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THE CONTRIBUTION OF MEMBRANE HYPERPOLARIZATION TO ADAPTATION AND CONDUCTION BLOCK IN SENSORY NEURONES OF THE LEECH

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SUMMARY

The factors underlying sensory adaptation and conduction block have been studied in cutaneous mechanoreceptor neurones of the leech. A touch-sensitive cell was activated by applying mechanical or electrical stimuli to its receptive field on the skin. Impulses were recorded extracellularly from its axons and intracellularly from its cell body, which is situated within the C.N.S.

1. Activation of the touch cell by mechanical stimuli revealed two distinct types of adaptation with characteristically different time courses. Sustained pressure on the skin caused a brief burst of impulses at the onset of the stimulus. This rapid adaptation to pressure was restricted to the part of the receptive field that had been stimulated mechanically. A second type of adaptation developed more slowly during the course of repetitive mechanical stimulation. It persisted for many seconds after the end of a train of impulses and appeared as an increase in the threshold to mechanical stimuli not only in the region of skin that had been rubbed but throughout the receptive field of the cell.

2. Impulses initiated in the cell body propagated antidromically towards the skin and also raised the threshold to touch, indicating that after-effects of impulse activity were responsible for the long-lasting threshold increase.

3. Repetitive mechanical stimulation could also produce a reversible conduction block in branches of the touch cell. The block occurred in discrete regions of low safety factor such as axonal branch points both within the ganglion and in the periphery. In some experiments impulses intermittently failed to reach one axonal branch yet continued to invade a separate branch of the same cell.

4. Several lines of evidence indicate that both conduction block and the slow component of adaptation are linked to a prolonged hyperpolarization that follows repetitive stimulation of the touch cell. Strophanthidin, which blocks the after-hyperpolarization in touch cells, reduced the adaptation

following trains of impulses and also relieved a conduction block previously established by repetitive stimulation. Furthermore, a comparison of the effects of hyperpolarizations produced by current injection and by repetitive firing showed that most of the threshold increase in the cell body after a train of impulses could be attributed directly to the membrane hyperpolarization.

5. These experiments suggest several ways in which repetitive activity can have pronounced and long-lasting effects on the performance of a highly branched sensory cell. Thus a relatively small number of impulses in a touch cell can markedly decrease its sensitivity to touch. The functional role of the conduction block observed during vigorous stimulation is not as clear because activity for many seconds or minutes is usually needed to establish a block in the larger branches of the cell.

INTRODUCTION

In many vertebrate and invertebrate neurones repetitive firing leads to a prolonged hyperpolarization that can persist for seconds or even minutes (Nakajima & Takahashi, 1966; Rang & Ritchie, 1968; Baylor & Nicholls, 1969*a*; see also Thomas, 1972). It is natural to wonder whether such a hyperpolarization might affect the performance of a cell by, for example, increasing the threshold for the initiation of impulses (Sokolove & Cooke, 1971). With a sufficiently large hyperpolarization there could also be a blockage of impulse conduction, particularly at branch points where the safety factor for propagation is low.

The nervous system of the leech offers several advantages as a preparation for studying the functional consequences of repetitive activity and the mechanisms that underly such effects. Each segment of the animal contains a number of mechanosensory neurones whose cell bodies lie within the segmental ganglion and whose axons innervate the skin in a stereotyped manner. The cell bodies of touch-sensitive cells can be recognized individually and impaled with micro-electrodes. Activity can also be monitored with external electrodes from axonal branches in connectives and nerve roots. Each touch cell can be activated by controlled mechanical stimuli to the skin and by electrical stimuli to the cell body or to various axonal branches. Repetitive firing in these cells is followed by a hyperpolarization of the cell body that can reach 30 mV in amplitude and last for several minutes; in touch cells the hyperpolarization is caused primarily by the activity of an electrogenic pump (Baylor & Nicholls, 1969*a*; Jansen & Nicholls, 1973). In this preparation it is therefore possible to monitor in a single cell the threshold to touch, the conduction of impulses along axons, and the membrane properties of the cell body during the hyperpolarization following trains of impulses.

METHODS

Many of the methods have been described in detail elsewhere (Nicholls & Baylor, 1968; Baylor & Nicholls, 1969*a*, *b*). Experiments were made at room temperature (20–24° C) on medicinal leeches (*Hirudo medicinalis*). An isolated ganglion, or a ganglion attached to a small patch of skin about 3 mm on each side, was mounted in a small chamber and viewed through darkfield illumination. The sensory cells in the ganglion are easily recognized. Pl. 1*A* shows a view of the ventral side of a ganglion in which the three pairs of touch cells are labelled (T).

The morphology of touch cells was determined by electrophoretic injection of the fluorescent dye Procion yellow M4RAN (Stretton & Kravitz, 1968). Dye was injected into the cell body while the ganglion was bathed in Ringer solution containing 10 mM-Ca and 20 mM-K. After the injection the dye diffused for 4 hr with the ganglion in 20 mM-Ca Ringer fluid. These solutions were used because they seemed to stabilize the cell membrane and decrease the leakage of dye following large current injections. In other respects the techniques for dye injection were the same as those used by Nicholls & Purves (1970). An example of a touch cell injected with Procion yellow is shown in Pl. 1*B*. From the monopolar cell body in the ganglion, axons run through the anterior and posterior ipsilateral nerve roots to the skin; other axons in the ipsilateral connectives lead to neighbouring ganglia. Many fine processes can be seen in the neuropile, where there are synaptic connexions with branches from other cells. The axons running in the connectives could be traced into adjacent ganglia, where they branched within the neuropile.

On each side of the ganglion the two touch cells innervating ventral and lateral skin have axons in both the anterior and posterior roots, while the third cell innervates the dorsal skin via a single axon in the dorsal branch of the posterior root. The peripheral axons divide into smaller branches as they approach the skin, so that the over-all receptive field for each cell covers an area of about 6×8 mm.

Stimulation and recording

Individual touch cells in the ganglion were impaled with a micro-electrode whose resistance was 50–120 MΩ when filled with 3 M-KCl or 4 M-K acetate. The output of the preamplifier was fed into an oscilloscope and a pen recorder. The pen recorder was useful for recording slow changes in membrane potential and also the presence or absence of impulses even though the action potentials were greatly attenuated in amplitude. A modified bridge circuit in the preamplifier permitted constant currents to be injected into the cell while simultaneously monitoring the resulting changes in membrane potential. A second intracellular micro-electrode was used for current injection in experiments where it was particularly important to have an accurate measurement of membrane potential while passing current into the cell body. Penetration with two micro-electrodes often injured the touch cell and reduced its input resistance; only cells with a relatively high input resistance (20–60 MΩ) were accepted for analysis.

Mechanical stimuli to the skin were applied with a stylus driven by a piezoelectric transducer. Two types of stylus were available, one of which moved perpendicular to the surface of the skin and was used for measuring the responses to light touch and to maintained pressure. The second type of stylus made larger excursions that were parallel to the skin; it was used to activate the touch cell repetitively by sweeping back and forth across a sensitive area at frequencies of 1–50/sec.

For electrical stimulation and recording from axonal branches a cut nerve or connective was drawn into a suction electrode filled with Ringer solution. The

electrode was made from capillary tubing drawn to a small tip opening (about 100 μm) at one end and connected to a syringe at the other. The internal and external leads could be connected either to an a.c. preamplifier for recording or to a stimulus isolation unit for stimulation. The suction electrode also could be used for electrical stimulation of sensory terminals by placing it over a sensitive spot on the skin. With the electrode positioned in this manner impulses are initiated in or near the sensory terminals and not in more central regions of the axon (Nicholls & Baylor, 1968). In some experiments it was necessary to record along an uncut length of nerve by running it across a partition insulated with silicone grease or by using a hook electrode that pulled a loop of the nerve into a small capillary. For recording the long-lasting hyperpolarization in the touch cell axon the nerve was placed across a narrow trough through which isotonic sucrose solution flowed. The nerve rested in notches in the sides of the trough, and the seal was completed by placing a greased piece of glass on top of the trough. Leads connected to opposite sides of the sucrose gap by KCl-agar bridges were fed into a differential d.c. preamplifier.

Solutions. The normal Ringer fluid contained (mM): NaCl 115, KCl 4, $CaCl_2$ 1·8, Tris-maleate buffered to pH 7·4 with NaOH 10, glucose 9. Isotonic KCl solution contained (mM): KCl 120, $CaCl_2$ 1·8, Tris-maleate buffered to pH 7·4 with NaOH 10, glucose 9. Isotonic sucrose solution contained 230 mM sucrose. High-Ca, high-Mg, and high-K Ringer solutions contained $CaCl_2$, $MgCl_2$, or KCl substituted for an osmotically equivalent amount of NaCl. In some experiments reflex contractions of subcutaneous muscles were prevented by bathing the preparation in Ringer solution containing 20 mM-$MgCl_2$, which blocks chemical synapses in the leech (Baylor & Nicholls, 1969*b*; Nicholls & Purves, 1970; Stuart, 1970). In such cases similar experimental results were obtained with preparations bathed in normal Ringer fluid. Strophanthidin (Sigma Chemical Co.) was initially dissolved in ethanol; the final concentration of strophanthidin was either $1{\cdot}3 \times 10^{-4}$ or 5×10^{-4} M in a Ringer solution containing 1 % ethanol. Control Ringer solution in these experiments also contained 1 % ethanol; the ethanol itself had no obvious effect on the properties of the touch cells. A flow system was used to make rapid changes in the composition of the bathing fluid while recording continuously from the cell.

RESULTS

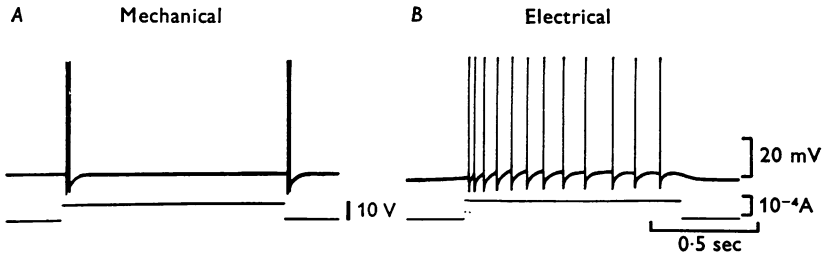
Adaptation

Adaptation to maintained pressure and to repetitive stimulation. The touch cell is sensitive to rather small deformations of the skin (30 μm or less) but adapts rapidly to a steady pressure (Nicholls & Baylor, 1968). In contrast the adaptation to repetitive stimulation develops more slowly, so that when a sensitive area of skin is rubbed the cell responds with a sustained discharge whose frequency declines with a time course of seconds (see, for example, Text-fig. 8). Experiments were made to see whether separate mechanisms underly the adaptation to pressure and to repetitive stimulation and to determine the role played by the hyperpolarization following activity.

Text-fig. 1*A* illustrates the intracellularly recorded responses of a touch cell to cutaneous pressure. Steady deformation of a sensitive spot on the skin (lower trace) caused a brief burst of impulses at the onset of the

stimulus and again upon release of the pressure (upper trace). The adaptation to pressure was usually complete in 20–100 msec, and a maintained discharge to steady pressure was never seen in touch cells. In contrast a prolonged train of impulses was set up by electrical stimulation of the same spot that adapted rapidly to pressure (Text-fig. 1 *B*).

During steady indentation of one sensitive area there was little or no change in the threshold of nearby unstimulated sensory endings of the same touch cell, except during the brief refractory period following the initial burst of impulses. Thus the adaptation to pressure was confined to the receptors directly affected by the mechanical stimulus.



Text-fig. 1. Response of a touch cell to mechanical and electrical stimuli applied to the skin. *A*, pressure was applied to the skin by a stylus driven by a piezoelectric transducer. Lower trace, stimulus to the transducer; upper trace, rapidly adapting response recorded intracellularly from the body cell; note the 'off-response' after release of pressure. *B*, an electrical stimulus was applied by passing current through a suction electrode placed over the same sensitive spot (lower trace, current through electrode). The same touch cell that adapted rapidly to pressure gave a maintained discharge (upper trace) to an electrical stimulus that caused no visible movement of the skin. A prolonged response also occurred when the stimulus polarity was reversed. The touch cell also gave a sustained response during depolarizing currents injected into the cell body through a micro-electrode or applied to its axon through a suction electrode around the root.

A slower form of adaptation developed over a period of seconds with repetitive mechanical stimulation. The possibility that after-effects of impulse activity were responsible for this gradual adaptation was tested by seeing whether impulses set up in one region of the touch cell affected the sensitivity to touch in other distant terminals of the same cell (Text-fig. 2). A brief indentation of the skin was delivered once every 2 sec by a stylus positioned over spot X, which was innervated by a branch of the anterior root (Text-fig. 2 *A*). The stimulus intensity was set just above the resting threshold level, so that each test pulse set up an impulse in the touch cell.

Intracellular response were monitored with a pen recorder (Text-fig. 2 *B*).

Conditioning trains of impulses were produced by a vibrating stylus that rubbed the skin at region Y, which was innervated through the posterior root. The geometry of the touch cell (Pl. 1*B*) is such that impulses initiated at spot Y propagate into the ganglion through the posterior root and back out through the anterior root to invade sensory terminals in the anterior region of the receptive field (Nicholls & Baylor, 1968). After four trains of impulses set up at Y the cell body became hyperpolarized by 10 mV, and the test stimuli at X which previously were above threshold now failed to initiate impulses (Text-fig. 2*B*). Twenty seconds after the end of repetitive stimulation the membrane potential had returned to its resting value and once again the test stimuli consistently elicited a response.

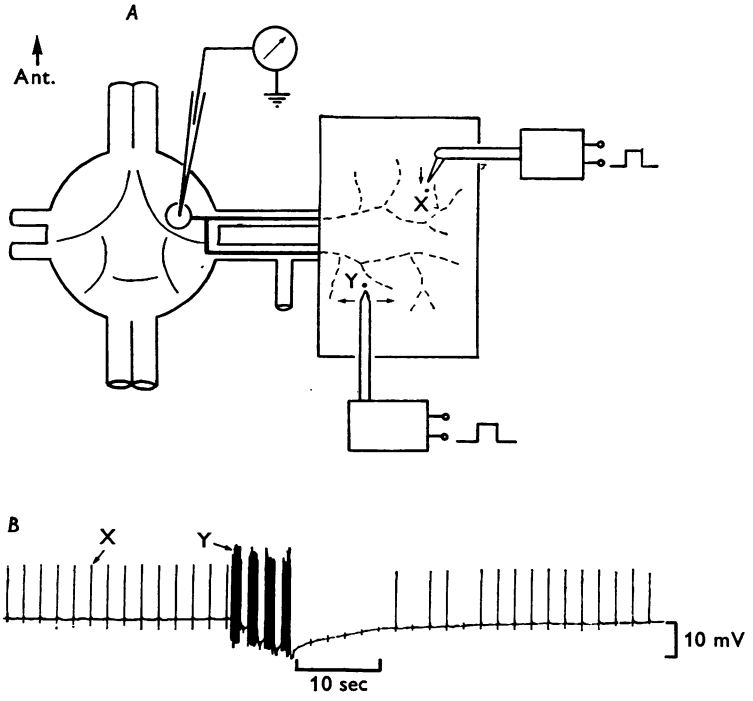
The increase in threshold produced by conditioning trains could be overcome simply by raising the intensity of the test stimuli at spot X. The threshold was highest immediately after the end of repetitive stimulation and declined slowly to its resting level. These observations make it unlikely that the apparent threshold increase was really due to a conduction block in the axon of the touch cell, because such a block presumably would not be affected by the intensity of the test stimuli. Both the threshold change in the periphery and the after-hyperpolarization in the cell body were increased in magnitude and duration when the number of conditioning trains was raised.

A long-lasting increase in the threshold of one spot on the skin could be produced by repetitive stimulation of any other part of the receptive field or by electrical stimulation of the cell body or any axonal branch of the touch cell. Thus trains of impulses decreased the general level of sensitivity of the cell, regardless of where the impulses were initiated.

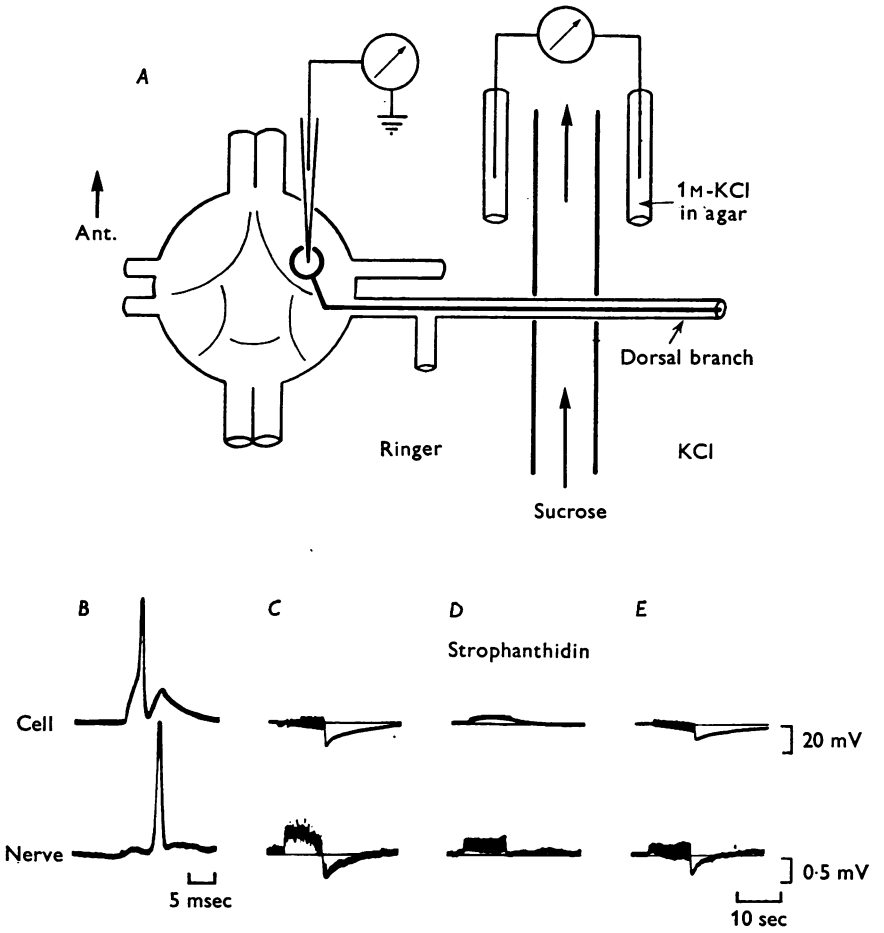
Mechanism of adaptation produced by repetitive stimulation. The idea that the adaptation following repetitive firing is linked to a membrane hyperpolarization would be strengthened if it could be shown that the hyperpolarization occurs not only in the cell body but also in peripheral branches of the touch cell. To test this possibility the dorsal branch of the posterior root, which contains the axon of a single touch cell, was placed across a sucrose gap (see Methods). Impulse conduction across the gap was blocked by bathing the cut distal end in isotonic KCl so that external electrodes placed on opposite sides of the gap recorded a small but constant fraction of the potential changes in the touch cell axon (Text-fig. 3*A*). An action potential initiated in the cell body (Text-fig. 3*B*, upper trace) was followed by a monophasic impulse recorded at the gap (lower trace); the size of the externally recorded impulse was only about 2 mV because of shunting by the rest of the nerve. A train of impulses produced a long-lasting hyperpolarization both in the peripheral axon and in the cell body (Text-fig. 3*C*). Text-fig. 3*D* and *E* show that the hyperpolarization was

reversibly abolished at both recording sites by bathing the preparation in fluid containing $1{\cdot}3 \times 10^{-4}$ M strophanthidin, which blocks the electrogenic pump in touch cells (Baylor & Nicholls, 1969*a*; Jansen & Nicholls, 1973).

One possible explanation of the results in Text-fig. 3 is that a current source within the ganglion produced a hyperpolarization that spread passively into the peripheral axon but failed to reach the receptor terminals. This was excluded by a direct test in which activity was monitored in a



Text-fig. 2. Effects of repetitive firing on the sensitivity to touch. *A*, a small stylus driven by a piezoelectric transducer was positioned over spot X on the skin. Once every 2 sec brief test stimuli were applied that were just above threshold for the touch cell. Conditioning trains of impulses were set up with a second stylus that rubbed spot Y, which was about 1 mm from X. Impulses in the cell body were monitored with a pen recorder that did not record the full size of action potentials. *B*, test stimuli at X that initially were above threshold did not consistently set up impulses for 20 sec following four conditioning trains of impulses initiated at Y (about 30 impulses/train). The preparation was bathed in Ringer solution containing 20 mM-Mg in order to abolish reflex contractions of subcutaneous muscles. Similar results were seen in the presence of normal Ringer fluid and also in experiments where test and conditioning stimuli were applied to an isolated patch of skin while impulses in the touch cell were monitored with a suction electrode around the cut distal end of the root.

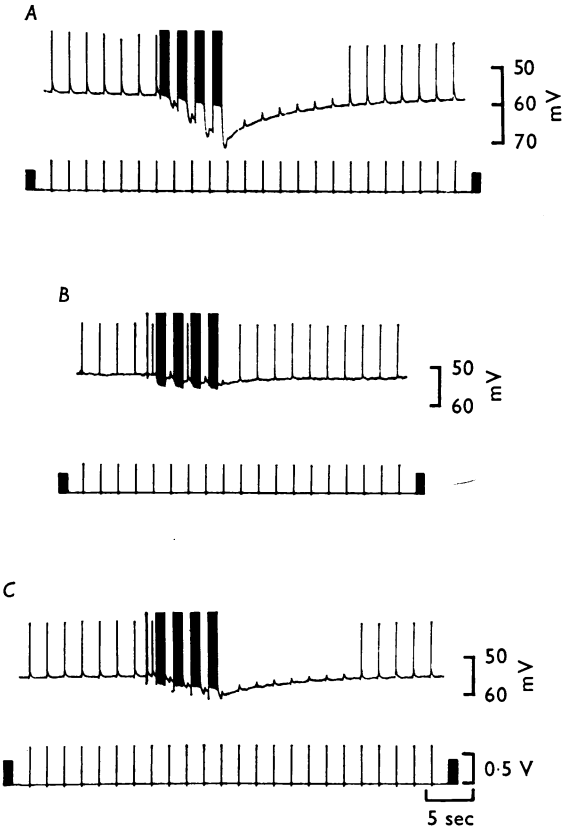


Text-fig. 3. Repetitive firing produces a long-lasting hyperpolarization in the axon of the touch cell. *A*, arrangement of electrodes for stimulation and recording. See Methods for a description of the sucrose gap. *B*, a depolarizing current pulse initiated an impulse in the cell body (upper trace) that propagated up to, but not across the sucrose gap (lower trace). *C*, in control Ringer solution, containing 1 % ethanol, a train of impulses (30/sec for 9 sec) initiated in the cell body caused a hyperpolarization recorded in the nerve and in the soma. Upper parts of action potentials are too faint to be seen. *D*, four minutes after application of $1{\cdot}3 \times 10^{-4}$ M strophanthidin a similar train of impulses failed to produce a hyperpolarization. *E*, the hyperpolarization was restored 16 min after returning to control Ringer solution. Hyperpolarizing current injected into the cell body caused a significant potential change recorded in the axon 2–3 mm from the ganglion. In this experiment the potential appeared to decay to 1/2 in about 2 mm, but an accurate determination of the length constant of the axon was not made.

nerve isolated from the ganglion but still connected to the skin. Rubbing a sensitive area of the skin elicited a train of impulses in the axon of the touch cell followed by an after-hyperpolarization similar in size and duration to that shown in Text-fig. 3. The absolute magnitude of the peripheral hyperpolarization can be estimated from the ratio of externally recorded amplitudes of the action potential and the peak after-hyperpolarization; this ratio was about 6:1 in Text-fig. 3*C* and *E*. If the amplitude of the impulse was between 60 and 90 mV within the axon, as it is in the cell body, then the peripheral hyperpolarization reached 10–15 mV, a value similar to that recorded in the soma.

If adaptation produced by repetitive firing is caused by hyperpolarization of the receptor terminals, then agents that eliminate the hyperpolarization should also abolish the adaptation. The next experiment shows that the long-lasting change in threshold following impulse activity was greatly reduced when the after-hyperpolarization was blocked by application of strophanthidin. Impulses were recorded from the cell body in a ganglion still connected to a patch of skin, and mechanical stimuli were delivered at 2 sec intervals by a stylus positioned over a sensitive spot on the skin. The test stimuli were adjusted so that in the control Ringer solution they were well above the resting threshold (Text-fig. 4*A*, lower trace). Following four conditioning trains of impulses initiated in the cell body, the test stimuli failed to set up impulses for 7 sec; during this time the cell body gradually recovered from a peak hyperpolarization of 14 mV (upper trace). The small deflexions seen when the touch cell failed to fire were synaptic potentials caused by impulses in another touch-sensitive fibre activated by the stimuli. When strophanthidin (5×10^{-4} M) was applied to the preparation, the cell body slowly depolarized by 6 mV. During this slow depolarization, the threshold to mechanical stimuli decreased transiently and then increased to a steady level slightly higher than the resting value in control solution. In the presence of strophanthidin the after-hyperpolarization in the cell body was only 3 mV, and test stimuli were able to initiate impulses almost immediately after the conditioning trains, even though these stimuli were not as far above the resting threshold as they were in control solution (Text-fig. 4*B*). After the strophanthidin was washed out the conditioning trains of impulses once again caused a long-lasting threshold increase (Text-fig. 4*C*). The incomplete return of the after-hyperpolarization (6 mV) may have resulted from injury caused by the micro-electrode or from a partially irreversible effect of prolonged application of strophanthidin (see Jansen & Nicholls, 1973).

A separate set of experiments was made to determine whether threshold changes could be accounted for entirely by membrane hyperpolarization, or whether additional after-effects of repetitive activity were involved.



Text-fig. 4. Strophanthidin blocks the increase in threshold to mechanical stimuli caused by repetitive firing. Brief test stimuli were delivered at 0·5/sec to a stylus placed over the skin. Lower trace, height of the thin lines marks the intensity of stimuli to the piezoelectric transducer that drove the stylus. Height of the dark bars before and after each record shows the threshold stimulus intensity; threshold was measured just before the conditioning trains and again about two minutes later. Upper trace, responses of the touch cell monitored with a pen recorder. Conditioning trains of impulses (thick lines in upper trace) were initiated in the cell body (30 impulses/train). *A*, in control Ringer solution (containing 20 mM-Mg and 1 % ethanol) test stimuli failed to set up impulses for 14 sec after the trains. *B*, after 5 min in 5×10^{-4} M strophanthidin the resting threshold was slightly elevated, but test stimuli were above threshold within 2 sec after the trains and the after-hyperpolarization in the cell body was greatly reduced. *C*, 14 min after returning to control solution, repetitive firing once again caused a long-lasting increase in threshold to mechanical stimuli.

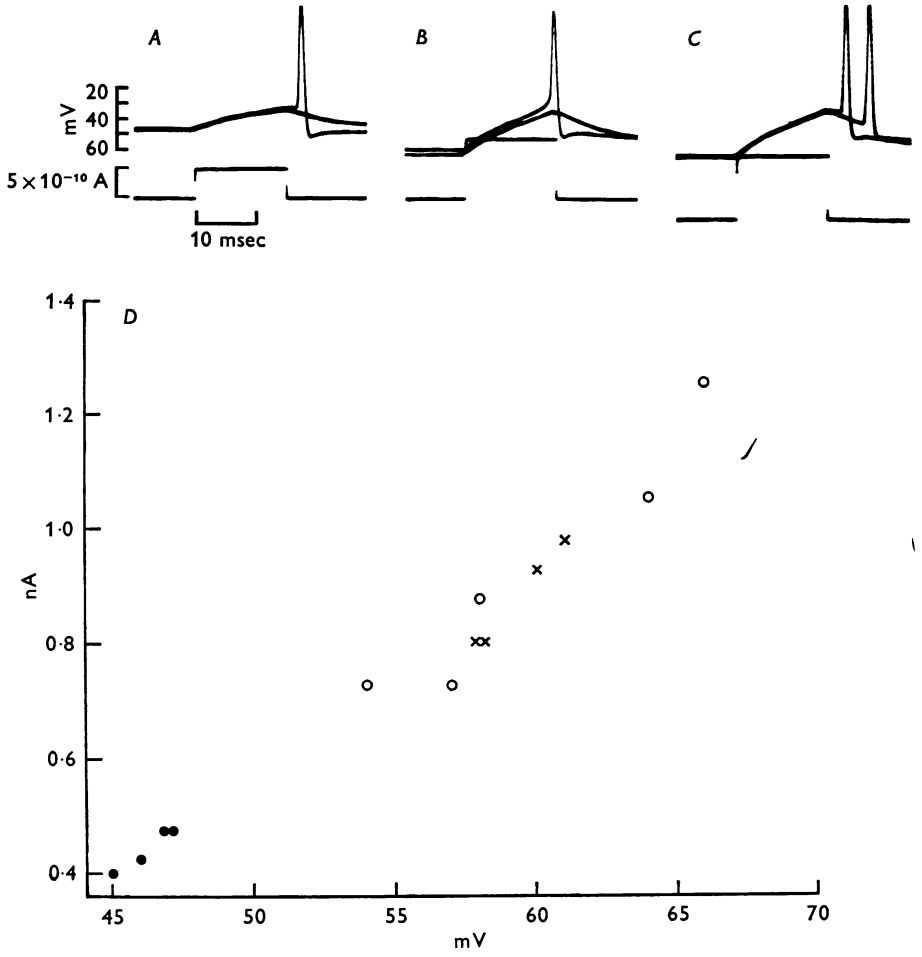
These experiments required accurate determinations of membrane potential at the site of impulse initiation, and for this reason the measurements were made on the threshold of the cell body to current injected through a micro-electrode. The touch cell was impaled with two micro-electrodes; one electrode recorded the membrane potential, while the other injected constant hyperpolarizing currents to change the steady membrane potential and also applied depolarizing test pulses to measure the threshold current. Each record in Text-fig. 5*A–C* shows two superimposed sweeps of the oscilloscope traces and demonstrates that the stimuli (lower traces) were close to threshold. The touch cell was at its normal resting potential in Text-fig. 5*A*; in *B* it was transiently hyperpolarized as a result of repetitive firing and in *C* it was hyperpolarized by steady current injection through the micro-electrode. The current required to set up impulses in *B* and *C* was increased markedly and to about the same extent with either method of hyperpolarizing the cell. Neither method of hyperpolarization was associated with a noticeable change in membrane resistance ($\pm 5\%$), as determined by the relationship between current and membrane potential. In record *C* the impulses arose after the end of the current pulse, suggesting that they were initiated in the axon at a distance from the soma. The critical membrane potential that resulted in impulse initiation was similar in all three records.

The complete results of the experiment are shown in Text-fig. 5*D*, where the threshold current is plotted for different values of the membrane potential. Hyperpolarization produced by current injection (open circles) was just as effective as hyperpolarization produced by repetitive firing (crosses) in raising the threshold current above its control level (filled circles). Five experiments of this type were successfully completed. In three cells all of the threshold increase ($\pm 10\%$) following trains of impulses could be attributed to membrane hyperpolarization. In the other two cells only 50–70 % of the increase was accounted for by the change in membrane potential. This variability may have resulted from varying degrees of injury associated with impalement by two micro-electrodes, or it may reflect normal differences in the properties of individual touch cells (see Discussion).

Conduction block

During repetitive stimulation of leech sensory neurones impulses sometimes fail to invade the cell body (Baylor & Nicholls, 1969*a*). An example of conduction block in a touch cell during mechanical stimulation is shown in Text-fig. 6. A ganglion was connected to a piece of skin by the posterior root while impulses were recorded from the cell body with an intracellular micro-electrode and from the peripheral axon with external electrodes (Text-fig. 6*A*). It was important to identify unequivocally the externally

recorded response associated with an impulse in the axon of the touch cell. This was done by initiating a single antidromic impulse in the cell body (Text-fig. 6*B*, upper trace) with a depolarizing current pulse (dark bar) and recording the propagated diphasic action potential in the root (lower



Text-fig. 5. For legend see facing page.

trace). When the touch cell was activated orthodromically by a vibrating stylus placed over the skin, each sweep of the stylus caused an impulse in the root which propagated into the ganglion and invaded the cell body (Text-fig. 6*C*). The orthodromic and antidromic impulses in the root were of opposite polarities because they were conducted in different directions, but their similarity in size and shape indicate that the orthodromic

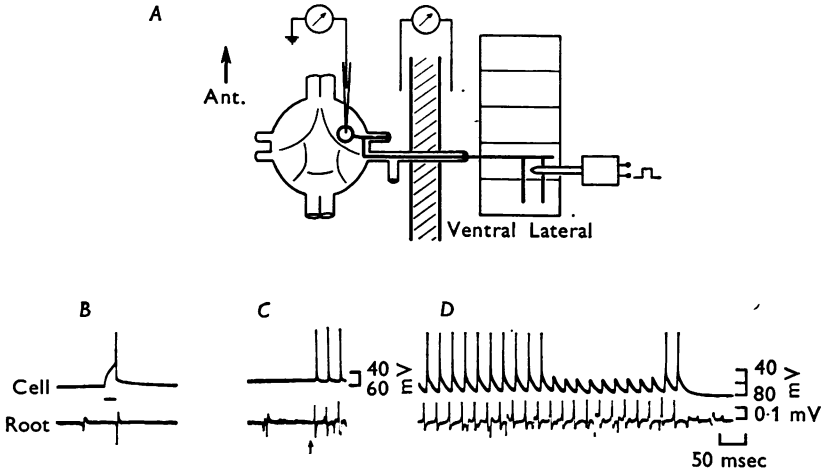
impulses were carried in the touch cell axon and that no other fibres with large externally recorded action potentials were directly activated by the mechanical stimuli.

As mechanical stimulation continued at a frequency of 44/sec, several related events took place. The cell gradually became hyperpolarized until the membrane potential reached 80 mV (an increase of 27 mV), and the action potential in the cell body acquired a prolonged falling phase that is associated with the hyperpolarization (Baylor & Nicholls, 1969*a*). After 10 sec of continuous stimulation some impulses that propagated past the recording site in the nerve root abruptly failed to invade the cell body, and after several minutes this intermittent conduction block was well established (Text-fig. 6*D*). The large humps recorded in the cell body during the block persisted in the presence of 20 mM-Mg, and thus did not appear to be synaptic potentials (see also Text-fig. 7). The results are most readily explained as a conduction block near the cell body that resulted in large electrotonic potentials spreading from the site of the block into the soma.

There was considerable variability in the frequency and duration of repetitive stimulation that was required to establish an intermittent block.

Text-fig. 5. Effects on threshold current of hyperpolarizations produced by repetitive firing and by current injection. One micro-electrode in the touch cell measured the membrane potential (upper traces), while another electrode injected current (lower traces). Each record shows two superimposed sweeps of the oscilloscope traces. *A*, the cell began at its normal resting potential of 47 mV, and one of two depolarizing test pulses ($4{\cdot}8 \times 10^{-10}$A) initiated an impulse at a critical threshold potential of 33 mV. *B*, the cell was hyperpolarized by impulse activity initiated by electrical stimuli to its peripheral axon. In the first of two sweeps the membrane potential began at 62 mV; a test pulse ($9{\cdot}7 \times 10^{-10}$A) depolarized the cell to 35 mV, but failed to set up an impulse. In the subsequent trace the membrane potential had recovered to 60 mV, and the same test stimulus was now above threshold. *C*, the cell was hyperpolarized to 64 mV by a steady current of $3{\cdot}4 \times 10^{-10}$A; the input resistance calculated from this was 50 MΩ. Both test pulses ($10{\cdot}5 \times 10^{-10}$A, measured from the $-3{\cdot}4 \times 10^{-10}$A base line) were just sufficient to fire an impulse, but other identical stimuli failed to set up impulses. The threshold potential in *C* was taken to be the peak depolarization at the end of the stimulus (35 mV), rather than the potential in the soma when the impulse began its regenerative rise. *D*, comparison of the increase in threshold current during hyperpolarization produced by repetitive firing and current injection. Filled circles, threshold current (ordinate) at the normal resting potential (abscissa), which declined by 2 mV during the experiment. Open circles, threshold current for hyperpolarizations produced by current injection. Crosses, threshold current for hyperpolarizations produced by repetitive firing. In this cell the two methods of hyperpolarization were equally effective in increasing the threshold current.

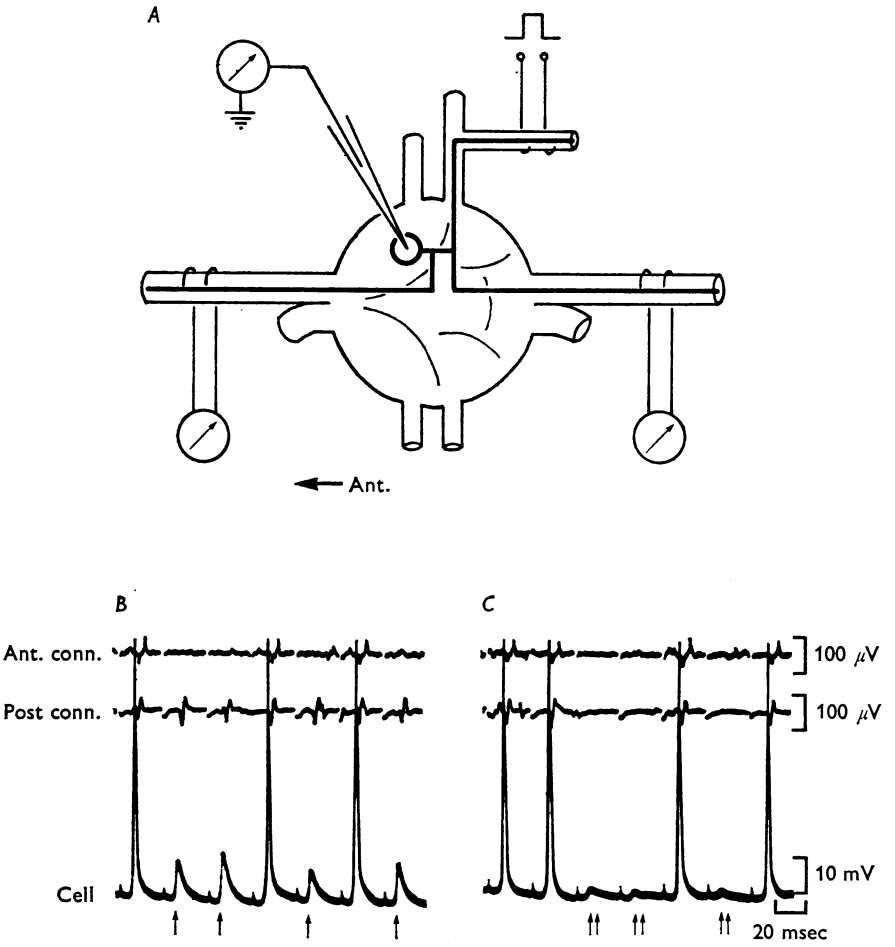
In some preparations the block occurred after only a few seconds of moderate activity (20–40 impulses/sec), while in other experiments the block was either not seen at all or only after many minutes of vigorous mechanical stimulation. Factors which may contribute to this variability are considered in the Discussion.



Text-fig. 6. Conduction block produced by repetitive firing in a touch cell. *A*, the touch cell was activated with a vibrating stylus driven by a piezoelectric transducer. The posterior root was placed across a narrow trough, covered with a glass partition and sealed with silicone grease. Major axonal branches of the touch cell are shown with heavy lines; axons of the touch cell in the connectives have been omitted. *B*, a depolarizing current pulse (dark bar) set up an impulse in the cell body (upper trace) which propagated antidromically past the recording electrode on the root (lower trace), thereby identifying the size and shape of the externally recorded touch cell impulse. Smaller impulses recorded in the root were caused by spontaneous activity in other fibres. *C*, stimulation of the touch cell by stroking the skin at 44/sec following a 3 min rest period. The resting potential was 53 mV. *D*, conduction failure near the cell body, occurring at a membrane potential of about 75 mV several minutes after the beginning of stimulation. The 20 mV humps recorded in the cell body are due to the electrotonic spread of current from the site of conduction block.

Conduction block in central and peripheral axonal branches. The observation that impulse conduction can fail during physiological stimulation of a touch cell brings up the question of how intermittent conduction might affect the transmission of sensory information to other cells in the nervous system. The failure of impulses to invade the soma does not bear directly on this question since the cell bodies of leech neurones do not make synaptic contact with other cells (Coggeshall & Fawcett, 1964). Therefore a test was made to see whether axons of the touch cell that are known to synapse

with other cells are also susceptible to conduction failure. Impulses in the axon of a single touch cell were set up by electrical stimuli to the dorsal branch of the posterior root (Text-fig. 7*A*). Since the touch cell axon has



Text-fig. 7. Conduction block in touch cell branches in the connectives. *A*, low intensity electrical stimuli were applied through a suction electrode to the dorsal branch of the posterior root, which contains the axon of only one touch cell. Impulses were recorded with additional suction electrodes around the ipsilateral connectives and with a micro-electrode in the cell body. The threshold of the externally recorded impulses was the same as that of the impulse in the cell body, indicating that they were carried in branches of the touch cell and not in other fibres. *B*, single arrows, conduction failure in the cell body and in the anterior but not the posterior connective after more than 1 min of stimulation at 40/sec. *C*, double arrows, conduction failure in the soma and in both connectives a few seconds later.

a low electrical threshold, it could be activated by stimuli that excited few, if any, other fibres. Activity was monitored by external electrodes on the anterior and posterior connectives leading to adjacent ganglia and by an intracellular micro-electrode in the cell body. The records of Text-fig. 7*B* and *C* were taken after the touch cell had been stimulated continuously at 40/sec for over 1 min. Many of the stimuli led to action potentials in the cell body (lower trace) that were followed by externally recorded impulses in the touch cell axons in the posterior connective (middle trace) and the anterior connective (upper trace). Other stimuli were followed by impulses which reached the posterior connective, but failed to invade the cell body or the axon in the anterior connective (Text-fig. 7*B*, single arrows). Several seconds later some impulses were blocked before reaching either of the connectives or the cell body (Text-fig. 7*C*, double arrows). The size of the electrotonic potential in the cell body was smaller during failure in both connectives than during failure in a single connective. This decrease in amplitude presumably reflects a shift of the site of conduction block from a region near the cell body, where the branch to the anterior connective takes off, to a place more remote from the soma, at or before the branch leading to the posterior connective; the results thus suggest that the block moved from one branch point to another (see below).

There was some consistency in the pattern of impulse failure in different branches of the touch cell. In four experiments conduction failed in the anterior connective while impulses still reached the posterior connective; no examples were seen of the reverse situation (posterior but not anterior block). Conduction failure occasionally was seen in the cell body but not in either connective, or in a connective but not in the soma.

It was possible to demonstrate conduction block in the touch cell before impaling the cell body by recording simultaneously from a connective and a peripheral nerve while repetitively stimulating the skin. Some impulses in the nerve failed to reach the touch cell axon in the connective, indicating that a block had occurred that was not caused by injury to the soma. Subsequently the cell was impaled, and direct stimulation through the micro-electrode verified that the impulses in the connective and the nerve originated in axons of the touch cell; moreover, conduction failure could still be established with mechanical stimulation while the micro-electrode was in the cell.

Conduction failure might be expected to occur not only within a ganglion, but also in peripheral branches of the touch cell. To test for this a length of peripheral nerve, isolated from the ganglion but still attached to a patch of skin, was placed across a sucrose gap with a branch point of the root close to the edge of the gap. Activity in the root was recorded with two electrodes placed on opposite sides of the gap. When the touch cell

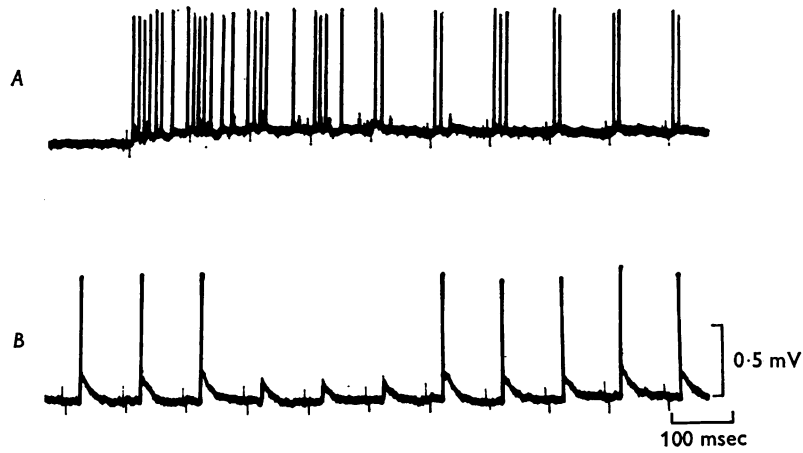
was activated by rubbing the skin with a stylus, monophasic impulses 1·5 mV in amplitude were recorded that gradually decreased in frequency (Text-fig. 8*A*). After several seconds of activity the externally recorded action potential developed a prolonged falling phase similar to that recorded in the cell body during a train of impulses. In addition some stimuli caused only a small hump which had the same latency as the touch cell impulse (Text-fig. 8*B*). The presence of these humps, similar to those seen in Text-fig. 6*D*, suggested that impulses had been blocked somewhere near the recording site.

A definitive test for peripheral conduction block was made in experiments where the root remained connected to both the ganglion and the skin (Text-fig. 9). Antidromic impulses initiated in the cell body were timed to collide with the small humps present during orthodromic stimulation which were suspected to be blocked impulses in the touch cell axon. Activity was monitored in the nerve and in the touch cell soma, and impulses were initiated by rubbing the skin and by injecting depolarizing current into the cell body. The skin was stroked repetitively at 11/sec, and in each record 5–10 consecutive sweeps of the oscilloscope traces are superimposed. In *A* and *C* each stimulus to the skin caused either a diphasic impulse in the nerve (lower traces) that propagated into the cell body (upper traces) or a smaller hump in the nerve that failed to reach the cell body. In *B* impulses set up in the cell body propagated across the sucrose gap and abolished both the diphasic impulses and the humps initiated by orthodromic stimulation; the arrow shows where the responses would have been but for the effect of the antidromic impulses. This experiment showed that the externally recorded humps were carried in the axon of the touch cell and were due to impulses blocked near the recording site.

In several experiments it was possible to look simultaneously for conduction block in both central and peripheral branches of the same touch cell. Sometimes the block was seen only at sites in the periphery (four experiments). In two other cells no peripheral block was seen but conduction failure did occur within the ganglion, and in three cells both central and peripheral block occurred intermittently during the same train of impulses. Thus there was no obvious difference in the susceptibility of central and peripheral branches to conduction failure.

Additional touch-sensitive fibres in the skin. The direct evidence for peripheral conduction block shown in Text-fig. 9 was particularly important because by using the sucrose gap one can record the presence of other fibres responding to touch besides the three pairs of touch cells previously identified in each ganglion (Nicholls & Baylor, 1968). Mechanical stimuli to the skin elicited diphasic impulses in the root that were about a fifth of the size of the externally recorded impulses of the touch cell axon. The

small impulses had nearly the same latency after a stimulus as the touch cell impulse, but they were not conducted in a touch cell axon since antidromic impulses in any of the touch cells did not collide with them. The two classes of touch fibres had different distributions of sensitive spots, although the boundaries of their receptive fields overlapped. The additional touch-sensitive fibres were rapidly adapting and were about as sensitive to mechanical stimuli as touch cells, and thus had properties that



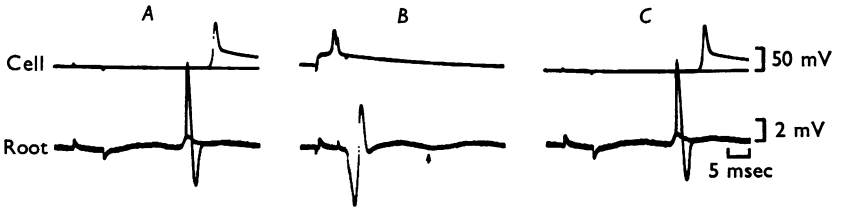
Text-fig. 8. Peripheral conduction block in the touch cell during natural activation of the skin. A sensitive region of ventral skin was rubbed by a stylus vibrating at 10/sec while impulses were monitored in the posterior root by recording across a sucrose gap that was situated close to a branch point of the nerve. Normal Ringer solution was placed on both sides of the gap, but the electrical resistance between the two solutions was high enough to prevent propagation of impulses across the gap. *A*, mechanical activation of the touch cell following a rest period. The response to continued stroking of the skin adapted over a period of seconds. *B*, conduction block occurring 15 sec after the beginning of stimulation. The small humps presumably represent electrotonic potentials spreading from the site of block. The externally recorded action potential acquired a prolonged falling phase similar to that seen in the soma during hyperpolarization. Small artifacts mark the stimuli to the transducer that drive the stylus.

were clearly distinct from those of pressure and noxious cells in the ganglion. The number of these fibres, the location of their cell bodies, and their possible sensitivity to other sensory modalities were not determined.

Mechanisms underlying conduction block. During conduction block the electrotonic potentials recorded in the cell body or from the root were usually constant in size, indicating that the block occurs at specific sites in the cell (see, for example, Text-figs. 8 and 9). Changes in amplitude of the electrotonic potentials usually occurred in discrete steps (Text-fig. 7).

Considering the geometry of touch cells (Pl. 1 *B*), the most likely regions of low safety factor are axonal branch points both in the periphery and within the ganglion, and also the region where the axon joins the monopolar cell body. The importance of geometrical factors is emphasized by the observation that the block consistently occurred more readily when stimulating a small branch that joined a larger axon than when the direction of conduction was reversed by stimulating the larger branch.

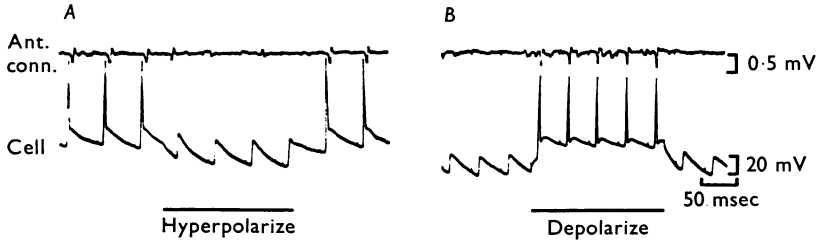
Several experiments support the idea that a hyperpolarization of the membrane is the primary factor underlying conduction block. The main piece of evidence is the observation that most of the increase in threshold



Text-fig. 9. Collision of antidromic and orthodromic responses in the root. Activity in the nerve was monitored across a sucrose gap while the skin was stroked by a stylus vibrating at 11/sec. Each record shows 5–10 superimposed sweeps. Artifacts mark the mechanical stimuli delivered in all three records. *A*, some mechanical stimuli set up impulses which propagated along the root (lower traces) and into the cell body (upper traces). Other stimuli caused monophasic responses in the root that failed to reach the cell body. *B*, shortly after record *A* was taken, appropriately timed antidromic impulses initiated in the cell body abolished both the action potentials and the small humps recorded from the root. *C*, intermittent conduction block was still present during continued mechanical stimulation a few seconds after record *B*. The slow oscillations on the lower traces are artifacts arising from fluid movements during mechanical stimulation of the skin.

following repetitive stimulation can be attributed to the hyperpolarization, at least for moderate levels of hyperpolarization and in the period of several seconds or more after trains of impulses (Text-fig. 5). In addition, hyperpolarizing and depolarizing currents were shown to have direct effects on impulse conduction. In Text-fig. 10 impulses in the touch cell were initiated by electrical stimuli to its peripheral axon and were recorded both in the cell body and in the anterior connective. A current pulse that hyperpolarized the cell body by about 18 mV prevented impulses from invading the soma (Text-fig. 10*A*, lower trace) and from reaching the axon in the connective (upper trace). On the other hand, a depolarization of about 30 mV relieved a conduction block in the cell body and in the connective that had been established by stimulation of the cell at a higher frequency (Text-fig. 10*B*).

In other experiments conduction failure was studied when the long-lasting hyperpolarization was reduced by poisoning the electrogenic pump. The peripheral axon of the touch cell was stimulated electrically until it was hyperpolarized by 22 mV and about one impulse in three failed to invade the soma (Text-fig. 11*A*). While stimulation continued, the external solution was switched from control Ringer to one containing 5×10^{-4} M strophanthidin, which reduces the after-hyperpolarization in touch cells

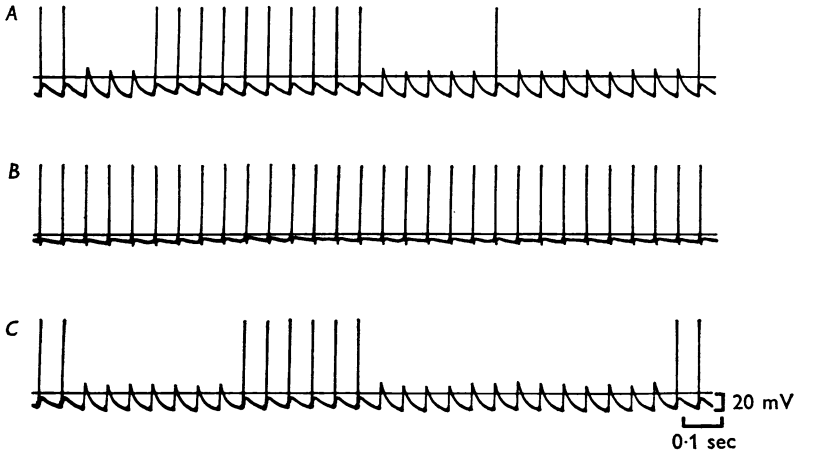


Text-fig. 10. Effects of current injection on impulse conduction. Activity was monitored in the anterior ipsilateral connective with a suction electrode (upper trace) and in the cell body with a micro-electrode (lower trace). *A*, a hyperpolarizing current pulse (bar) blocked conduction into the soma and the anterior connective during stimulation of the peripheral nerve at 20/sec. *B*, a depolarizing current pulse relieved a block established by stimulation at 26/sec. The ganglion was bathed in 10 mM-Ca-Ringer fluid, but similar results were seen with preparations bathed in normal Ringer solution.

(Baylor & Nicholls, 1969*a*). Within 11 sec of the solution change the cell was depolarized by 3 mV and the intermittent block was completely relieved. Over the next 2 min the cell gradually depolarized by 16 mV from its peak membrane potential, while impulses continued to invade the soma without fail (Text-fig. 11*B*). Following a 10 min rest period while the strophanthidin was washed out, conduction block was re-established after less than 1 min of stimulation (Text-fig. 11*C*).

Effect of synaptic potentials on impulse conduction. Touch cells in the leech are known to receive excitatory and inhibitory inputs from other neurones in the same and adjacent ganglia (Baylor & Nicholls, 1969*b*). The function of synapses that are made on central branches of a sensory neurone, far from the site of normal impulse initiation in the skin, is not known. One possibility was that synaptic potentials might influence conduction block. In several experiments the unexpected observation was made that a synaptic pathway whose normal effect is inhibitory can facilitate impulse conduction in a hyperpolarized cell, as illustrated in Text-fig. 12. Low intensity electrical stimuli to the anterior root set up inhibitory synaptic potentials in a touch cell by a pathway that has not been identified (Text-

fig. 12*A*). Impulses in the touch cell were initiated by electrical stimuli to its axon in the dorsal branch of the posterior root. After the cell was hyperpolarized by repetitive stimulation, the synaptic potentials were reversed in sign because the membrane potential was more negative than the reversal potential for the ions that move during the action of the inhibitory



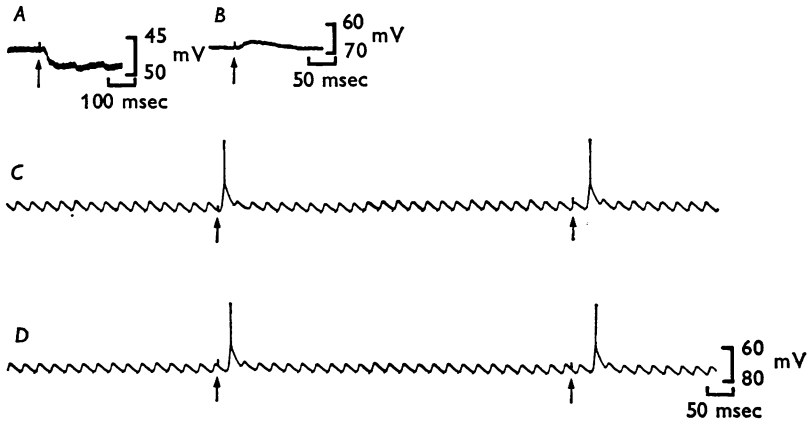
Text-fig. 11. Effect of strophanthidin on intermittent conduction. Electrical stimuli were delivered at 17/sec through an external electrode around the posterior root. The initial resting potential in the cell body was 53 mV (continuous lines in *A–C*). *A*, conduction block occurring in control Ringer fluid after 1 min of stimulation; membrane potential about 75 mV. *B*, absence of conduction failure 1 min after switching to Ringer fluid containing 5×10^{-4} M strophanthidin; the membrane potential at this time was 61 mV, but the block had been relieved since 11 sec after application of strophanthidin, when the potential was still 72 mV. *C*, return of conduction block 13 min after return to control Ringer. A 10 min rest period was interposed between *B* and *C* while the strophanthidin was washed out.

transmitter (Text-fig. 12*B*). The two traces in Text-figs. 12*C* and *D* are a continuous record from the touch cell beginning after a maintained conduction block was established by stimulation at 40/sec. During this period stimuli to the anterior root (arrows) elicited synaptic potentials that transiently relieved the conduction block.

DISCUSSION

Mechanisms of adaptation and conduction block

Two types of adaptation were distinguished in touch cells. Adaptation to pressure was a rapid event restricted to the receptors under the mechanical stimulus. The touch cell fired impulses both at the onset and upon release of pressure, and gave a sustained response to a steady current applied through an external electrode on the skin. These observations are consistent with the idea that a transient generator potential occurs in sensory terminals at the beginning and end of steady pressure and suggest that mechanical factors may contribute to the rapid component of adaptation, as at Pacinian corpuscles (Hubbard, 1958; Loewenstein & Mendelson, 1965; Ozeki & Sato, 1965).



Text-fig. 12. Synaptic potentials in the touch cell relieve a previously established conduction block. *A*, an electrical stimulus to the anterior root (arrow) elicited a series of hyperpolarizing synaptic potentials in the touch cell. *B*, the same stimulus caused a depolarizing synaptic potential after the touch cell was hyperpolarized by repetitive stimulation of its axon in the dorsal branch of the posterior root. *C*, *D*, maintained conduction failure was established by stimulating the touch cell at 40/sec. Each stimulus to the anterior root (arrows) consistently resulted in an impulse invading the cell body. The ganglion was bathed in 12 mM-Ca-Ringer, which made the block easier to establish and may also have increased the size of the synaptic potentials.

The second type of adaptation developed in response to trains of impulses regardless of where or how the impulses were initiated. The threshold increase during and after repetitive firing had a similar time course to the hyperpolarization that was recorded both in the cell body and in the peripheral axon. Both the threshold increase and the hyperpolarization

were greatly reduced in the presence of strophanthidin. Trains of impulses also increased the threshold current needed to initiate impulses in the cell body, and most of this threshold increase could be attributed directly to the membrane hyperpolarization. Together these experiments suggest that hyperpolarization of sensory terminals is the primary factor underlying the adaptation that follows repetitive stimulation. Evidence that an electrogenic pump contributes to sensory adaptation has been presented for the crayfish stretch receptor (Sokolove & Cooke, 1971) and the frog muscle spindle (Landowne, 1970).

A similar series of observations link the hyperpolarization to the conduction block that occurs during high frequency stimulation of the touch cell. Injection of hyperpolarizing currents could prevent impulses from invading the cell body and certain axonal branches. Artificial depolarization, both by current injection and by strophanthidin application could relieve a conduction block previously established by repetitive firing. As mentioned above, most of the threshold increase following moderate levels of impulse activity could be accounted for by hyperpolarization of the cell. Additional mechanisms may also be involved in conduction block, but the hyperpolarization is likely to be the most important factor.

The long-lasting hyperpolarization in leech sensory neurones is caused both by the activity of an electrogenic pump and by a prolonged increase in K conductance (Baylor & Nicholls, 1969*a*; Jansen & Nicholls, 1973). The relative importance of these two mechanisms is not the same in the three modalities of sensory cells. In touch cells the electrogenic pump appears to be most important, and an increase in K conductance plays a minor role. There also appears to be some variability among individual touch cells in the effects of repetitive firing on membrane resistance. In some cells there is no significant change in input resistance following a train of impulses (see, for example, Text-fig. 5); in other cells either an increase or a decrease in input resistance is seen (Baylor & Nicholls, 1969*a*; Jansen & Nicholls, 1973). These individual differences may to some extent reflect variations in the quality of micro-electrode penetrations, and they may account for some of the variability in the degree to which passive hyperpolarization could account for the threshold changes following activity.

Functional consequences of adaptation and conduction block

Conduction block and the two types of adaptation may each have distinctive effects on the way in which sensory information is transmitted to the C.N.S. The rapid component of adaptation insures that the touch cell will respond only transiently to sustained pressure on part of its receptive field without affecting the threshold of nearby receptors. In addition the general level of sensitivity throughout the receptive field will be influenced

by the rate of firing of the cell in the preceding seconds or minutes. Similar observations have been reported for cutaneous mechanoreceptors in vertebrates (Cattell & Hoagland, 1931; Tower, 1940; Lindblom, 1958).

The functional role of conduction block is difficult to assess without knowing the levels of activity in sensory cells produced by stimuli that the leech encounters in its natural environment. Touch cells in an isolated preparation are very sensitive to mechanical stimuli and even respond to movements of fluid over the skin. This suggests that touch cells are activated during normal movements of the animal, such as swimming and crawling, but it is not known just how vigorous these responses are. Several factors may contribute to the variability in the ease with which conduction block could be detected in the present experiments. The block was seen most readily in experiments where the micro-electrode penetration was of high quality, as estimated by a high input resistance and a large action potential; it occurred less often in cells that had been slightly damaged by the micro-electrode. In some preparations a block at peripheral branch points may have limited the impulse traffic reaching more proximal recording sites where a block could have been recorded. In addition there may be variations in the properties of individual cells that affect the susceptibility of branch points to conduction failure.

Conduction block at peripheral branch points of the touch cell would act as a filter that limits the impulse traffic reaching the ganglion. Within the C.N.S. a block might have a more selective effect on the distribution of impulses to specific axonal branches of the cell. Synaptic potentials in the touch cell can influence the conduction of impulses into the cell body; such effects might be more important in the fine branches within the neuropile, where both the potential changes and conductance changes associated with synaptic activity are larger than in the soma.

Intermittent conduction has been described for neurones in several vertebrate species (Barron & Matthews, 1935; Barron, 1940; Chung, Raymond & Lettvin, 1970; Katz, 1950; Krnjević & Miledi, 1959; Parnas, 1970). It has not been possible to establish the specific mechanisms underlying conduction block in these other preparations because extracellular recordings or other indirect methods were used to demonstrate the block. While the mechanism of conduction failure may not be the same in all cells it is nevertheless interesting to speculate on the ways that intermittent conduction might influence integrative processes in the nervous system.

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REFERENCES

BARRON, D. H. (1940). Central course of 'recurrent' sensory discharges. *J. Neurophysiol.* **3**, 403–406.

BARRON, D. H. & MATTHEWS, B. H. C. (1935). Intermittent conduction in the spinal cord. *J. Physiol.* **85**, 73–103.

BAYLOR, D. A. & NICHOLLS, J. G. (1969*a*). After-effects of nerve impulses on signalling in the central nervous system of the leech. *J. Physiol.* **203**, 571–589.

BAYLOR, D. A. & NICHOLLS, J. G. (1969*b*). Chemical and electrical synaptic connexions between cutaneous mechanoreceptor neurones in the central nervous system of the leech. *J. Physiol.* **203**, 591–609.

CATTELL, M. & HOAGLAND, H. (1931). Response of tactile receptors to intermittent stimulation. *J. Physiol.* **72**, 392–404.

CHUNG, S., RAYMOND, S. A. & LETTVIN, J. Y. (1970). Multiple meaning in single visual units. *Brain Behav. Evol.* **3**, 72–101.

COGGESHALL, R. E. & FAWCETT, D. W. (1964). The fine structure of the central nervous system of the leech, *Hirudo medicinalis*. *J. Neurophysiol.* **27**, 229–289.

HUBBARD, S. J. (1958). A study of rapid mechanical events in a mechanoreceptor. *J. Physiol.* **141**, 198–218.

JANSEN, J. K. S. & NICHOLLS, J. G. (1973). Conductance changes, an electrogenic pump and the hyperpolarization of leech neurones following impulses. *J. Physiol.* **229**, 635–655.

KRNJEVIĆ, K. & MILEDI, R. (1959). Presynaptic failure of neuromuscular propagation in rats. *J. Physiol.* **149**, 1–22.

KATZ, B. (1950). Depolarization of sensory terminals and the initiation of impulses in the muscle spindle. *J. Physiol.* **111**, 261–282.

LANDOWNE, D. (1970). The role of the sodium pump in adaptation in the frog muscle spindle. *Biol. Bull. mar. biol. Lab. Woods Hole* **139**, 406.

LINDBLOM, U. (1958). Excitability and functional organization within a peripheral tactile unit. *Acta physiol. scand.* **44**, suppl. 153, 1–84.

LOEWENSTEIN, W. R. & MENDELSON, M. (1965). Components of receptor adaptation in a Pacinian corpuscle. *J. Physiol.* **177**, 377–397.

NAKAJIMA, S. & TAKAHASHI, K. (1966). Post-tetanic hyperpolarization and electrogenic sodium pump in stretch receptor neurones of crayfish. *J. Physiol.* **187**, 105–127.

NICHOLLS, J. G. & BAYLOR, D. A. (1968). Specific modalities and receptive fields of sensory neurons in the C.N.S. of the leech. *J. Neurophysiol.* **31**, 740–756.

NICHOLLS, J. G. & PURVES, D. (1970). Monosynaptic chemical and electrical connexions between sensory and motor cells in the central nervous system of the leech. *J. Physiol.* **209**, 647–667.

OZEKI, M. & SATO, M. (1965). Changes in the membrane potential and the membrane conductance associated with a sustained compression of the non-myelinated nerve terminal in Pacinian corpuscles. *J. Physiol.* **180**, 186–208.

PARNAS, I. (1970). Differences in blocking frequency of presynaptic terminals of an axon innervating different crayfish muscles. *Biol. Bull. mar. biol. Lab. Woods Hole* **139**, 432.

RANG, H. D. & RITCHIE, J. M. (1968). On the electrogenic sodium pump in mammalian non-myelinated nerve fibers and its activation by various external cations. *J. Physiol.* **196**, 183–221.

SOKOLOVE, P. G. & COOKE, I. M. (1971). Inhibition of impulse activity in a sensory neuron by an electrogenic pump. *J. gen. Physiol.* **57**, 125–163.

STRETTON, A. O. W. & KRAVITZ, E. A. (1968). Neuronal geometry: determination with a technique of intracellular dye injection. *Science, N.Y.* **162**, 132–134.

STUART, A. E. (1970). Physiological and morphological properties of motoneurones in the central nervous system of the leech. *J. Physiol.* **209**, 627–646.

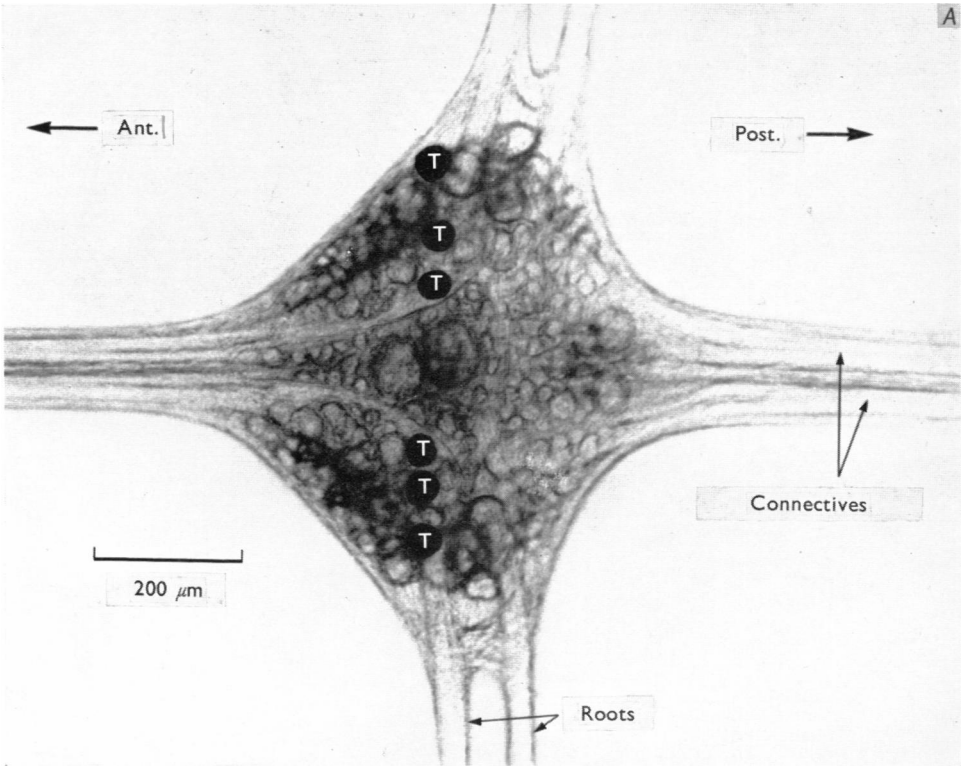
THOMAS, R. C. (1972). Electrogenic sodium pump in nerve and muscle cells. *Physiol. Rev.* **52**, 563–594.

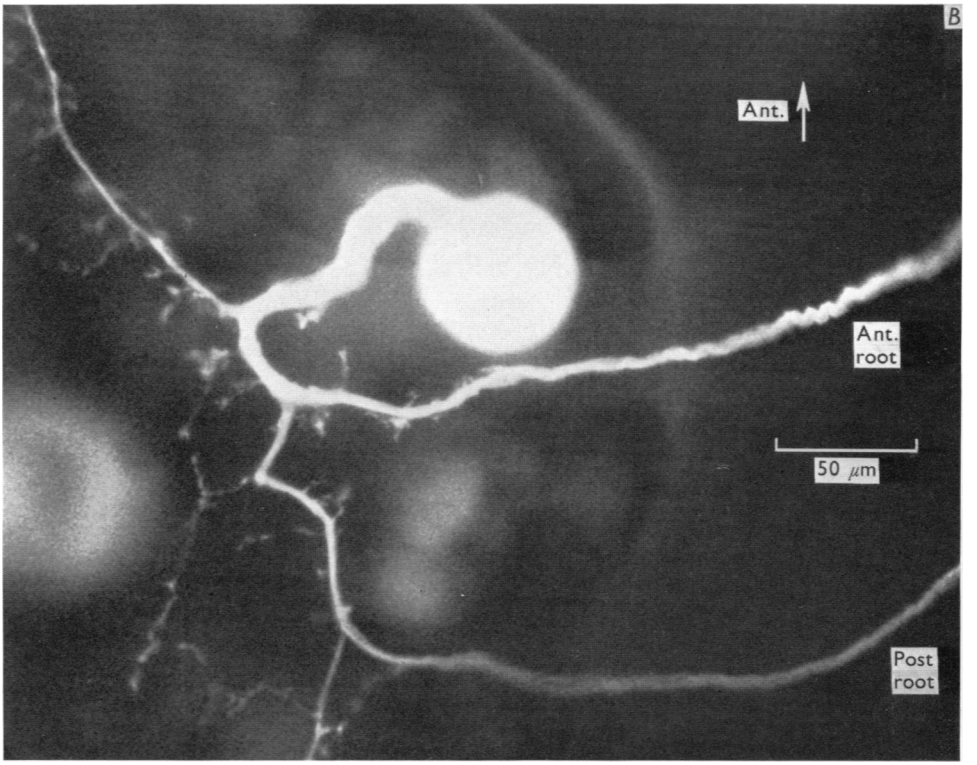
TOWER, S. S. (1940). Unit for sensory reception in cornea. *J. Neurophysiol.* **3**, 486–500.

EXPLANATION OF PLATE

A, photomicrograph of the ventral side of a segmental ganglion viewed with transmitted light. The roots innervate the body wall, and the paired connectives run to adjacent ganglia. The three pairs of touch cells have cell bodies (T) situated in the anterior half of the ganglion.

B, photomicrograph of a touch cell injected with the fluorescent dye Procion yellow. The preparation is a whole mount, and not all parts of the cell are in focus. Axons run out of the anterior and posterior roots towards the skin, and other branches lead to anterior and posterior ipsilateral connectives. Fine branches of the touch cell are present in the neuropile. Several autofluorescent cells also can be seen in the ganglion.





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