more strongly in segments toward which a mechanostimulus is moving.

DISCUSSION

The results presented in this paper demonstrate that identified primary mechanoreceptors and a first-order interneurone in the leech are responsive to nearfield water disturbances. Both the sensory neurones and the interneurone have subthreshold excitatory inputs to motor neurones which cause a simple avoidance behaviour: longitudinal contraction of the body wall (Nicholls & Purves, 1970; Gardner-Medwin *et al.* 1973).

(1) *Comparison to other detectors of nearfield water disturbances*

Receptors found in decapod crustaceans and certain fish and amphibians are also sensitive to nearfield water disturbances (Wiese, 1976; van Bergeik, 1967). This sense in the leech is more primitive in several respects. In Crustacea and lower vertebrates, the nerve endings of receptors are associated with special transduction elements, usually sensory hairs. The 'touch' cells of the leech are not known to be associated with special transducing elements: although touch to some areas of the skin is a more effective stimulus than at other spots (Nicholls & Baylor, 1968), no associated structures have been observed. However, they might be the spindle-shaped epidermal sensillae described by Autrum (1959) that terminate at the outer end in fine sensory hairs which project 10 μm beyond the cuticle and at the inner end are associated with sensory nerve fibres. They occur singly and in groups over the body surface.

The threshold sensitivity of the leech touch cells has not been determined. A travelling surface wave caused by a single drop of saline hitting the surface 10 cm from a submerged leech can cause activity in the sensory neurones and interneurone lasting for hundreds of milliseconds. This suggests that the T-cells are extremely sensitive.

The nearfield water disturbance receptors in arthropods and lower vertebrates code for directionality, and information from receptors responding to one vector are segregated into separate channels within the CNS (Wiese, Calabrese & Kennedy, 1976). The T-cells are not directionally selective (Nicholls & Baylor, 1968), and RF responds to several stimulus modalities. This does not, however, rule out the possibility that the T-cells mediate the leech's orientation toward water currents and disturbances. The directional coding could take place at an integrative level higher than the receptors.

(2) *Repetitive activity in the T-cells*

The results presented here show that a single impulse in each of the peripheral axons of the three T-cells of a hemiganglion result in synaptically driven activity lasting for hundreds of milliseconds, both in T-cells of the stimulated hemiganglion and in the ipsilateral T-cells of the adjacent ganglion. T-cell excitation was tested only in ganglia directly adjacent to the ganglion stimulated, but the work of other authors can be interpreted to suggest that T-cells are excited as far as six ganglia from the site of stimulation: T-cell stimulation lowers the RF threshold to direct electrical stimulation applied at a distance of six ganglia (Bagnoli *et al.* 1975). The following model of repetitive excitation, which incorporates the known synaptic connexions of the

T-cells, considers the case of three ganglia, where the posterior root of the middle ganglion is excited at T-cell threshold.

(A) *Model of repetitive T-cell activity and its termination.* The three T-cells activated directly by the root stimulus excite one another via their electrical connections. A T-cell impulse plus afterhyperpolarization lasts for approximately 20 ms. (Baylor & Nicholls, 1969*b*), while the coupling potential caused by a single presynaptic impulse lasts for at least 50 ms (Baylor & Nicholls, 1969*b*). It is not known whether a postsynaptic impulse 'resets' the synaptic membrane. If not, intraganglionic connexions could account for brief repetitive firing. An appropriate delay line to secure long lasting T-cell depolarization is provided by the link to the ipsilateral cells in adjacent ganglia. T-cell impulses which travel to the neighbouring ganglia require 10 ms to reach them (Baylor & Nicholls, 1969*b*). Further delays are provided by the time required to initiate impulses in the postsynaptic cells (50–100 ms) and by conduction time back to the middle ganglion.

Excitation reverberates between ganglia until (1) synaptic potentials, because of increasing asynchrony, fail to reach threshold, (2) synaptic inhibition prevents cells from reaching threshold, (3) hyperpolarization caused by Na^+-K^+ pump activity becomes great enough to uncouple the cells (Baylor & Nicholls, 1969*a*), or (4) the Ca^{2+}-dependent increase in K^+-conductance, which increases with repetitive activity, short-circuits the synaptic excitation (Jansen & Nicholls, 1973).

Synaptic inhibition was observed at the termination of some impulse trains and could result from inhibitory interneurones driven by summated T-cell inputs. Inhibition of the T-cells in one hemiganglion would be adequate to terminate the positive feedback. Baylor & Nicholls (1969*b*) have described inhibitory potentials which are mediated by an unidentified polysynaptic pathway and follow two or more T-cell impulses with a long and variable latency. Further, synchronous IPSPs are observed in the T-cells of the same hemiganglion, suggesting they are mediated by the same interneurone. Negative feedback from an inhibitory interneurone may well be the mechanism by which repetitive activity is terminated.

Although groups of electrically coupled cells and their repetitive firing behaviour have been studied in a number of preparations (Getting, 1974; Getting & Willows, 1974; Gardner, 1971; Kater, 1974; Levitan, Tauc & Segundo, 1970), this is the first example where synaptic inhibition has been suggested as the mechanism of burst termination. In electrically coupled interneurones ('trigger cells') in the marine mollusc *Tritonia*, where the electrical synapses are non-rectifying, repetitive firing is terminated when cells fire synchronously, effectively eliminating the loading by the electrical synapses and allowing the full afterhyperpolarization to develop (Getting, 1974). This mechanism cannot account for the termination of repetitive firing in the T-cells since their synapses allow only depolarizing current to pass between cells.

(B) *Functional significance of repetitive T-cell activity.* A classic problem in neurophysiology is to explain how a brief stimulus can cause a prolonged motor output. The time course of synaptic events is too brief to account for motor events which continue for seconds or minutes. Two kinds of mechanisms have been suggested. Lorente de Nó (1938) suggested that recurrent feedback loops could provide sustained facilitation to motor neurones. More recently, it has been suggested that prolonged output may be

explained by the membrane properties and neuronal geometry of single cells (Maynard, 1969; Wine, 1975). Both the T-cells and RF have subthreshold electrical synapses onto motor neurones. The repetitive activity of these cells, by a mechanism not unlike that envisaged by Lorente de Nó, provides facilitating inputs to motor neurones for up to 2 s following a brief mechanostimulus.

(C) *Facilitation of inhibition as a possible mechanism of response decrement with repetitive stimulation.* This scheme does not explain why there should be a decrease in the number of T-cell impulses with *repetitive* stimulation. Possible mechanisms are (1) facilitation of the inhibition which terminates repetitive firing, (2) hyperpolarization caused by the Na^+-K^+ pump, known to follow repetitive activity (Baylor & Nicholls, 1969*a*; Jansen & Nicholls, 1973; van Essen, 1973), and (3) increased refractoriness. It is unlikely that increased refractoriness or hyperpolarization caused by a pump are important at low frequencies of stimulation; T-cells can fire at high frequencies, and many impulses are required before the Na^+-K^+ pump produces significant hyperpolarization (see van Essen, 1973). In many cases, synaptic hyperpolarization that terminated repetitive firing increased in strength and shortened in latency over the first few stimulus trials. In the later stimulus trials, synaptic inhibition was weaker, and individual IPSPs were observed. In Fig. 12(A), where hyperpolarization during the stimulus series blocked impulses, one can separate more clearly synaptic and non-synaptic sources of inhibition. The cell was held hyperpolarized below the reversal potential for the IPSPs, so they are seen as depolarizing potentials. The depolarization increased over the first three stimulus trials. In the last two trials, it is weaker than in the second or third but still greater than in the first. These results are consistent with the notion that the inhibitory feedback facilitates between successive stimuli, at least over the first few trials. This may partially account for the decrease in the number of T-cell impulses in response to repeated low frequency stimuli to the roots. Increase of inhibition (rather than decrease of excitation) has been suggested previously as a mechanism of habituation (Moruzzi, 1959). However, in the few cases where the mechanism of response decrement to repeated stimulation has been investigated directly, homosynaptic depression across excitatory synapses is the underlying mechanism (e.g. Bruner & Tauc, 1966; Zucker, 1972; see Kandel, 1976). Nevertheless, it seems premature to assume that homosynaptic depression is the universal basis of habituation.

(D) *Functional significance of doubly rectifying synapses.* A function of the double rectification can now be suggested. If these cells were connected by non-rectifying electrical synapses, then intense or long-lasting stimulation of the receptive field of a single cell (which causes a massive hyperpolarization due to Na^+-K^+ pump activity and increased gK) could cause the whole network to become hyperpolarized and therefore unresponsive. The doubly rectifying synapses ensure that excitation is spread throughout the network but that cells hyperpolarized by repetitive activity are uncoupled from the network and are unable to lower its threshold.

(E) *Lateralization of repetitive T-cell activity.* It was observed that the root stimulus causes greater excitation to ipsilateral T-cells of adjacent ganglia than to contralateral T-cells of the same ganglion. This has no obvious integrative significance for RF. However, the T-cells have other outputs, e.g. to motor neurones (Nicholls & Purves, 1970), and laterality of some responses can be preserved.

(3) *The mechanoreceptor-interneurone pathway*

The long and variable latency of the RF response to T-cell stimulation (observed with natural stimulation, stimulation via a single T-cell, and stimulation of the peripheral axons of T-cells) may at first seem a curious finding in view of the fact that T-cells synapse directly onto RF (Gardner-Medwin *et al.* 1973). Several results presented in this paper suggest that the long latency response to T-cell stimulation requires both spatial and temporal summation of T-cell inputs. The three simultaneous T-cell impulses driven by a root stimulus provided clearly subthreshold excitation to RF because no short latency response was observed. The long and variable response latency suggests that either (1) interneurones excited by the T-cells must summate with the T-cell inputs or (2) additional T-cells must be activated by the recurrent excitatory pathways described in the preceding section. The former case is analogous to the crayfish lateral giant escape circuit. Primary afferents synapse electrically onto the premotor interneurone (the lateral giant) but are unable to excite it to threshold. The primary sensory neurones also synapse onto sensory interneurones that in turn excite the premotor interneurone. These sensory interneurones can drive the premotor interneurone to threshold (see Zucker, Kennedy & Selverston, 1971).

(A) *The RF response to intracellular T-cell stimulation.* The results presented here confirm those of Bagnoli *et al.* (1975) that intracellular stimulation of a single T-cell can excite RF. However, the results differ in two respects. First, Bagnoli and his co-workers report a latency between T-cell activity and a RF impulse of 60 ms, while it is shown here that the response latency is quite variable, from 30 to 350 ms. Second, in their experiments a burst of T-cell impulses was required, while in this study a single T-cell spike was occasionally adequate to fire RF. If the model of repetitive T-cell activity presented here is correct, the variability in latency can be accounted for by variability in the strength of T-cell stimulation, number of segments intact, and the level of tonic excitation both in RF and in the T-cells in the preparation.

(B) *Receptive field of RF.* The results of electrical stimulation experiments show that RF has a bilaterally symmetrical receptive field within each segment. Like certain crayfish multisegmental interneurones (Kennedy & Mellon, 1964*a*, *b*), it receives excitatory inputs from fibres entering the central nervous system in adjacent ganglia. It is not known whether sensory cells of one segment have direct synaptic contacts with RF in adjacent segments. However, an alternative pathway for this excitation has been demonstrated: sensory cells in one segment excite sensory cells in adjacent segments, which in turn excite the interneurone. As pointed out by Kennedy & Mellon (1964*a*), this organization allows summation of subthreshold stimuli over several segments. Like crayfish interneurones, RF is more strongly excited by inputs to the posterior segments than by inputs to the anterior segments.

(C) *Response decrement to repetitive stimulation.* The number of impulses elicited in RF by a travelling wave stimulus or by a brief electrical shock to a segmental root decreases with repetitive stimulation (Figs. 3, 9, and 11). It has been argued above that the T-cells mediate both these responses and has been shown that the T-cell response decreases with repetitive stimulation. Whether the RF response decrement is due entirely to T-cell response decrement, or whether unidentified interneurones are involved, cannot be determined from the data presented here. The fact that T-cells

over three or more ganglia can be excited by stimulation in a single ganglion suggests that decrement will not be strictly site-specific.

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