

RECEPTIVE FIELDS, GEOMETRY AND
CONDUCTION BLOCK OF SENSORY NEURONES IN THE
CENTRAL NERVOUS SYSTEM OF THE LEECH

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SUMMARY

1. In segmental ganglia of the leech, the cutaneous mechanosensory neurones responding to touch innervated the skin of their own segment and of part of the anterior and posterior adjacent segments. Each touch receptive field could be divided into three non-overlapping areas: a central part innervated by the branches of the cell which ran in the nerve roots of the ganglion containing the cell body, and anterior and posterior parts innervated by its branches which ran in the nerve roots of the anterior and posterior adjacent ganglia.

2. Impulses originating from the anterior and posterior parts of the receptive fields were susceptible to conduction block within the central nervous system when the touch cells fired repetitively at frequencies that could readily be elicited with weak mechanical stimulation. In contrast, impulses originating from the central part of the receptive fields were less susceptible to block.

3. The morphology of touch cells revealed by intracellular injection of horseradish peroxidase suggested that conduction block occurred at specific bifurcation points where small cell processes joined the main process. Different physiological experiments supported this conclusion.

4. In some touch cells, bifurcation points with particularly low safety margins of conduction operated as low-pass filters, limiting the frequency of impulses capable of invading certain branches.

5. The results suggest that mechanical stimuli which would likely be encountered by the animal can lead to conduction block within its central nervous system and as a result modify its integrative activities.

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INTRODUCTION

Although there are numerous descriptions of conduction block in neurones of both vertebrates and invertebrates (e.g. Barron & Matthews, 1935; Krnjević & Miledi, 1959; Tauc & Hughes, 1963; Bittner, 1968; Raymond & Lettvin, 1969; Parnas, 1972; Zucker, 1972; Grossman, Spira & Parnas, 1973), its general significance in the normal functioning of the nervous system is still obscure. One difficulty resides in determining the natural conditions in which conduction block occurs and also the exact blocking sites in a neurone. In leech mechanosensory cells it has been shown that conduction block can take place as the result of a prolonged hyperpolarization developed after repetitive firing (Van Essen, 1973). This hyperpolarization is caused partly by the activation of a Na pump and partly by an increase in K conductance (Baylor & Nicholls, 1969; Jansen & Nicholls, 1973). The advantages of studying conduction block in these sensory neurones are that their detailed branching geometry is known and that they can be activated by natural stimuli applied to the skin of the animal.

The present study has been undertaken to determine whether conduction block plays a role in signalling by the leech sensory cells in normal physiological conditions. The investigation was prompted by two new findings. The first is that the receptive fields of the sensory cells are more extensive than previously described (Nicholls & Baylor, 1968); the second is that the impulses originating from the more remote parts of receptive fields are very susceptible to conduction block. Moderate firing from mild stimulation of the skin would thus be expected to modify the extent of the receptive field of a sensory neurone.

METHODS

All experiments were done on the medicinal leech, *Hirudo medicinalis*, at room temperature (20–25° C). The experimental arrangement and recording techniques have been described elsewhere (Nicholls & Baylor, 1968; Van Essen, 1973).

A chain of two or three segmental ganglia, each still innervating the corresponding body segment, was dissected from the animal. The mechanosensory cells studied were those that responded to touch on the skin (Nicholls & Baylor, 1968). There are three such neurones on each side of a segmental ganglion, with characteristic positions as shown in Fig. 1. They innervate the ventral part, the lateral part and the dorsal part of the skin respectively. The receptive fields of these cells were mapped by stimulating the skin mechanically with a stylus moved either manually or by a piezoelectric crystal (Nicholls & Baylor, 1968). In experiments where a touch cell had to be stimulated vigorously, a small vibrating brush (2 mm × 3 mm) driven piezoelectrically was brought against its receptive field. Extracellular recording was made from a nerve root by lifting the nerve into mineral oil with single or paired platinum hooks, or by leading it through a narrow partition filled with silicone grease and recording across the partition.

Unless otherwise stated, all experiments were conducted in normal leech Ringer fluid, containing (mM): NaCl, 115; KCl, 4; CaCl₂, 1.8; Tris maleate buffered to pH 7.4 with NaOH, 10; glucose, 9. In Ringer fluid containing Mg²⁺, MgCl₂ was substituted for an equivalent concentration of NaCl.

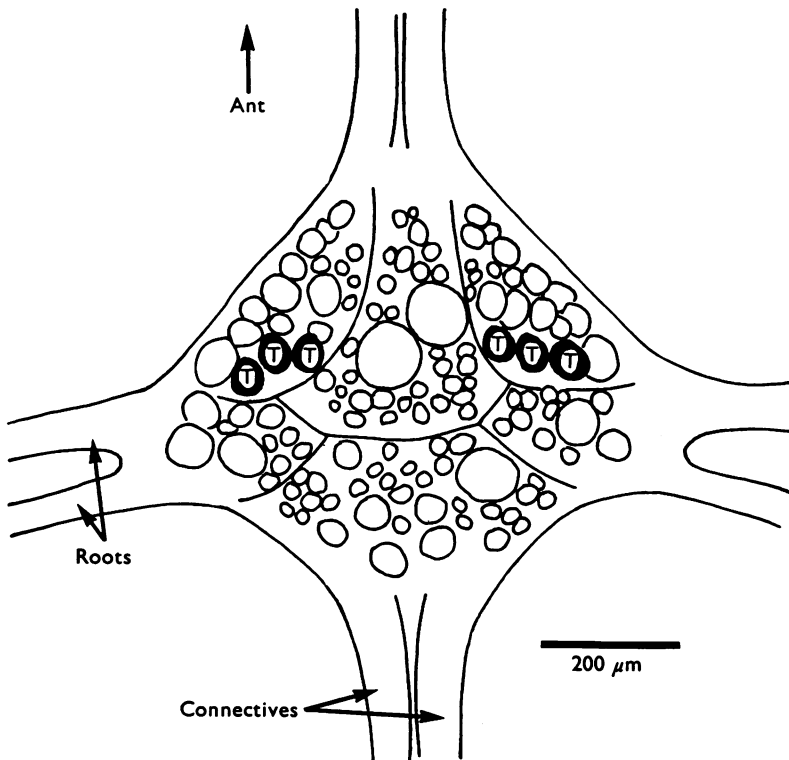


Fig. 1. Drawing of a leech segmental ganglion seen from its ventral side. The cutaneous mechanosensory neurones that respond to touch (T) are shown in their characteristic positions in the anterolateral packets of the ganglion. They innervate ventral skin, lateral skin and dorsal skin respectively. The large neurones at the centre of the ganglion are the Retzius cells. Not all cell bodies on the ventral side of the ganglion are shown.

The technique of intracellular horseradish peroxidase injection and the histological procedures were similar to those described by Muller & McMahan (1976) and Yau (1976). In order to trace cell processes from one ganglion to another the injected preparations were left in 50–75% hypotonic Ringer fluid at 4° C for periods up to 48 hr before fixing.

Cell geometry

RESULTS

Nicholls & Baylor (1968) reported that each touch cell innervated an area of skin which covered its own segment and also small parts of adjacent segments (each segment comprises five rings or annuli along the

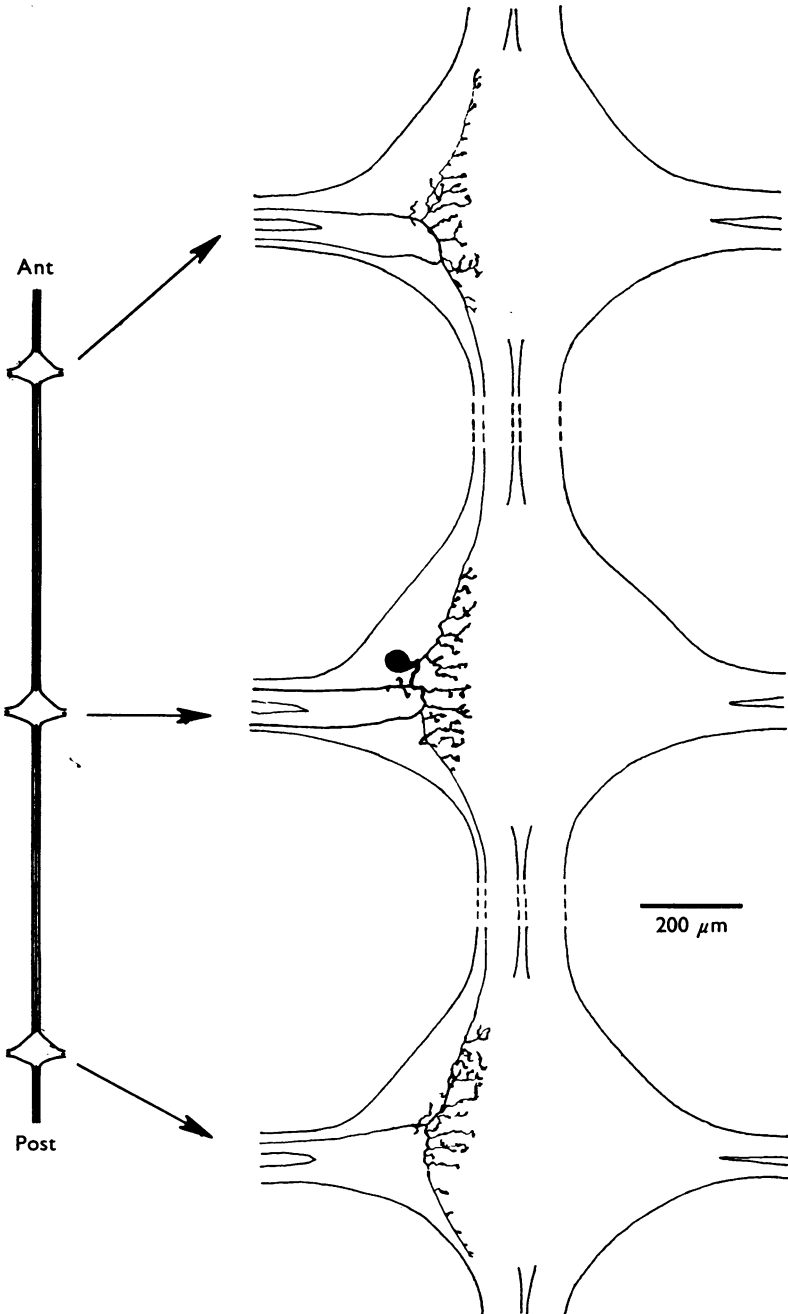


Fig. 2. Camera lucida drawing of an injected touch cell that innervated ventral skin. The entire extent of arborization of the cell spanned three segmental ganglia. The processes that left the C.N.S. through the roots of the anterior and posterior adjacent ganglia were more slender than those that went through the roots of the cell's own ganglion. Note the asymmetry in branching in the anterior and posterior directions.

leech's cylindrical body). This receptive field was supplied by fibres that left the central nervous system through the nerve roots of the ganglion containing the touch cell body. Although with cell staining and electrical recording they had noticed slender branches of touch cells running along

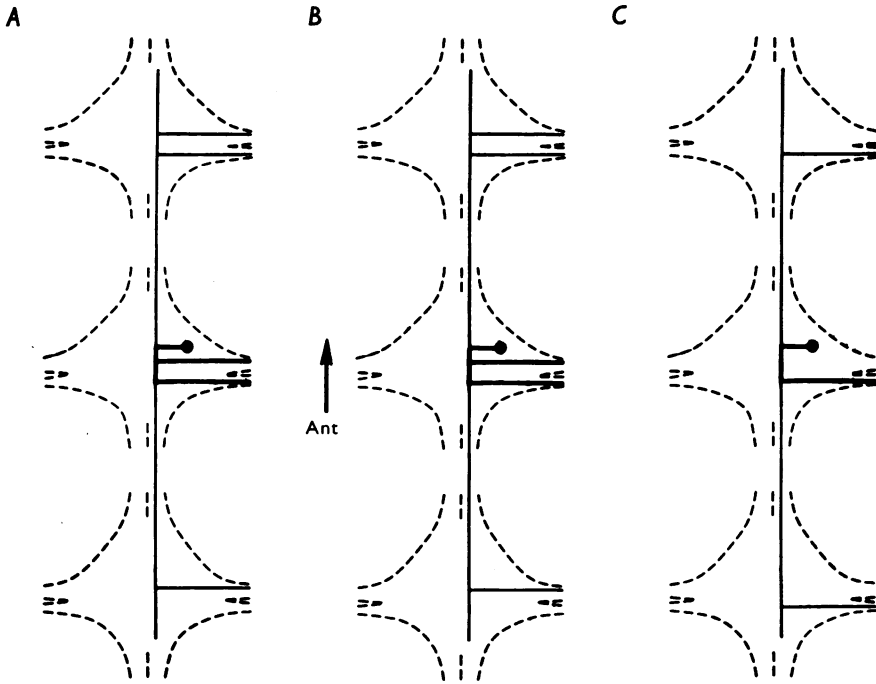


Fig. 3. Diagrammatic drawings of the main branching characteristics of the three touch cells on each side of a segmental ganglion. They innervated respectively *A*, ventral skin, *B*, lateral skin and *C*, dorsal skin. The cells were very similar in appearance, except the one that innervated dorsal skin had only one process going out of each ganglion. These branching patterns were constant along the main length of the body.

the connectives to adjacent ganglia, they failed to detect any processes that left these ganglia to innervate additional areas of skin. In the present study, however, such processes became evident as a result of the improved technique of tracing cell arborizations for long distances by injecting horse-radish peroxidase intracellularly. Fig. 2 is the camera lucida drawing of an injected touch cell that innervated ventral skin. The cell body gave a single stem process which early on branched into four main processes. Two of these processes went to the roots and left the ganglion. The other two processes, which were more slender, ran respectively through the anterior and posterior connectives to the adjacent ganglia, where they arborized and sent branches to the periphery. The extent of arborization

of the cell therefore spanned three segmental ganglia. The patterns of arborization in the three ganglia were very similar. The branching asymmetry, that two processes ran peripherally through the roots of the anterior adjacent ganglion but only one through a root of the posterior adjacent ganglion, agreed with physiological experiments to be described below. The processes in the roots of these adjacent ganglia were much smaller than those in the roots of the cell's own ganglion. The extent and general characteristics of branching of all three touch cells on either side of a segmental ganglion were very similar although they innervated different parts of the skin, with the exception that the cell innervating dorsal skin sent fewer branches to the periphery which all ran in the posterior roots (Fig. 3). The branching was also constant along the main length of the animal's body.

Receptive fields

As expected from their branching geometry, the touch cells had receptive fields that were more extensive than described previously (Nicholls & Baylor, 1968). Each receptive field covered the cell's own segment and also all or part of the anterior and posterior adjacent segments, resulting in large overlaps between receptive fields centred on adjacent segments. Each field was always a smoothly continuous area that was roughly oval or rectangular in shape, with the anterior and posterior boundaries tending to run along annular margins (Fig. 4). By severing ganglionic nerve roots one after another and noting changes in size of a touch receptive field, it was possible to divide the total field into subfields, each innervated by a different nerve root. Fig. 5 shows such subdivisions of a receptive field on lateral skin. There were a total of five subfields, in accord with the morphological finding that a touch cell innervating lateral skin sent branches through five nerve roots to the periphery. The transverse boundaries between subfields also tended to run along annular margins, and there was little overlap between adjacent subfields. This was also demonstrated in an intact preparation by a discrete jump in the otherwise smoothly varying latency of action potentials recorded from the soma when an applied mechanical stimulus was moved across the boundary between two adjacent subfields (see Fig. 5).

For convenience, the part of a receptive field innervated by branches leaving the ganglion containing the touch cell body will be called the major field, and those parts innervated by branches running through the connectives and out through adjacent ganglia will be called the anterior and posterior minor fields. The major and minor receptive fields of a cell had the same relative positions on the skin with respect to their being ventral, lateral or dorsal, and their relative sizes and boundary positions were

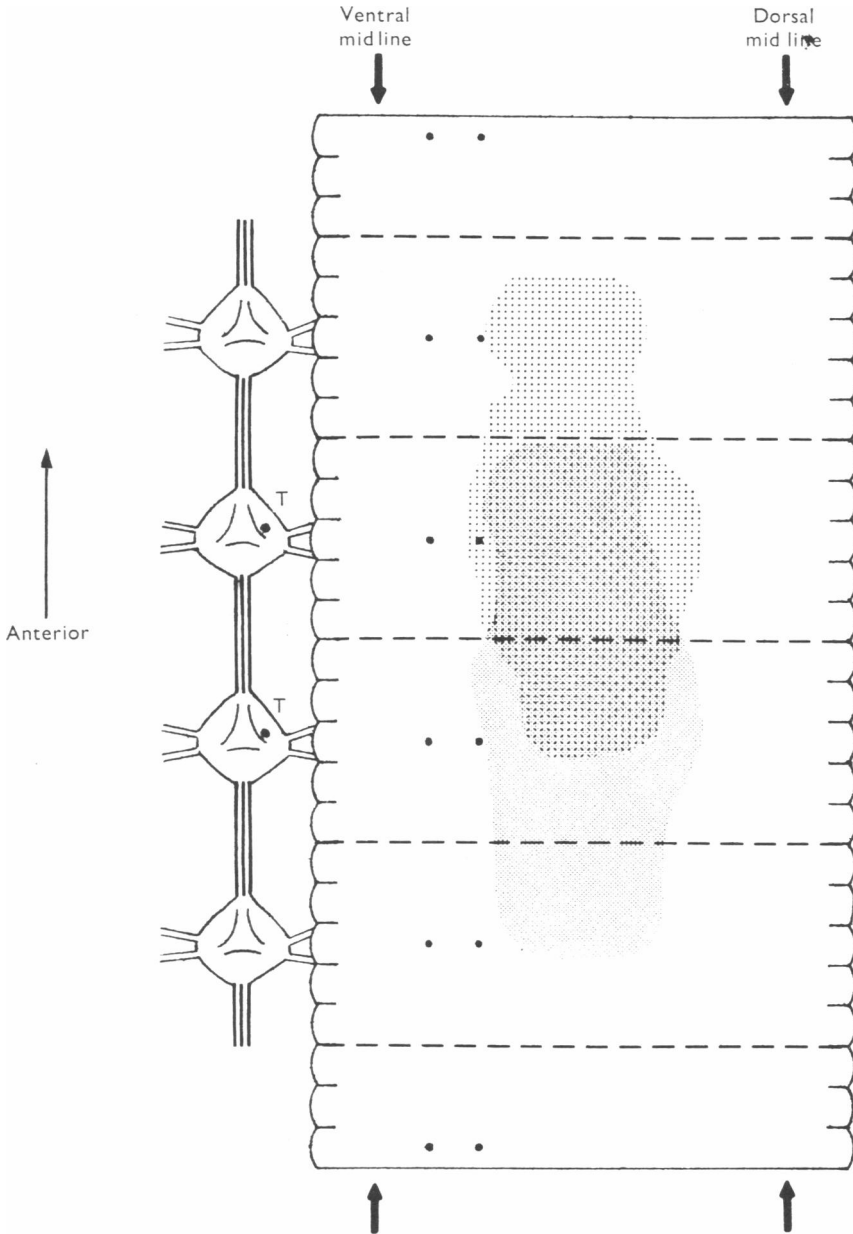


Fig. 4. Receptive fields of two touch cells in adjacent ganglia. They both innervated lateral skin. Each receptive field spanned about twelve to thirteen annuli, and were larger than previously reported (Nicholls & Baylor, 1968). Note the extensive overlap between the receptive fields. The size and appearance of touch receptive fields on ventral and dorsal skin were very comparable.

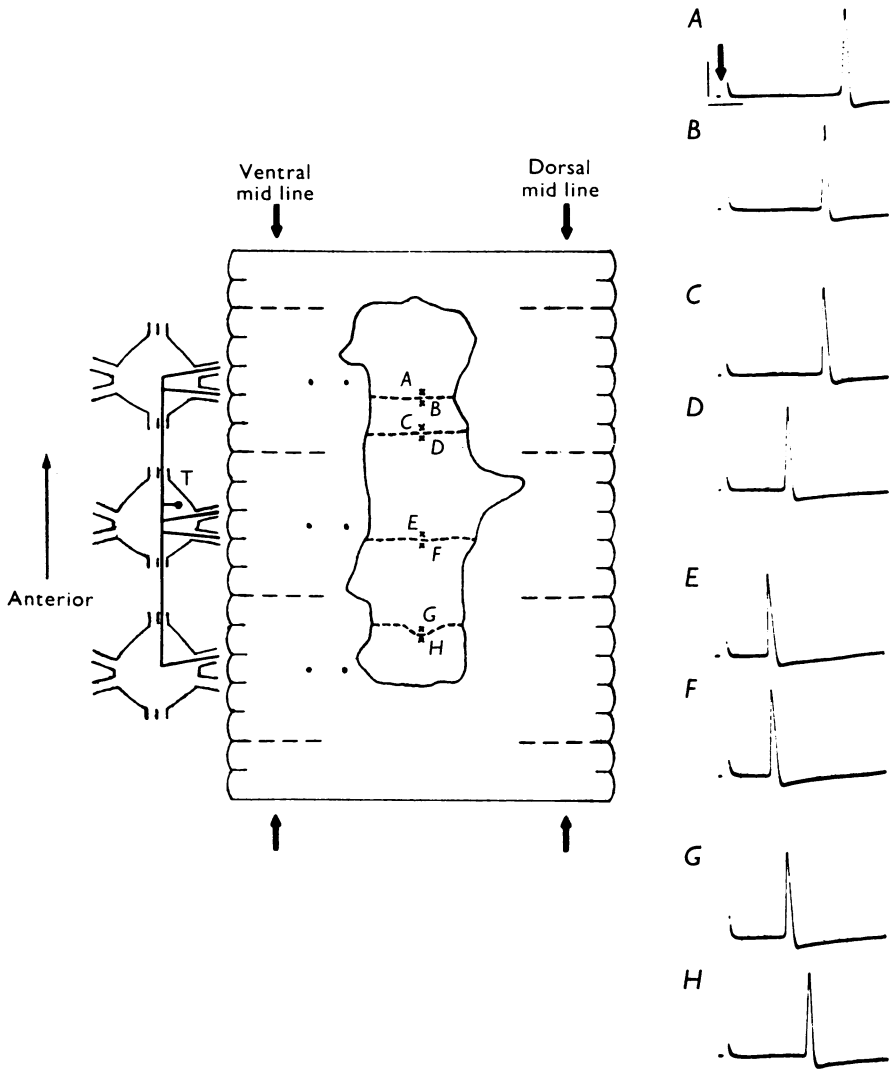


Fig. 5. Subfields of a touch cell that innervated lateral skin. Each subfield was innervated by a separate branch of the cell passing through either a root of its ganglion or that of an adjacent ganglion. Adjacent subfields had negligible overlap with each other, as indicated by discrete jumps in the time delay of intracellularly recorded action potentials (records on the right) when a mechanical stimulus was moved across the boundary between two adjoining subfields. Vertical calibration, 20 mV. Horizontal calibration, 10 msec. Arrow indicates the time when the mechanical stimulus was applied.

quite constant. Apart from being smaller in size the minor fields had a lower density of sensory endings than the major field. The density peaked at the centre of the major field (Nicholls & Baylor, 1968) and gradually decreased toward the periphery and smoothly into the minor fields. Moreover, although the major and minor fields of one touch cell superposed on those of touch cells in the anterior and posterior adjacent ganglia, the sensory endings of two cells were physically separate so that with a sufficiently fine stylus each cell could be independently stimulated.

To examine whether impulses originating from the minor receptive fields of a touch cell were indeed conducted centrally in the branches issuing from adjacent ganglia, the pathways taken by these impulses were followed electrophysiologically. Fig. 6 illustrates such an experiment for impulses from the anterior minor field. A touch cell that innervated ventral skin was recorded from intracellularly while external electrodes were used to pick up activity from both the connective and a root of the anterior adjacent ganglion. Stimulation at the cell body with depolarizing current generated an action potential which propagated to the connective and the root, and was recorded by the external electrodes (Fig. 4*A*). By touching on the anterior minor field an action potential was again recorded successively at the root, the connective and the cell body (Fig. 4*B*). The time delays between the action potentials at the recording sites were constant whether the cell was stimulated at the cell body or from the periphery. These observations were not affected by the presence of Mg^{2+} (up to 20 mM), which is known to block chemical synapses in the segmental ganglia (Nicholls & Purves, 1970). The outgoing and incoming action potentials could also annihilate each other by collision when they were initiated within a critical time interval (Fig. 4*C*). The results verified that the touch cell innervated the anterior minor field directly through its branches in the anterior adjacent ganglion revealed by intracellular horseradish peroxidase injection. Experiments on the posterior minor field have led to a similar conclusion.

With their pathways established, the fate of impulses in touch cells can be followed based on cell geometry (Fig. 2) and known subfield boundaries (Fig. 5). A single impulse initiated by mechanical stimulation of any point on a touch receptive field would be expected to propagate centrally in a defined nerve root and eventually spread into the neuropiles of three segmental ganglia. In the animal, the actual spatial and temporal distribution of impulses in a touch cell would be more complicated because action potentials initiated simultaneously or in close succession from different parts of the receptive field and propagating centrally in different nerve roots would sooner or later collide with and annihilate each other at various points in the cell's branch network.

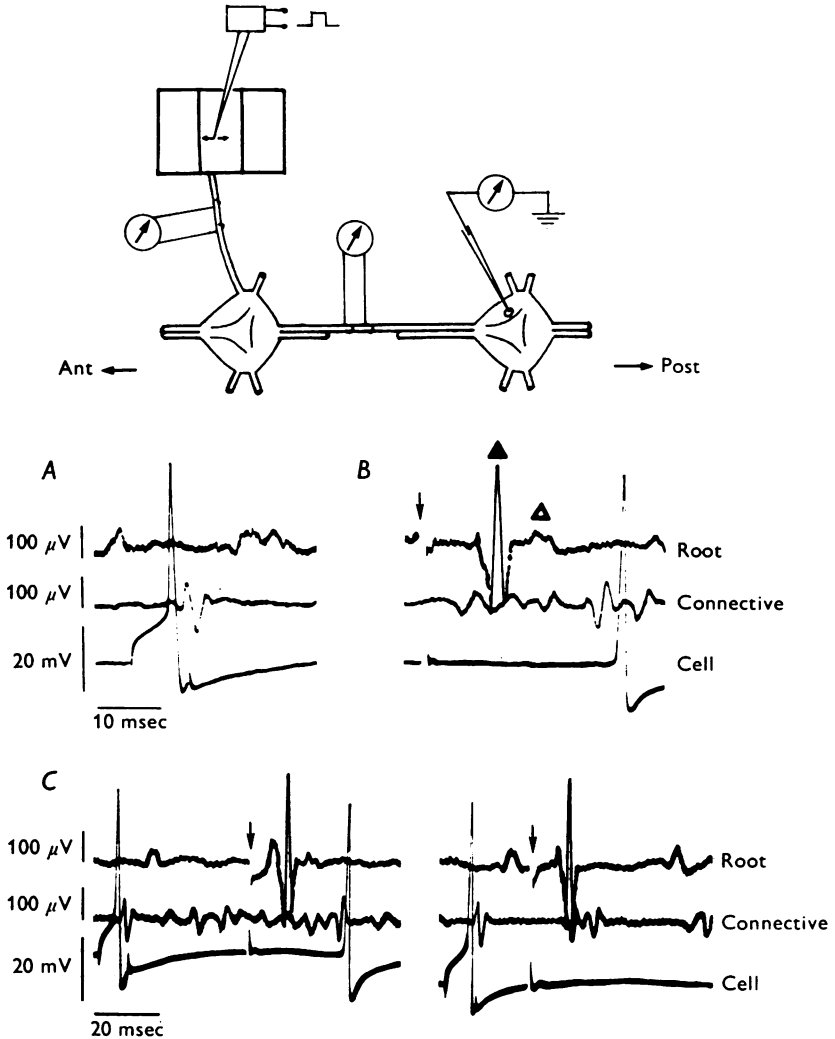


Fig. 6. Simultaneous recordings from root, connective and cell body of a touch cell that innervated ventral skin. Description, see text. The experiment verified that impulses from the minor fields of a cell were conducted centrally in its processes. The arrows in *B* and *C* indicate the instant when mechanical stimulus was applied to the skin. The large impulse in the extracellular root recordings (marked in *B* by \blacktriangle) was from the axon of another touch cell situated in the anterior ganglion, and the small impulse (marked in *B* by \triangle) was associated with the touch cell being studied.

Conduction block of touch impulses from the minor receptive fields

When the receptive fields of touch cells were mapped it was observed that impulses originating from their minor receptive fields frequently failed to invade the cell bodies. This suggested that there were sites along the conduction pathways of these impulses that had low margins of safety. Experiments undertaken to study this showed that conduction block occurred even after the touch cells fired at moderate frequencies for brief periods. Fig. 7 illustrates this phenomenon. It shows intracellular recording from the soma of a touch cell while mechanical stimulus was applied on its anterior minor receptive field at the rate of 10/sec with a vibrating stylus. After only 5 sec of firing, impulses failed to invade the soma actively (traces *C, D*). This occurred when the cell was hyperpolarized by about 8 mV. The site of conduction block was close enough to the soma for electrotonic potential changes to be recorded. The reasons for believing that these potential changes represented blocked action potentials rather than synaptic potentials had been discussed elsewhere (Van Essen, 1973). Briefly, they were as follows. (i) These potentials had a steep rising phase, a fast decay phase, and amplitudes consistently over 10 mV. None of the synaptic potentials observed in these cells had such characteristics. Moreover, the time delays of these potentials corresponded exactly to those of unattenuated action potentials. (ii) The potentials sustained a constant amplitude even at high frequencies of stimulation. This amplitude was neither affected by external Mg^{2+} nor sensitive to changes in the cell's membrane potential in the same way as synaptic potentials. (iii) The potentials could be annihilated by collision with properly timed action potentials initiated at the soma and travelling in the opposite direction. (iv) Conduction block could be created artificially by injecting hyperpolarizing current into the cell, and this produced exactly the same electrotonic potential changes as observed during natural block. As Van Essen (1973) also observed, the blocked and unblocked action potentials usually came in bursts. This was probably due to small, slow fluctuations of the membrane potential during the prolonged hyperpolarization. The constancy in the amplitude of the blocked impulses was striking, especially since the same amplitude was observed in different touch cells. It suggested that block of impulses from the anterior minor field invariably occurred at a characteristic point in touch cells.

The important point was that conduction block occurred in touch cells with only moderate firing and hence little hyperpolarization, since the two are directly related (Baylor & Nicholls, 1969; Jansen & Nicholls, 1973). In some touch cells, such as the example already described, activities of 5–10 impulses/sec for a few seconds (resulting in hyperpolarization

of only a few millivolts) were sufficient to cause conduction block; in other cells, activities of 10–30 impulses/sec for 30 sec or longer (resulting in hyperpolarization of 10 mV or more) were necessary. These ranges of activities, however, could readily be elicited by mild mechanical stimulation

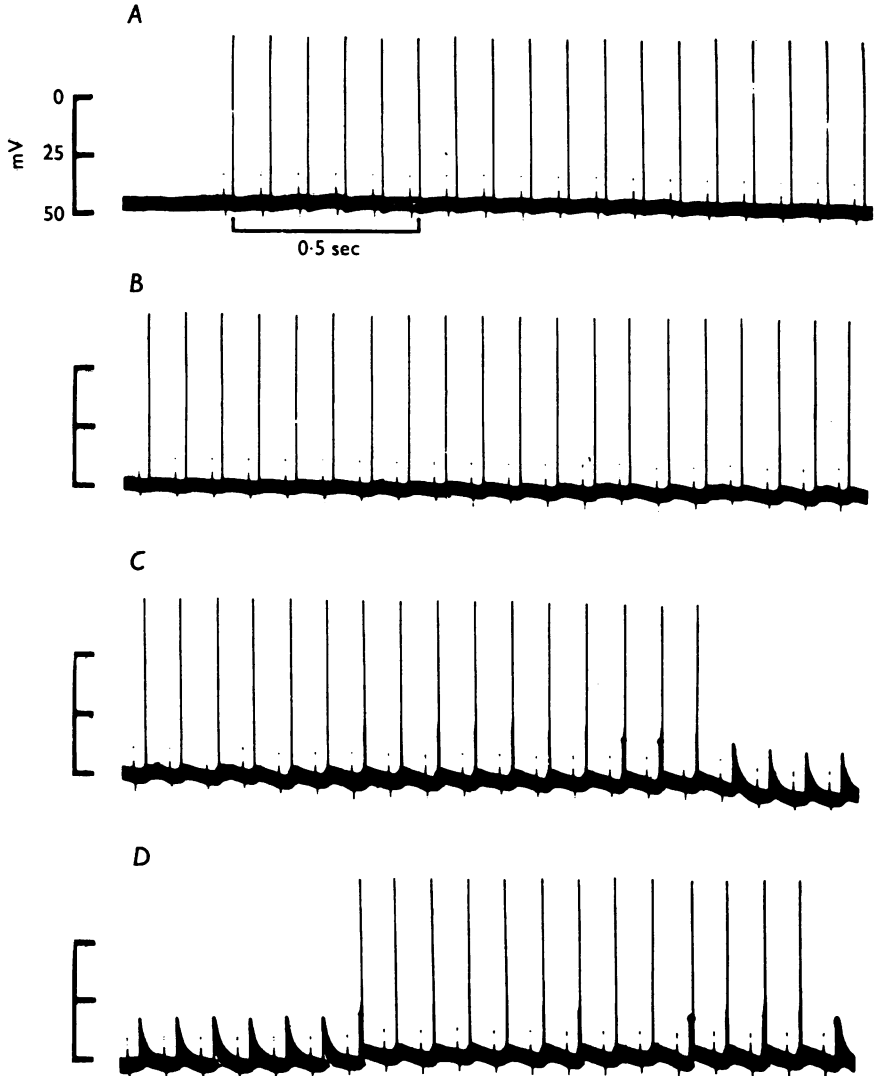


Fig. 7. Conduction block of impulses from the anterior minor field of a touch cell. Traces A to D were continuous in time. The cell was stimulated from the periphery to fire at 10 impulses/sec, and conduction block occurred after about 5 sec of firing, during which the cell became hyperpolarized by about 8 mV.

of the skin. Conduction block also occurred for impulses from the posterior minor field after the touch cells fired at 10–30 impulses/sec for 1–2 min.

In the experiment of Fig. 7, the action potentials recorded from the cell body showed a characteristic inflexion point at their rising phase just before conduction block occurred (this inflexion point is more apparent in Fig. 9). This was seen in a number of experiments; occasionally the action potential was delayed by as much as 4–5 msec. Although difficult to interpret, this appeared to indicate conduction difficulty and had also been observed in other invertebrate neurones (Eyzaguirre & Kuffler, 1955; Tauc & Hughes, 1963) and vertebrate spinal motoneurones (Coombs, Curtis & Eccles, 1957; Fuortes, Frank & Becker, 1957).

Relative block susceptibilities of impulses from major and minor receptive fields

The experimental results described so far extended those of Van Essen (1973), who found that vigorous repetitive firing was usually necessary to cause conduction block of impulses from the major receptive field of touch cells. It thus appeared that impulses from the minor fields were much more susceptible to block than those from the major field. To determine whether this differential block was consistent, the fates of impulses originating from the minor and major fields were examined in the same touch cell (Fig. 8). Intracellular recording was made from the cell while mechanical stimulation was applied alternately to the minor and major fields. At start, punctate test stimuli were applied to the minor field with a vibrating stylus, generating impulses at 1/sec each of which invaded the cell body (trace *A*). The cell was then stimulated to fire vigorously by a small vibrating brush on its major field for a period of about 1 min, during which time the cell became hyperpolarized by about 25 mV (traces *B*, *C*). When test stimuli were again applied at 1/sec on the anterior minor field (trace *C*) the impulses were blocked. Simultaneous stimulation of the major and anterior minor fields showed that impulses from the anterior minor field were preferentially blocked (trace *D*). Similar experiments showed that impulses from the posterior minor field of touch cells were also more susceptible to block than those from the major field, although the difference in susceptibility was not as pronounced.

It thus appeared that in the course of repetitive firing a touch cell could at the most sustain uninterrupted conduction of only those impulses originating from its major field. Successful volleys from the major field, on the other hand, would have the effect of inducing or sustaining conduction block of impulses from the minor fields, because membrane hyperpolarization depended only upon impulse activity and not upon the direction of propagation.

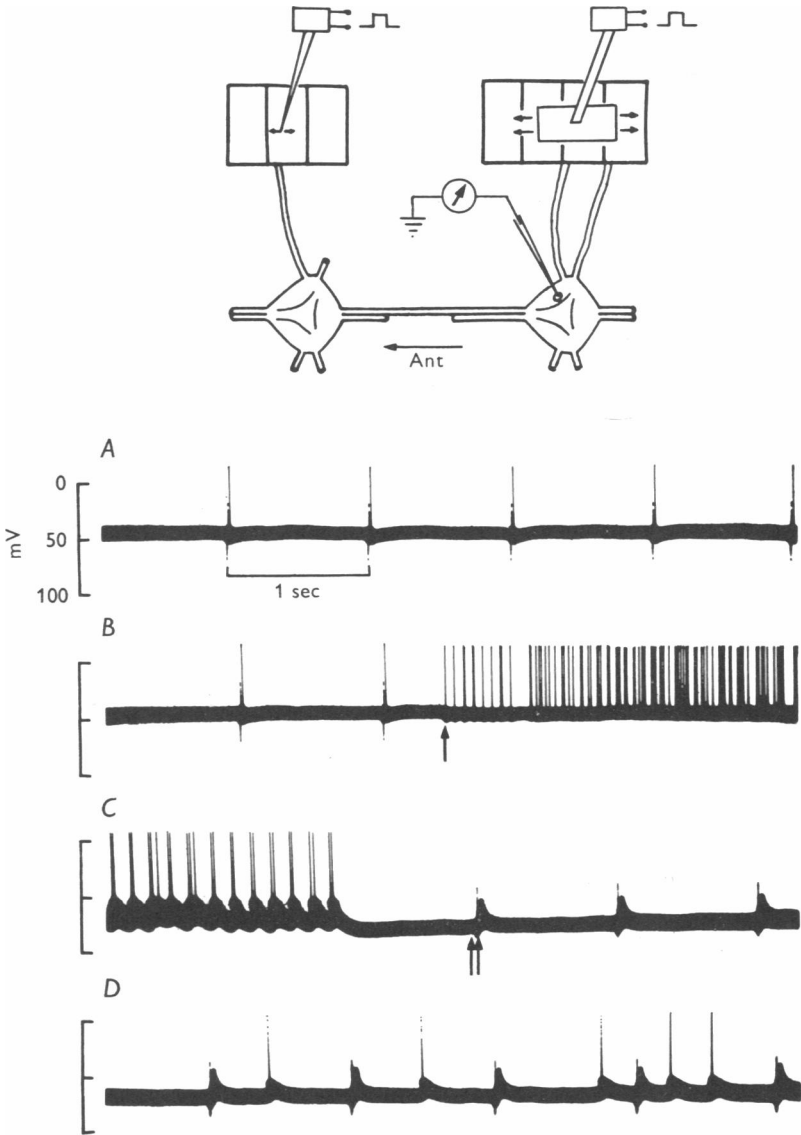


Fig. 8. Comparison of conduction block of impulses from the anterior minor field and the major field of a touch cell. Traces *A-B*, *C-D* were continuous in time. The cell was first stimulated to fire at 1 impulse/sec from the minor field (trace *A*). At the instant indicated by arrow on trace *B*, the cell was caused to fire vigorously by a vibrating brush on the major field. After about 1 min of firing the cell hyperpolarized by about 25 mV. When the cell was again stimulated from the minor field (double arrows, trace *C*) the impulses were blocked. When both areas of skin were stimulated, only impulses from the minor field were blocked (trace *D*).

Sites of conduction block in touch cells

To locate the sites of conduction block in touch cells the pathways taken by the impulses were followed in more detail (Fig. 9). The recording arrangement was identical to that described earlier (Fig. 4), with intracellular recording made from a touch cell body and extracellular recordings

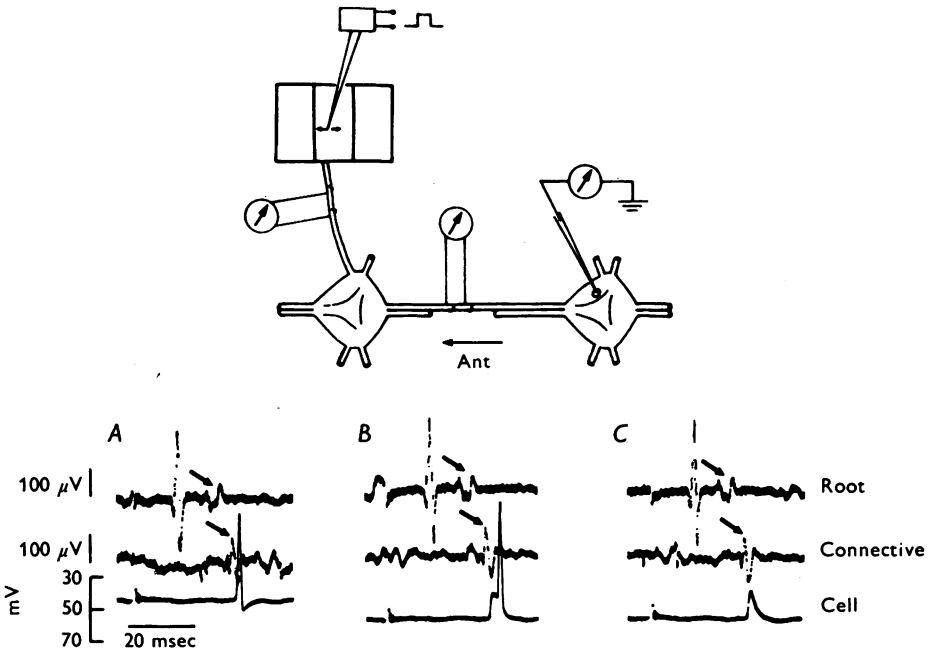


Fig. 9. Locating the site of block of impulses from the anterior minor receptive field of a touch cell. Simultaneous recordings from the root, the connective and the cell body. *A*, touch on the skin initiated a single impulse that passed through the recording sites at the root and the connective to invade the cell body (arrows). *B*, *C*, repetitive firing of the touch cell resulted in hyperpolarization and eventually conduction block, during which an impulse still succeeded in reaching the recording site at the connective. The blocking site was thus somewhere between this point and the cell body.

made from a root and the ipsilateral connective. For impulses initiated from the anterior minor field, the experiment showed that they succeeded in reaching the recording site at the connective whether or not conduction block occurred, implying that the blocking site was situated between the recording site at the connective and the cell body. It was unlikely that this was along the connective because during conduction block impulses could be observed at the connective regardless of the position of the external

electrodes. The blocking site must therefore be within the segmental ganglion where the touch cell body was situated. Similar experiments indicated that impulses originating from the posterior minor field must also be blocked within this ganglion.

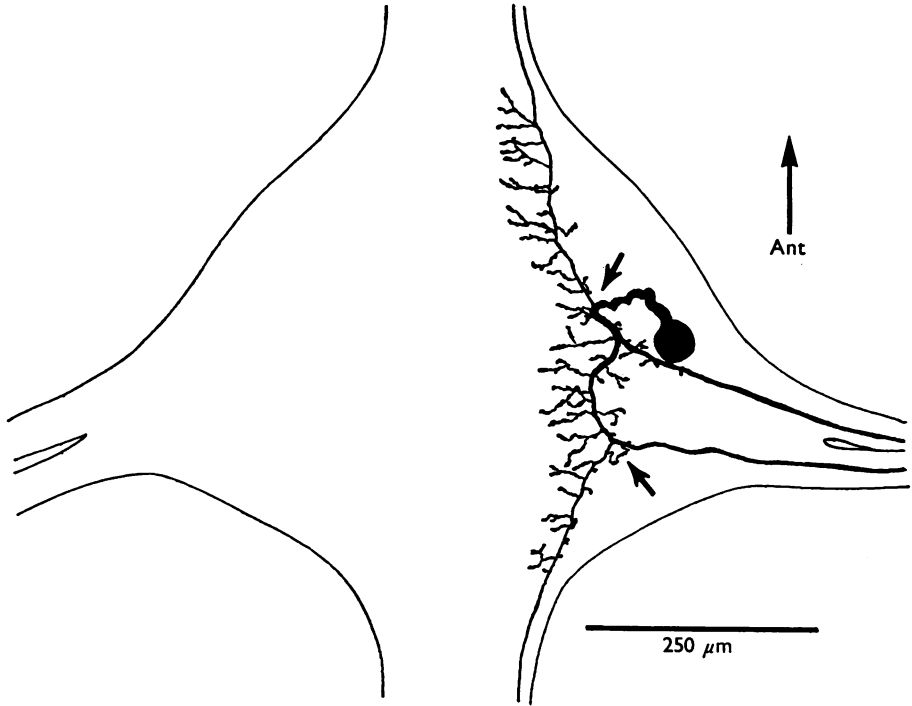


Fig. 10. Camera lucida drawing of a touch cell injected with horseradish peroxidase. The cell innervated lateral skin. Note the fineness of processes in the anterior and posterior connectives compared to the stem process. The branch points where these met (arrows) were the probable sites of conduction block. The same branch points in touch cells that innervated ventral and dorsal skin would be expected to block conduction.

Cell geometry revealed by intracellular horseradish peroxidase provided further clues about the blocking sites in touch cells. Fig. 10 is a high-magnification camera lucida drawing of an injected touch cell that innervated lateral skin. The geometry of the touch cell innervating ventral skin was identical, while that of the touch cell innervating dorsal skin was also comparable except for a lack of process in the anterior root of the ganglion (cf. Fig. 3). The fine process which ran in the anterior connective had no obvious anatomical discontinuities or major branching until it met the much larger stem process arising from the cell body (upper arrow). The anatomy therefore suggested that when an action potential passed

from the small anterior connective process to the large stem process, there would be a low safety margin of conduction because of a sharp increase in load on the action currents. As the cell became hyperpolarized from repetitive firing, the membrane potential would be shifted further away from threshold and conduction block could occur.

To further examine the above arguments, impulses were recorded from the cell body intracellularly and from the posterior ipsilateral connective with external electrodes (Fig. 11). The cell was stimulated as usual by a vibrating stylus on its anterior minor receptive field (Fig. 11*A*). A single impulse initiated by the stylus invaded both the cell body and the posterior connective (Fig. 11*B*). On repetitive stimulation the cell gradually became hyperpolarized and eventually conduction block occurred (Fig. 11*C, D*). In the course of hyperpolarization impulses continued to invade the posterior connective, but failed abruptly when block occurred. The results thus suggested that impulses were blocked at branch point α (Fig. 11*A*), i.e. the point where the anterior connective process met the stem process.

Similar reasoning based on the disparity of fibre diameters led to the conclusion that conduction block of impulses from the posterior minor field probably occurred at the junction between the small posterior connective process and the large stem process (lower arrow in Fig. 10). Cell geometry also explained why impulses from the major receptive field were relatively much less susceptible to block. These impulses propagated centrally in branches of large diameter and would encounter a smaller increase in load when they crossed over the branch points to the stem fibre. A larger hyperpolarization was therefore necessary to precipitate conduction block at these points. In fact, in moderate firing conditions, these impulses not only had little difficulty in invading the cell body, but they would also invade the anterior and posterior connectives because the same branch points which impeded propagation of impulses from the minor fields lost this property in the reverse direction.

Branch points as the only sites of block

One way of testing whether branch points were the only likely blocking sites in a touch cell was to examine artificial block precipitated by electrical hyperpolarization of the cell (Fig. 12). In Fig. 12*B*, a touch cell innervating lateral skin was stimulated by a stylus on its anterior minor receptive field while being hyperpolarized by current through the intracellular electrode (left record). At a critical level of hyperpolarization, conduction block occurred. As the cell was hyperpolarized further, the amplitude of the electrotonic potential remained unchanged, suggesting that as far as the hyperpolarization could spread, there was only one point along this

pathway that was likely to block conduction. Simultaneous intracellular and extracellular recordings indicated that at the instant when block occurred no associated impulse was detected from the posterior connective

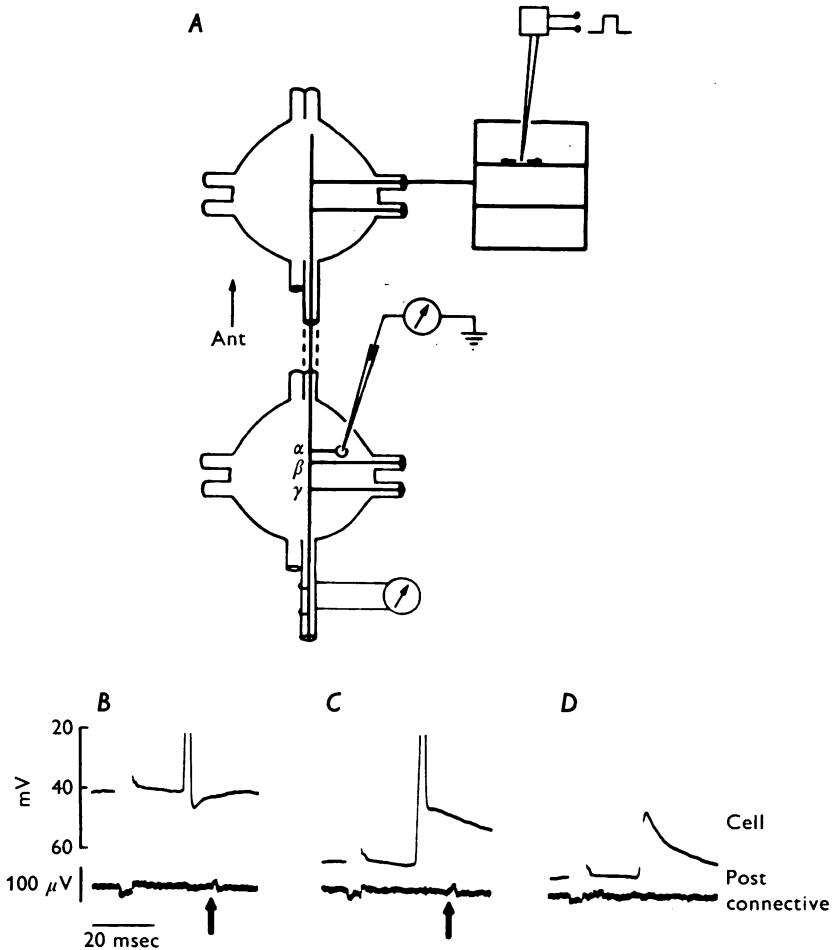


Fig. 11. Simultaneous recordings from a touch cell body and the posterior connective to verify the blocking site suggested morphologically. The cell innervated lateral skin. *B-D*, cell activated from its anterior minor field. The experiment showed that impulses succeeded in invading the posterior connective (arrows) but failed abruptly when block occurred. This supported the suggestion that conduction block occurred at branch point α (see text).

(Fig. 11*B*, two records on right). These results could be explained by assuming that only branch point α (Fig. 12*A*) was susceptible to block. For comparison, the cell was stimulated on the posterior part of its major

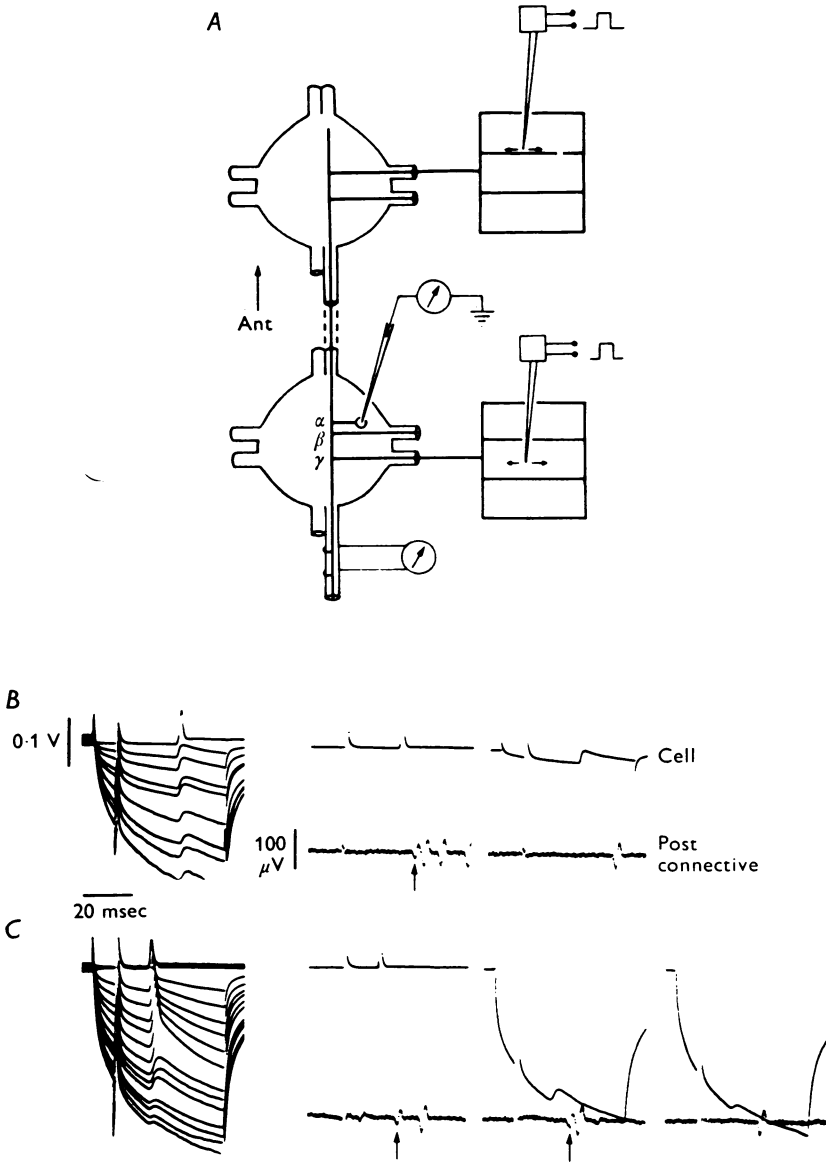


Fig. 12. Conduction block of impulses in a touch cell induced by current injection into the cell body. The cell innervated lateral skin. *B*, impulses initiated from the anterior minor field were blocked at only one site. Simultaneous recordings from the cell and the posterior connective indicated that impulses failed abruptly to invade the connective when block occurred (extreme right record). *C*, impulses initiated from the posterior part of the major field were blocked at two sites. Only at the second site of block did impulses fail to invade the posterior connective (extreme right record). The arrows indicate the impulses recorded in the posterior connective that were from the touch cell being studied.

receptive field, so that the impulses propagated centrally by way of the posterior root of the ganglion (see Fig. 5). When the cell was hyperpolarized to the same degree as before an action potential still succeeded in invading the cell body. Further hyperpolarization, however, brought about two stages of conduction block, as evidenced by two discrete sizes of electrotonic potentials recorded from the cell body (Fig. 12*C*, left record). Extracellular recordings indicated that only at the second stage of block did the impulse fail to invade the posterior connective (Fig. 12*C*, three records on right). These results could be explained by assuming that conduction block was possible at two branch points in this conduction pathway. The second stage of block most likely occurred at branch point γ (Fig. 12*A*), because the impulse also failed to invade the posterior connective. The location of the first stage of block could not be determined, although it was more likely to be at branch point β than branch point α because, as already pointed out, geometry suggested that branch point α was only unfavourable to impulses arriving from the anterior connective process.

The above experiment had the limitation that the electrical hyperpolarization decayed with distance from the cell body so that only the parts of the processes adjacent to it could be studied. Also, under the conditions of the experiment, not all points of discontinuities would be revealed because, depending on their relative susceptibilities to conduction failure and their relative positions in a pathway, one discontinuity point could mask the presence of others. Within these limitations, however, the experiment did not reveal any conduction discontinuity unaccountable by branch points.

Filter property of branch points

Conduction block of impulses from the anterior minor receptive field of touch cells sometimes occurred without continuous firing of the cells; instead, one action potential which closely followed another was blocked. Apparently in these cases the branch point between the anterior connective process and the stem process of the cell was so unfavourable to conduction that even the relative refractoriness following an action potential could block the propagation of closely succeeding ones. Fig. 13 demonstrates this phenomenon. In Fig. 13*A*, two action potentials were initiated in close succession from the anterior minor receptive field of a touch cell by mechanical stimulation, and were recorded both intracellularly from the cell body and extracellularly from the posterior connective (indicated by arrows). The second action potential, however, showed an inflexion point that was suggestive of conduction difficulty. As the time interval between the two action potentials was gradually shortened, the second action potential became more delayed (Fig. 13*B*) and eventually blocked,

at which instant it failed to invade both the cell body and the posterior connective (Fig. 13C), suggesting that block occurred at the branch point between the anterior connective process and the stem process. The time delay between the two action potentials when this occurred was about 8 msec. In other words, the branch point acted as a low-pass filter and would not transmit firing frequencies of higher than about 125 impulses/sec.

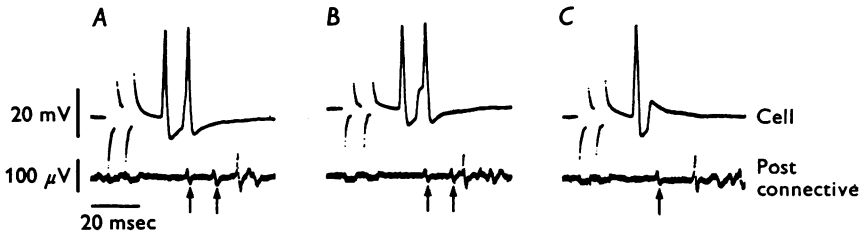


Fig. 13. Filter property of branch point in a touch cell. Intracellular recording from the cell body and extracellular recording from the posterior connective (same recording situation as Fig. 11). The cell was stimulated from its anterior minor field. *A*, two action potentials initiated about 10 msec apart succeeded in invading the cell body and the posterior connective (arrows in lower trace). Note the inflexion point at the rising phase of the second action potential. *B*, shortening the interval between the two action potentials made the inflexion point on the second action potential more obvious. *C*, shortening the time interval to about 8 msec induced conduction block of the second action potential. At the same time, it failed to invade the posterior connective, suggesting that block occurred at the branch point between the anterior connective process and the stem process of the cell.

Branch points with such a filtering characteristic were not frequently encountered (three out of ten preparations). In experiments which demonstrated this phenomenon, however, the time delay between successive action potentials at which conduction block began to occur was from 7 to 12 msec.

DISCUSSION

Cell geometry and receptive fields

The complex arborization of touch sensory neurones revealed by horseradish peroxidase injection was reflected by their elaborate receptive fields. Each cell innervated not only an area on the skin through branches that ran in the roots of the ganglion containing its cell body (the major receptive field) but also anterior and posterior adjacent areas through smaller branches than ran in the roots of adjacent ganglia (the minor receptive fields). In an earlier study Van Essen (1973) had noticed certain fibres in the roots of segmental ganglia which responded to touch but did

not belong to the touch cells within the same ganglia. From the results of the present experiments, it appeared that the fibres he noticed corresponded to the branches of touch cells which innervated the minor fields. In both cases, the amplitudes of extracellularly recorded action potentials in these fibres were only about a fifth of what were normally recorded from 'regular' touch fibres in the roots. This could be explained by the finding that the touch fibres innervating the minor fields were finer than those innervating the major field.

The present results confirmed two earlier findings by Nicholls & Baylor (1968). The first was that whereas the receptive fields of different touch cells in the same and adjacent ganglia overlapped extensively, the sub-fields of a given cell hardly overlapped with each other. By mechanisms yet unknown the branches of a cell recognized and avoided invading each other's territory. The other was that the density of sensitive spots on the receptive field of a touch cell peaked near the field centre and gradually dropped off towards its periphery, where there was superposition with the receptive fields of two other touch cells. In other words, a cell had the highest density of innervation over an area where others had the lowest. At this stage it is not possible to explain the mechanisms underlying this innervation pattern without other pieces of information such as whether the total density of sensitive spots (i.e. summed over all cells) is uniform over a body segment, and what the structure of the sensory endings is like.

The extents of branching and receptive fields of touch cells were invariant along the main length of the animal. However, in the few segmental ganglia near the head and the tail (anterior to 12th segment and posterior to 24th segment, see Mann, 1961, for numeration of segments in the leech) the touch cells sent processes beyond the adjacent ganglia and gave out branches to the periphery through the nerve roots of more distant ganglia (K.-W. Yau, unpublished). It appeared that there was a gradient along the leech body that governed the branching of touch cells such that toward the head and the tail their arborization became more extensive. It is not known how this is related to specializations in structure and function of the head and tail regions, but it might imply greater emphasis on mechanosensory reception by the two ends of the animal.

Sites of conduction block

In his experiments, Van Essen (1973) showed that conduction block of impulses from the major receptive field occurred at branch points of peripheral and central processes of touch cells. The results of the present experiments have led to the same conclusion for the central block of touch impulses from the minor receptive fields. For example, the susceptibility of a branch point to block impulse propagation in a given direction

as measured physiologically agreed with the morphologically observed disparity in fibre diameters encountered by the impulses at that point, and the discontinuities along a specific conduction pathway detected from current injection experiments could be accounted for by branch points in that pathway.

Although conduction block at branch points has been described or inferred in various studies (see Grossman *et al.* 1973 for brief review), the quantitative dependence of impulse propagation on the parameters of a branch point has only been analysed for the Hodgkin-Huxley model of the squid giant axon (Berkenblit, Vvedenskaya, Gnedenko, Kovalev, Kholopov, Fomin & Chailakhyan, 1971). In the simpler situation of conduction along a fibre of non-uniform diameter, mathematical computation again based on the Hodgkin-Huxley theory and using the squid giant axon as a model has shown that an action potential would pass through an abrupt fivefold widening with difficulty, and would be completely blocked by a sixfold widening (Khodorov, Timin, Vilenkin & Gul'ko, 1969). In the leech, it is difficult to make a quantitative analysis because, apart from the complicated over-all geometry and the unknown cable properties of touch cells, it is impossible to record from the immediate neighbourhood of a branch point. None the less, one can convert the situation of conduction across a branch point into that of conduction across an abrupt widening of an unbranched fibre and make a rough estimate of the equivalent increase in diameter for any branch point. As an example, consider the branch point where impulses from the anterior minor field were observed to fail without appreciable hyperpolarization (branch point α in Figs. 11 and 12). Examination of different touch cells injected with horseradish peroxidase (e.g. Fig. 10) indicated that at this branch point the impulses had to cross over from a small fibre to two other fibres whose diameters were between 2 and 4 times as large. With the simplifying but reasonable assumption that the situation was closely approximated by that of an infinitely long cylinder branching into two other infinitely long cylinders, then since the input impedance of an infinitely long cylinder is proportional to the reciprocal of (diameter)² (Dodge & Cooley, 1973), the branch point was equivalent to a small fibre sharply expanding into one with 3-6 times the original diameter. A branch point with equivalent widening closer to threefold would require a larger hyperpolarization and hence higher repetitive activity in order to block conduction (e.g. Fig. 11), whereas a branch point with equivalent widening closer to sixfold would require a smaller hyperpolarization and hence little or no repetitive activity to block (e.g. Figs. 7, 13). Interestingly, this equivalent widening of about 6 for branch points extremely susceptible to block is similar to that theoretically derived for the squid axon as quoted earlier.

Effect of conduction block on signalling by touch cells

The present experiments demonstrate that conduction block occurs in touch cells with moderate mechanical stimulation of the skin, suggesting that this phenomenon probably plays a physiological role in the daily life of the animal, such as when it is crawling or swimming. The morphology of touch cells (Fig. 5) indicates that when impulses from the minor fields are blocked they no longer invade most of the branches of these cells and will therefore fail to reach many sites of synaptic contacts between touch cells and other neurones. The result, however, is not a simple shrinkage of the receptive field down to the size of the major field because impulses from the anterior minor field still invade the anterior adjacent ganglion and those from the posterior minor field still invade the posterior adjacent ganglion. Furthermore, the situation is asymmetrical because the rectifying nature of branch point blockage always permits impulses from the major receptive field to invade the neuropiles of all three segmental ganglia without interruption. The effect of conduction block in touch cells is thus not functionally to eliminate their minor fields but, rather, to minimize their relative significance. This change will be transmitted to post-synaptic cells, with effects depending on the nature and location of the synapses. The possible significance of branch points in the differential channelling of information has also been emphasized in other invertebrate and vertebrate neurones (Tauc & Hughes, 1963; Scheibel & Scheibel, 1970; Grossman *et al.* 1973).

Filter effect of branch points

An occasional observation is that a branch point can act as a low-pass filter. In the touch cells where this occurred, the minimal temporal delays between successive action potentials that were allowed across the branch point was found to be about 10 msec. In other words, the instantaneous frequency of action potentials which was permitted across such a branch point could not be higher than about 100 impulses/sec. This is a significant effect considering that touch cell fibres are capable of firing at over 200 impulses/sec (Nicholls & Baylor, 1968). Frequency filters are not peculiar to touch cells. In other leech mechanosensory cells that respond to pressing on the skin (Nicholls & Baylor, 1968), frequency filters have been observed which limited firing rates to below 25–50 impulses/sec (K.-W. Yau, unpublished). These rates are again well under the firing capability of these cells. The presence of branch points acting as frequency filters in the sensory neurones introduces a new variable which affects primarily the temporal aspect rather than the spatial distribution of impulses in their branch networks.

The observation that the relative refractory period of one impulse could result in blockage of a succeeding impulse at a branch point suggested the possibility of conduction block brought about by inhibitory synapses. It is conceivable that inhibitory synapses situated in the vicinity of a vulnerable branch point might generate hyperpolarizing potentials or shunting effects large enough to block propagating action potentials. The reverse kind of interaction, namely that inverted inhibitory synaptic potentials can relieve conduction block resulting from a large prolonged hyperpolarization, has already been demonstrated in the touch cells (Van Essen, 1973).

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