J. Physiol. (1969), **203**, *pp.* 591–609
With 2 plates and 12 text-figures
Printed in Great Britain

CHEMICAL AND ELECTRICAL SYNAPTIC CONNEXIONS BETWEEN CUTANEOUS MECHANORECEPTOR NEURONES IN THE CENTRAL NERVOUS SYSTEM OF THE LEECH

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(*Received* 5 *February* 1969)

SUMMARY

Experiments have been made to study the synaptic connexions between sensory cells in the C.N.S. of the leech. Each segmental ganglion contains six neurones that respond specifically to light touch applied to the skin; each of these 'touch cells' innervates a discrete area on the surface of the body and has a characteristic set of properties by which it can be recognized. Using intracellular electrodes it has been shown that these sensory cells interact with one another through chemical and electrical synapses by way of a stereotyped set of pathways.

1. Action potentials occurring in one touch cell gave rise to synaptic potentials in the five other touch cells in the same ganglion and also in the three ipsilateral touch cells in the adjacent ganglia. Thus, synaptic interactions took place between sensory cells whose receptive fields lay within the same segment and on the same side of adjacent segments.

2. The post-synaptic potentials consisted of a short-latency coupling potential, followed by an excitatory potential and a number of inhibitory potentials. These delayed synaptic potentials occurred inconsistently and with a variable latency; they could also be recorded in the cell which had been stimulated. All of the touch cells appeared to be equally effective in initiating synaptic potentials.

3. The short-latency coupling potential was shown to be mediated through an electrical synapse by observing a voltage change in one touch cell when current was injected into its neighbour. It was not abolished by

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high concentrations of Mg in the bathing fluid, which blocked chemical synapses in this ganglion. This electrical synapse displayed remarkable rectification; a depolarization could spread from cell to cell in both directions, while a hyperpolarization could spread in neither.

4. The inhibitory potentials were reversed by injecting Cl into the cell. In Cl-free Ringer solution this effect was so marked that the reversed IPSPs caused long trains of impulses in touch cells, which tended to excite each other by a process of positive feed-back.

5. Synaptic potentials evoked by activation of a touch cell did not usually reach threshold since excitation and inhibition tended to cancel. The connexions between touch cells that mediated the delayed excitatory and inhibitory potentials are polysynaptic; the interneurones have not yet been found but some of their connexions could be inferred from electrical recordings.

6. Action potentials in sensory cells of a different modality (responding to pressure) also initiated synaptic potentials in the same family of touch cells.

7. The possible significance for integration of these synaptic interactions between sensory cells is discussed.

INTRODUCTION

The C.N.S. of the leech consists of a chain of ganglia, each containing about 300–400 neurones. The nervous organization of a ganglion is specifically concerned with the sensory and motor innervation of a segment of the body. In addition, each ganglion has well organized connexions with other parts of the C.N.S. A relatively simple nervous system of this type, containing few cells, seems particularly suitable for studying the connexions that underlie co-ordinated reflex movements. An understanding of the integration of sensory inputs to a ganglion with each other and with descending or ascending influences could reveal principles of organization, which in turn might help in the analysis of similar problems in the more complex systems of vertebrates.

Leech ganglia offer a number of advantages for this type of analysis because several sensory and motor cells are now known (Nicholls & Baylor, 1968; Stuart, 1969). For example, there are three sensory cells on either side of each ganglion that convey information about light touch applied to the skin (see Plate 1). Each of these cells innervates a discrete, oval shaped territory, situated dorsally, laterally or ventrally on the surface of the body. The boundaries of the field supplied by a particular touch cell are remarkably constant from segment to segment and from animal to animal. Similarly, there are two cells responding specifically

to pressure and two to noxious mechanical stimulation; each of these neurones supplies an area of skin covering about half of the body wall on one side (i.e. the dorsal or the ventral quadrant). Thus, the pattern of sensory innervation of the skin resembles a quilt made up of oval patches which overlap at their edges and have sizes and positions that are different for touch and for pressure (or noxious) modalities. Motoneurones can also be recognized in leech ganglia; each cell consistently supplies a discrete region of the musculature of the body wall (Stuart, 1969). In this preparation one can therefore hope to find out some of the rules which govern the patterns of connexions of sensory cells of known modality with each other, with motoneurones of the same segment, and with cell populations of other segments. Of equal advantage is the possibility of simultaneously studying excitatory and inhibitory mechanisms and the way in which they are organized to bring about a set of well defined integrative actions.

As a first step we have traced the synaptic connexions between the primary neurones responding to touch. It will be shown in this paper that touch cells with adjacent receptive fields influence each other reciprocally by electrical and chemical synaptic connexions that are intricate but stereotyped. Thus, an action potential, which has been initiated in the peripheral terminals of one touch neurone, propagates along an axon to the C.N.S. where it gives rise to synaptic potentials in other sensory cells. This raises a more general problem concerning the function that such interactions between sensory neurones might perform in the discrimination or further analysis of cutaneous stimuli by higher order cells in the nervous system. Preliminary accounts of some of these observations have been published elsewhere (Baylor, Nicholls & Stuart, 1967; Baylor & Nicholls, 1968).

METHODS

The experiments were made on medicinal leeches (*Hirudo medicinalis*) with conventional recording techniques using micro-electrodes to impale individual cell bodies and external electrodes to stimulate or record from the roots or connectives (see Nicholls & Baylor, 1968, for a full description). Leech Ringer fluid of the following composition was used: (mM/l.) NaCl 115; KCl 4; $CaCl_2$ 1·8; Tris-maleate, neutralized with NaOH 10; glucose 11. In solutions containing high Mg, NaCl was replaced by an osmotically equivalent amount of $MgSO_4$. In certain experiments ganglia were bathed in SO_4-Ringer fluid of the following composition (mM/l.) Na_2SO_4 57·5; K_2SO_4 2; $CaCl_2$ 1·8; Tris-maleate 10; glucose 11; sucrose 76 (Nicholls & Kuffler, 1964).

RESULTS

Description of sensory cells in leech ganglia. The preparation and procedure for identifying individual nerve cells according to a number of criteria have been described elsewhere (see Nicholls & Baylor, 1968). In the living ganglion one can recognize a number of neurones on the basis of

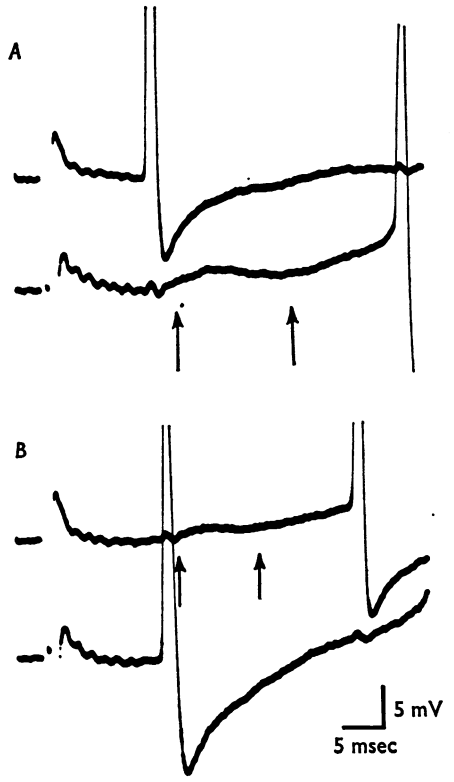
their shapes, sizes, positions and physiological properties. Plate 1 is a photograph of a leech ganglion showing the six touch cells, labelled T. A single process extends from the cell body to a central neuropile where it branches extensively to make synapses with the processes of other neurones. There are no synapses on the cell body itself. The axons run to the periphery through the ipsilateral roots and to neighbouring ganglia through the ipsilateral connectives (see Pl. 2). When the receptive field in the skin is touched action potentials propagate along the axon and invade the cell body within the ganglion. P and N represent cells responding to pressure and noxious mechanical stimulation of the skin.

Description of intra- and interganglionic connexions. The touch cells within a ganglion are connected to one another in a highly stereotyped manner, so that an action potential in any one cell evokes similar synaptic potentials in the other five. The principal features of the post-synaptic response are (*a*) a short-latency potential which occurs with each action potential in the presynaptic cell and will be shown to be due to an electrical synapse, (*b*) a longer latency depolarizing potential that follows only intermittently and may occasionally reach threshold, and (*c*) hyperpolarizing inhibitory potentials (IPSPs), that usually arise after a brief train rather than a single impulse in the presynaptic cells. Delayed depolarizing and hyperpolarizing synaptic potentials can also be recorded in the cell that had been stimulated, indicating that there are pathways for recurrent excitation and inhibition.

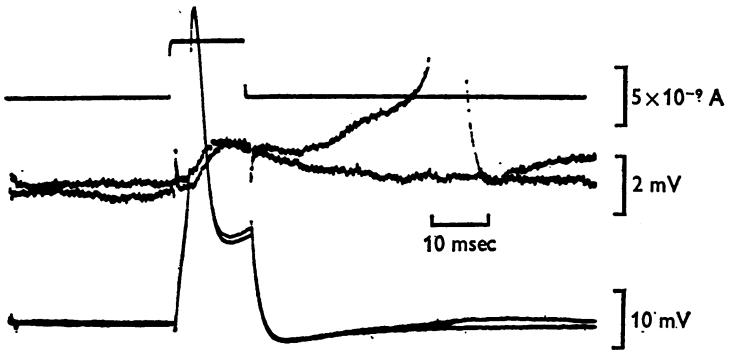
An example of the synaptic interaction between two adjacent touch cells is shown in Text-fig. 1*A* and *B*. In Text-fig. 1*A* one touch cell (upper trace) was activated by a brief mechanical pulse delivered to the skin within its receptive field. The action potential recorded from the cell body rose abruptly with a latency of about 10–15 msec after the stimulus artifact. In contrast, the same mechanical pulse did not activate the neighbouring touch cell (Text-fig. 1*A*, lower trace) by way of its axon, since its receptive field lay in a more lateral position on the animal. Instead, the arrows in this recording indicate the short- and the long-latency depolarizing synaptic potentials in response to the impulse in the first cell; eventually the synaptic potentials gave rise to an action potential in the second cell. The reciprocal nature of the interaction can be seen in Text-fig. 1*B*. Here the touch stimulus was moved to an adjacent region of the skin so that the second touch cell was activated directly, while the first was driven synaptically. The coupling potential and the delayed depolarizing potential can also be seen in Text-fig. 2, where the touch cell was stimulated by current injected through the micro-electrode. The latency of inhibitory potentials was too long for them to be seen in these records.

Similar reciprocal connexions are also present between the ipsilateral

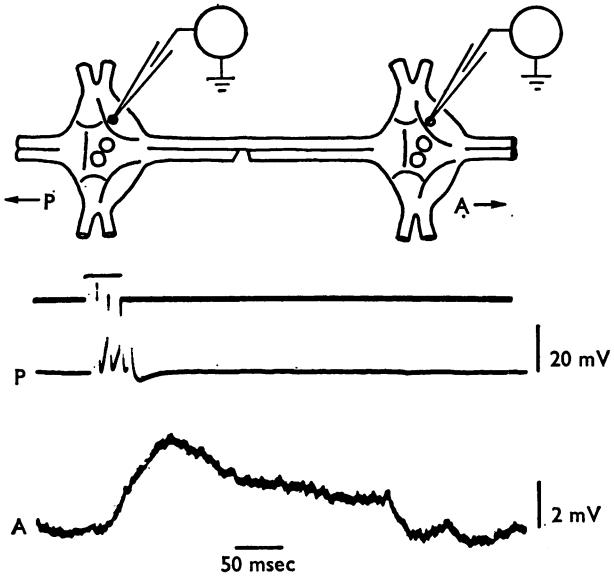
touch cells of adjacent ganglia. The lower trace in Text-fig. 3 illustrates the synaptic potentials evoked in an ipsilateral touch cell of the anterior ganglion by directly stimulating a cell in the neighbouring posterior ganglion. With the slow sweep speed used, the short- and long-latency depolarizing potentials cannot be distinguished from one another, but two delayed IPSPs can be clearly seen following three action potentials in the posterior touch cell. The latency of the inhibitory synaptic potentials was variable and frequently shorter than in the example shown here, so that they tended to interact with the depolarizing potential. Coupling potentials,



Text-fig. 1. *A* and *B* are intracellular recordings made from two adjacent touch cells, with electrodes filled with K acetate, 3 M/l. In *A* a brief touch was applied within the receptive field of the cell whose action potential is recorded on the upper trace (the top of the action potential is not seen). The first deflexion in each record is the artifact produced by the current pulse passing through the 'toucher' on the skin. Notice the short latency and the long latency depolarizing synaptic potentials (marked by arrows) in the adjacent cell (lower trace) that eventually gave rise to an impulse. In *B* the stimulus was applied to the area of skin supplied by the cell whose action potential is seen in the lower trace; synaptic potentials now occurred in the upper trace. The action potentials evoked directly by touching the skin show no 'step' but rise abruptly from the base line.



Text-fig. 2. Intracellular recordings from adjacent touch cells to show the coupling potential and the delayed depolarizing potential. Two successive action potentials were set up by transmembrane current pulses (upper trace) in one cell (lower traces) and gave rise to coupling potentials in its neighbour (middle traces). On the first occasion the coupling potential was followed by a delayed synaptic potential that gave rise to an action potential. Failures of the delayed potential were observed frequently. The micro-electrodes were filled with K citrate, 2 M/l.



Text-fig. 3. Interganglionic connexions: The upper part of this Text-fig. shows the arrangement of the stimulating and recording micro-electrodes (filled with K acetate, 3 M/l.) in touch cells of the posterior (P) and the anterior (A) ganglia. In this experiment the lower connective was cut (see diagram) disconnecting the touch cells on that side of the preparation. The top trace is a record of the current delivered to the posterior cell through the micro-electrode. This gave rise to three action potentials (middle trace). In a touch cell of the anterior ganglion (bottom trace, note the higher gain) the depolarizing synaptic potentials were followed by two IPSPs after a long latency. Frequently, the latency for the IPSPs was far shorter so that they interrupted the depolarizing potentials (see Text-fig. 8). Similar effects were observed in the posterior touch cell when the anterior was stimulated.

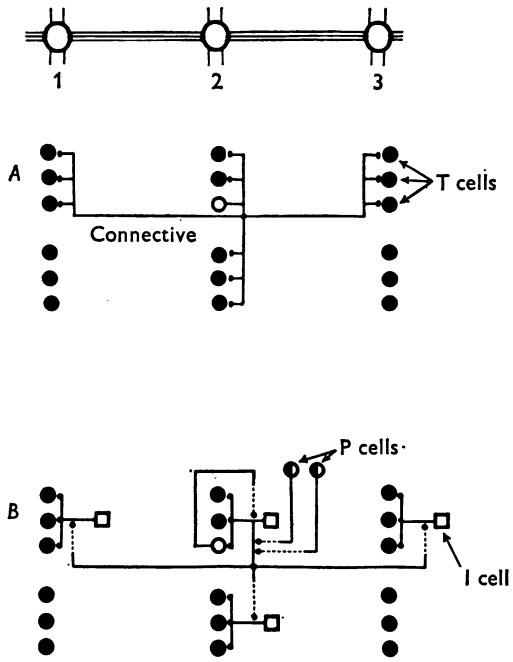
delayed depolarizing potentials and IPSPs were also seen in the posterior touch cell after stimulation of the anterior cell.

The axons mediating these interganglionic connexions run to the adjacent ganglion on either side through the ipsilateral connectives, which are completely sheathed by connective tissue and do not branch (Coggeshall & Fawcett, 1964). If a single connective was cut (see Text-fig. 3) the touch cells on that side became disconnected, while those on the side opposite remained connected. Plate 2 is a photomicrograph kindly provided for us by Dr D. Purves, of a touch cell which had been injected with Procion Yellow according to the technique described by Stretton & Kravitz (1968). One axon can be clearly seen to enter the ipsilateral connective on the left; others enter the roots and approach the connective on the right. Because the preparation is a whole mount some parts are out of focus. Under the microscope, however, one can follow the axon for some distance in the connective running to the next ganglion. Electrical recordings show that this axon conducts at about 0·5 m/sec.

In summary, action potentials in one touch cell give rise to synaptic potentials in eleven other touch cells, five in the same ganglion and three in each adjacent ganglion. Under the conditions of our experiments each individual touch cell seemed equally effective in initiating synaptic potentials in the others. A simplified diagram of the connexions of a typical touch cell (open circle) with other touch cells (filled circles) is shown in Text-fig. 4*A*. In general this scheme corresponds to the pattern of the electrical connexions that mediate the short-latency coupling potential (see below). The pathways for recurrent excitatory and inhibitory potentials are more complex (Text-fig. 4*B*) and will be described in the next section.

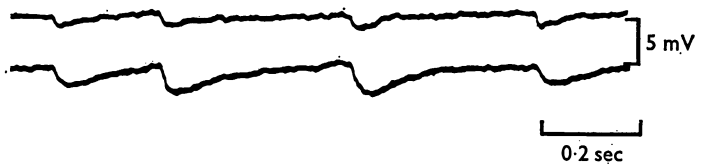
Pathways for recurrent synaptic potentials. In contrast to the short-latency coupling potentials, which were electrical and appeared to be monosynaptic (see below), the properties of the delayed depolarizing potentials and inhibitory potentials suggested that they were mediated through di- or polysynaptic pathways. They failed to follow presynaptic action potentials in a one-to-one manner, they were usually initiated by two impulses or better still by a train (see Text-fig. 3), and their latency was long (over 10 msec) and variable.

We have little additional information about the properties of the delayed depolarizing potential, but there is evidence from electrical recordings that the three touch cells in one side are supplied by a common inhibitory cell. Thus, spontaneous IPSPs (which often occurred in bursts) were synchronous in adjacent touch cells; a typical record is shown in Text-fig. 5. The pathway for the recurrent inhibition may also involve a common cell, perhaps the same one that gives rise to spontaneous IPSPs.



Text-fig. 4. Schematic diagrams to illustrate the connexions of one touch cell (○) with its homologues (●) in the same ganglion (2) and in neighbouring ganglia (1 and 3). *A* shows the group of cells that are interconnected: Note that ipsilateral cells in adjacent ganglia are connected by way of the ipsilateral connective. For convenience the synapses are shown on the cell body, whereas in fact all synaptic connexions are made on processes within the neuropile. This scheme represents the electrical synapses that are probably monosynaptic (see later).

B shows a tentative scheme for the connexions of touch cells with inhibitory cells (□). The interrupted lines indicate pathways that are not known to be monosynaptic. The three cells on one side are supplied by a common inhibitory cell and the two sides are somehow linked so that partial synchrony can be achieved. For this scheme the assumption has been made that the same inhibitory neurone is responsible for the spontaneous IPSPs as well as those evoked by action potentials in touch cells. The effect of P cells (see Pl. 1) in initiating inhibitory potentials is described later (see Text-fig. 8).



Text-fig. 5. Spontaneously occurring IPSPs recorded in adjacent touch cells with micro-electrodes filled with K acetate, 3 M/l. Such IPSPs were always synchronous in adjacent cells and this suggests that they are initiated by a common interneurone (see Discussion).

Thus, IPSPs evoked by impulses in one touch cell occur synchronously in the stimulated cell and in its neighbours.

In contrast to these results obtained with adjacent cells, only partial synchrony of IPSPs was observed in (*a*) touch cells on opposite sides of the same ganglion, or (*b*) ipsilateral touch cells in adjacent ganglia. A record taken simultaneously from two touch cells in adjacent ganglia is shown in Text-fig. 6. Though some spontaneous IPSPs occur in synchrony others plainly do not. In other experiments the degree of synchrony was more obvious with brief bursts of IPSPs occurring in the two cells at a constant latency. Text-figure 7 provides evidence that the connexions mediating

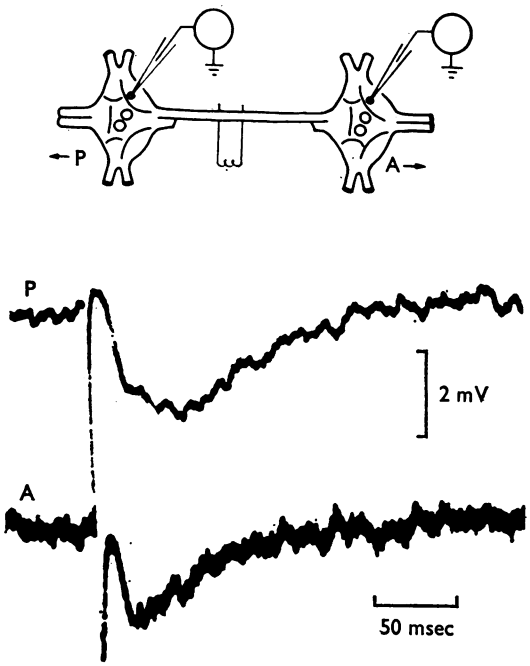
Text-fig. 6. Spontaneous IPSPs recorded in ipsilateral touch cells of adjacent ganglia (electrodes arranged as in Text-fig. 3). Note that although many IPSPs occur synchronously there are extra IPSPs which do not arise simultaneously in both cells. Similar, partial synchrony is observed when recordings are made from touch cells on opposite sides of the same ganglion.

the IPSPs run in the ipsilateral connectives. For this experiment one connective was cut, as in Text-fig. 3, and the other stimulated electrically. Each stimulus now gave rise to a unitary IPSP of invariant latency and amplitude in the ipsilateral touch cells.

The simplest scheme that one can devise from these results is shown in the diagram of Text-fig. 4*B*; it implies that there are at least two inhibitory interneurones for the six touch cells in a ganglion. Each is activated by the touch cells of one side, and feeds back on them. It also produces IPSPs in the contralateral touch cells and in those of neighbouring ganglia on the same side, but the absence of complete synchrony of the IPSPs in these cells suggests that the connexions may not be so simple and might involve other interneurones or electrical synapses (see Discussion). It will be shown later than an alternative explanation for the synchrony of IPSPs, current spread through an electrical synapse, is unlikely because of rectification.

Cross-connexions of sensory cells with different specific modalities. We could not observe synaptic potentials in sensory neurones responding to pressure or noxious stimuli (P and N cells in Pl. 1) as a result of impulses initiated in touch cells. In contrast, the touch cells were inhibited by activity originating in cells of another modality, the 'pressure' cells. Text-figure 8 shows the effect on a touch cell of two impulses produced in a pressure cell

by stimulation through the micro-electrode. The first action potential gave rise to a depolarizing potential which was not invariably present; the second gave rise to inhibitory potentials preceded by a depolarizing potential. Once again, the IPSPs were synchronous in the three ipsilateral touch cells; inhibitory potentials were also seen in ipsilateral touch cells of adjacent ganglia. The pathways are indicated in Text-fig. 4*B*. Action

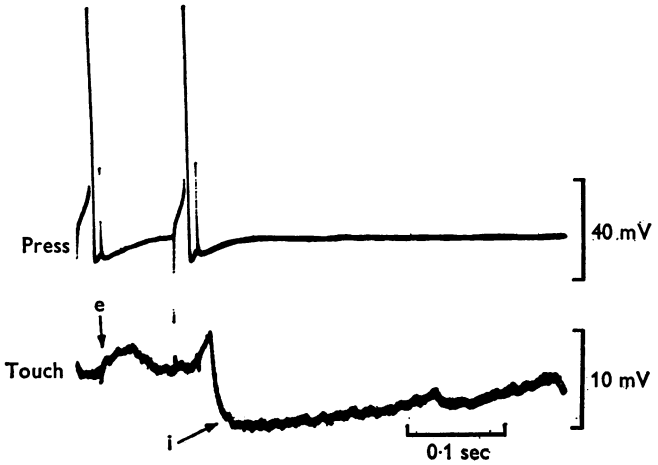


Text-fig. 7. Synchronous IPSPs evoked in touch cells of adjacent ganglia by stimulation of a connective. The arrangement of stimulating and recording electrodes is shown above. Note that the lower connective has been cut. The latency and amplitude of these IPSPs were constant. This type of experiment suggests that axon(s) mediating the IPSP runs in the ipsilateral connective.

potentials in a pressure cell gave rise to synaptic potentials in cells of another modality, the N cells. Here, however, the responses were always depolarizing, never IPSPs as in the touch cells.

Effectiveness of synaptic potentials for the initiation of impulses. At present we do not know what role the synaptic interactions between touch cells play in sensory integration. Action potentials are normally initiated at the peripheral processes of a touch cell when the skin is stroked; it therefore seemed natural to wonder how effective the excitatory potentials were in initiating impulses in central processes within the ganglion. To investigate this problem, experiments were made in which we recorded intracellularly from one touch cell while another touch cell was stimulated by indenting

the skin of its receptive field at different frequencies. Under the conditions of our experiments, the synaptic potentials did not usually reach threshold so that an action potential was not initiated in the post-synaptic cell (see Text-figs. 3 and 10). For example when one cell was made to fire at rates of 10–50/sec for a few sec by repeatedly touching the skin, the neighbouring touch cell gave at most one or two action potentials. Even when two adjacent cells were made to give prolonged high frequency bursts by



Text-fig. 8. Synaptic connexions of cells with different sensory modalities. Intracellular recordings from a pressure cell (upper trace) and a touch cell (lower trace). A single action potential gave rise to an excitatory potential (e) in the touch cell, whereas two or more action potentials were followed by inhibitory potentials (i). These impulses in P cells evoke only depolarizing potentials in the N cells (see text). Stimulation of a touch cell does not give rise to obvious synaptic effects in P or N cells

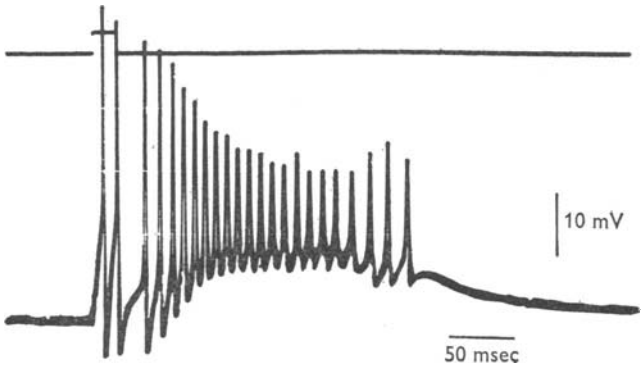
stroking the skin over both their fields, a third touch cell showed trains of synaptic potentials but few, if any, impulses. In general we found that the synaptic potentials reached threshold only at very low rates of stimulation, as in the records shown in Text-figs. 1 and 2, where a single impulse in one cell occasionally gave rise to an impulse in its neighbour. A simple explanation for these results would be that the inhibitory potentials, which are brought in with trains of action potentials, usually prevent the depolarizing potentials from reaching threshold. It will be shown later that when inhibitory potentials were reversed by the intracellular injection of Cl, repetitive firing did occur in touch cells.

Mechanisms of synaptic transmission

In addition to tracing connexions, we have investigated the ionic basis of the inhibitory potentials and the properties of electrical synapses

between touch neurones. These findings are relevant to an understanding of the function of the synaptic connexions and are described in the following sections.

Ionic basis of IPSPs. We have shown in an earlier paper that the IPSPs generated within the neuropile are reversed when the cell body of a touch neurone is hyperpolarized by 5–10 mV (see Fig. 3 in Baylor & Nicholls,



Text-fig. 9. Repetitive firing of a touch cell due to reversed inhibitory potentials. The micro-electrode contained KCl 3 M/l., while the bathing fluid was Cl-free, SO_4-Ringer fluid. Under these conditions spontaneous IPSPs were reversed in sign. When two action potentials were initiated by a brief depolarizing current pulse a high frequency train followed, presumably as the result of depolarizing potentials that summed and reached threshold. Effects of this type were not seen when Mg was used to block synaptic transmission.

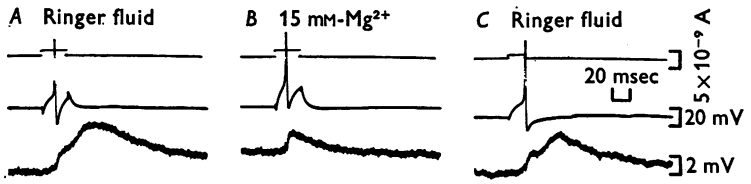
1969). All the records shown so far in this paper were made with electrodes filled with K acetate or K citrate. When KCl electrodes were used the IPSPs became reversed within a few minutes, and set up depolarizing local potentials that often caused the cells to fire. The situation was even more pronounced when KCl electrodes were used to impale cells in ganglia bathed in Cl-free, SO_4-Ringer fluid. An example is shown in Text-fig. 9, where two action potentials in a touch cell set up a whole train of impulses. Longer trains of higher frequency could be produced after injecting Cl into the cell with a hyperpolarizing pulse.

The reversal of IPSPs seen with Cl electrodes implies that the inhibitory transmitter increases the Cl conductance of the touch cells. The simplest explanation for the burst of impulses occurring in SO_4-Ringer fluid with a KCl electrode is that recurrent excitation occurred. In the absence of Cl in the extracellular fluid all the recurrent synaptic potentials would tend to depolarize. Hence, one or two action potentials would give rise to synaptic potentials that reached threshold; this in turn would lead to further excitation and so the process could continue until terminated by some

other mechanism. These effects were abolished by Mg, 8–20 mM/l. in the bathing fluid, which blocks synaptic transmission (see below).

These experiments, while establishing a role for Cl, do not rule out an additional contribution of K to the IPSP.

Evidence for electrical coupling between touch cells. The following evidence indicates that the earliest synaptic event is a 'coupling' potential mediated through electrical synapses between the touch cells:



Text-fig. 10. Effect of Mg on coupling potentials and delayed synaptic potentials. Intracellular recordings (electrodes filled with KCl 3 M/l.) were made from two adjacent touch cells in normal Ringer fluid (*A*, *C*). An action potential in one cell (middle trace) gave rise to a coupling potential and a delayed depolarizing potential in its neighbour (bottom trace). In this pair cells of the delayed potential failed only occasionally in Ringer fluid. Without removing the electrodes, Mg, 15 mM/l., was allowed to flow past the preparation (*B*), abolishing the delayed potential but not the coupling potential. After replacing Mg with normal Ringer fluid (*C*) the delayed potential returned. The top traces are the current injected through the microelectrode to stimulate the cell of the middle trace.

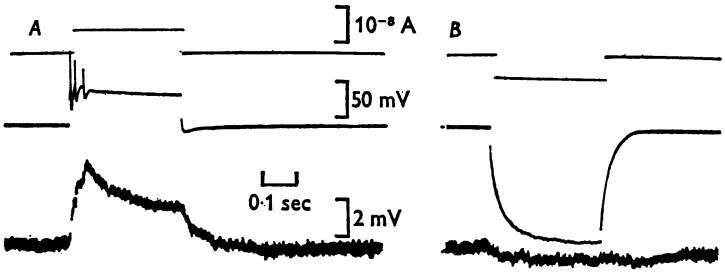
(*a*) The *latency* of the coupling potential was very brief (0·5 msec or less); there was no obvious delay between its rising phase and that of the presynaptic action potential; its peak was reached after 1–2 msec (Text-fig. 2).

(*b*) A coupling potential invariably accompanied every presynaptic action potential even at high frequencies (e.g. 50/sec). In contrast the delayed depolarizing potentials and IPSPs often failed to appear (Text-fig. 2).

(*c*) The coupling potential was unaffected by bathing the preparation in Mg, 15–20 mM/l. (Text-fig. 10). This was so for the interganglionic as well as the intraganglionic connexions of touch cells. Mg characteristically has no effect on the spread of current between cells that are known to be electrically coupled (e.g. the Retzius cells (Hagiwara & Morita, 1962; Eckert, 1963)). On the other hand, the delayed depolarizing potentials and IPSPs in touch cells were completely abolished in these concentrations of Mg (Text-fig. 10). We can conclude from our studies and from those of Stuart (1969) that Mg effectively blocks chemical synapses in leech ganglia, as in other preparations (Martin, 1965).

(*d*) The presence of an electrical synapse could be demonstrated directly by observing the spread of current between adjacent touch cells. With Mg, 20 mM/l., in the bathing fluid, depolarization of any one of the three

touch cells on one side of a ganglion caused a depolarization of its neighbours. Text-figure 11*A* shows the spread of the depolarization and the steep attenuation that occurred; a depolarization of about 40 mV in the presynaptic cell gave rise to about 2 mV depolarization in the post-synaptic cell (lower trace). This post-synaptic potential was not an artifact, since withdrawing from the cell either the stimulating or the recording micro-electrode abolished the effect. The brief peaks in the initial part of this record are coupling potentials set up by the three impulses in the 'presynaptic' cell. In other experiments, where the presynaptic cell did



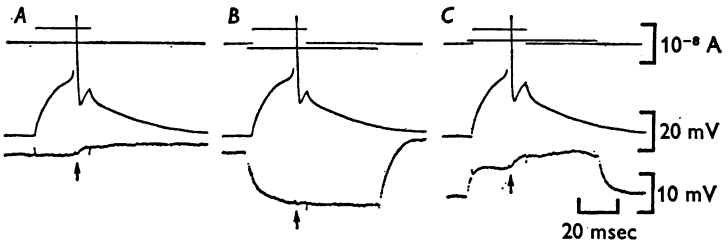
Text-fig. 11. Spread of current between adjacent touch cells. The upper trace shows the depolarizing (*A*) and the hyperpolarizing (*B*) current pulses injected into one cell (middle traces, low gain). The bottom trace in *A* shows the depolarization recorded in the adjacent touch cell. Each action potential in the presynaptic cell gave rise to a coupling potential; current spread could also be observed in the absence of action potentials. The records on the right show the considerably greater attenuation which occurred when one cell was hyperpolarized. Similar rectification was observed when the stimulating and recording conditions were reversed. The electrodes were filled with K acetate, 3 M/l., and the bathing fluid contained Mg, 20 mM/l., to block chemical synapses.

not fire during the depolarization, a smooth 'electrotonic' potential was observed. The spread of current could not be demonstrated directly between cells on opposite sides of the ganglion, or in adjacent ganglia presumably because the distances between the cell bodies are too great (see Pl. 1).

Rectification at electrical synapses between touch cells. A remarkable type of rectification occurred at the electrical synapse; hyperpolarization of any one touch cell led to little or no detectable change in the membrane potential of its neighbours (Text-fig. 11*B*). The attenuation was so steep that a hyperpolarization of 100 mV gave rise to a change of less than 1 mV in the post-synaptic cells. Although a depolarizing current could flow between adjacent cells in either direction, a hyperpolarizing current failed in both directions. Thus, the cells behaved as if they were not coupled when hyperpolarized.

Another demonstration of the rectification is shown in Text-fig. 12. In

this experiment one action potential in the touch cell gave rise to a coupling potential in its neighbour (Text-fig. 12*A*). When the post-synaptic cell was hyperpolarized the coupling potential became smaller and eventually disappeared (Text-fig. 12*B*). Hyperpolarization of the post-synaptic cell gave rise to a similar reduction in the amplitude of 'electrotonic' potentials of the type shown in Text-fig. 11, which were caused by the injection of a depolarizing current. Conversely, depolarization of the post-synaptic cell increased the effectiveness of the coupling (Text-fig. 12*C*). The significance of these results for signalling and one possible explanation for the bi-directional rectification will be discussed below.



Text-fig. 12. Effect of changes in the membrane potential of the post-synaptic cell on coupling potentials. Top traces indicate current injected into pre- and post-synaptic touch cells. In *A* a presynaptic action potential gave rise to a coupling potential (arrow) in the adjacent touch cell.

B. When the post-synaptic cell (bottom trace) was hyperpolarized by a long current pulse, the amplitude of the coupling potential decreased.

C. The coupling potential was increased when the cell was depolarized by a current pulse. These results are consistent with the idea that the coupling potential is mediated through an electrical, rectifying synapse. (Electrodes filled with K acetate, 3 M/l.)

DISCUSSION

Patterns and possible functions of connexions between touch cells. A striking feature of our observations is that sensory cells interact with one another in a complex but stereotyped manner in the C.N.S. of the leech. It has been shown in earlier experiments that impulses in these cells are initiated at their peripheral terminals by gently touching or stroking the skin (Nicholls & Baylor, 1968); each cell has a clearly delineated receptive field at a particular position on the skin surface. The individual touch cells that are interconnected in the neuropile have receptive fields close to each other in the same segment of the body and in adjacent segments. Our experiments have so far provided no evidence for a hierarchy within this group of neurones. It is clear, however, that intracellular recordings made from the cell body could not reveal the finer details of the connexions, which are likely, from anatomical evidence, to be more intricate and specific

than the scheme shown in Text-fig. 4. For example, the coupling potentials, excitatory potentials and inhibitory potentials could all arise in different regions of the same neurone. Furthermore, some processes of the touch cells, which arborize extensively within the neuropile (Retzius, 1891; Nicholls & Baylor, 1968), must in addition end on higher order neurones; these presumably integrate the inputs from a number of fields, so that the leech can discriminate between different tactile stimuli applied to the skin.

What is not at present clear is the part that is played by the synaptic interactions we observed between these lower-order sensory cells. The central processes of the touch cells are invaded by impulses that propagate inward from the periphery. Do the synaptic potentials modulate the effects of these impulses or do they themselves set up action potentials within the C.N.S.? These questions may be relevant for our understanding of integration in more complex sensory systems, because there is evidence that interactions of a similar type occur between primary sensory neurones in the mammalian spinal cord (Eccles, 1961; Mendell & Wall, 1964). In the leech, however, the real significance of these connexions will probably be understood only when one can identify the higher order cells to which the sensory cells project. In the absence of direct evidence, a number of alternative functions can be considered.

One possibility is that the excitatory synaptic potentials sum to give rise to propagated impulses. Under the conditions of our experiments this was not so, since excitation and inhibition tended to cancel; in the intact animal, however, the number of excitatory inputs might be sufficient to reach threshold and initiate action potentials. An alternative function of the synaptic potentials might be to influence transmitter release by the terminals of touch cells. In the squid giant synapse Katz & Miledi (1967) have shown that hyperpolarization of the presynaptic terminal increases the amount of transmitter released in response to a subsequent depolarization. Accordingly the hyperpolarizing and depolarizing synaptic potentials might facilitate or reduce (see Dudel & Kuffler, 1961) the effectiveness of an action potential that propagates into the terminal of a touch cell from the periphery. A mechanism similar to this has been postulated for the interactions that have been observed between certain afferent fibres in the mammalian spinal cord (Eccles, 1961; Mendell & Wall, 1964).

An attractive speculation is that the synaptic potentials might counteract the after-effects of impulses in touch cells. In the preceding paper (Baylor & Nicholls, 1969) we have shown that a neurone can be hyperpolarized by up to 30 mV following a burst of action potentials induced by stroking the skin; the potential gradually returns to normal over a period of several minutes. It is possible that such hyperpolarizations could

prevent impulses from propagating in processes within the neuropile, since it is known that a hyperpolarization of this magnitude can prevent impulses from invading an axon or the cell body (Nicholls & Baylor, 1968). Consequently, if a touch cell had been firing because of a stimulus applied to its receptive field, a fresh stimulus occurring shortly after might give rise to impulses that failed to invade regions of low safety factor within the C.N.S. If, however, an adjacent field were now stimulated by a moving tactile stimulus, the hyperpolarized cell would be bombarded by coupling potentials, depolarizing potentials and IPSPs. The net effect of these synaptic potentials would be to increase the membrane conductance of fine processes in the neuropile and short out the hyperpolarization. We have in fact frequently observed IPSPs of reversed polarity in cells hyperpolarized by activity. According to this hypothesis, then, each cell, when activated by natural stimulation, evokes synaptic potentials in a family of cells with nearby receptive fields. If a cell has not recently been active the excitatory and inhibitory effects will cancel; if on the other hand, the cell has just fired, the synaptic potentials will tend to drive the membrane potential towards the resting value. The peculiar rectification in the electrical synapse (see below) might seem designed to allow coupling potentials, but not hyperpolarization, to spread from cell to cell.

Experiments are now in progress to test these speculations. In particular we hope to determine whether the hyperpolarization induced by natural stimulation does lead to a failure of impulse propagation in some processes of a touch cell and whether this can be prevented by synaptic activity.

Electrical synaptic connexions. We have observed electrical synapses in great abundance in leech ganglia (D. A. Baylor & J. G. Nicholls, unpublished observations). Apart from the well known example of the Retzius cells (Hagiwara & Morita, 1962; Eckert, 1963), and the Leydig cells (see Retzius, 1891) electrical connexions have been seen between P cells and between N cells (see Plate 1 and Nicholls & Baylor, 1968). Thus an action potential in one pressure cell invariably gives rise to coupling potentials, that are not affected by Mg, in the three other pressure cells in the same ganglion and the two ipsilateral pressure cells in each adjacent ganglion. In addition, on the dorsal surface of the ganglion, where many of the motoneurones lie (Stuart, 1969) almost all of the cells are coupled by non-rectifying synapses.

A property that so far appears to be uniquely present in the touch cells is the peculiar rectification that works in both directions: current will spread between two touch cells only when one is depolarized and the other not hyperpolarized. This implies that the current can flow in either direction, provided that the membrane potential is not increased. The effect could not be achieved by connecting the cells through two parallel

rectifying synapses (of the type occurring in the crayfish giant synapses (Furshpan & Potter, 1959)), in opposite directions. With this arrangement each synapse would allow current to flow in only one direction, but it would not matter whether the cells were depolarized or hyperpolarized to achieve the voltage gradient. Electrical synapses with properties resembling those of touch cells have recently been found between retinular cells in the eye of *Limulus* (Borsellino, Fuortes & Smith, 1965).

At present we have little information about how the rectification could occur in two directions. One possible mechanism might be an increase in the electrical resistance of the junctional membrane at the synapse with hyperpolarization; if this were so, less current could flow from cell to cell if the membrane potential of either increased. This is an attractive idea because we have shown elsewhere (Baylor & Nicholls, 1969) that the current–voltage relationship in touch cells is non-linear and shows considerable rectification; at the resting potential the input resistance is about 6 MΩ, but it increases to about 40 MΩ when the membrane is hyperpolarized by 30 mV. Thus, the junctional membranes would behave like those surrounding the rest of the neurone with respect to rectification. The fact that the synaptic contacts occur between small, inaccessible processes within the neuropile makes it difficult to make a direct test of this hypothesis and other possible mechanisms.

We are grateful to Dr D. Purves who kindly gave us the picture of the injected cell of Plate 2, and to Dr Ann E. Stuart who collaborated in several of the early experiments and provided many valuable suggestions. We are also indebted to Mr H. Fein for stimulating discussions and criticism. J.G.N. was supported by USPHS Grant No. NB 06145.

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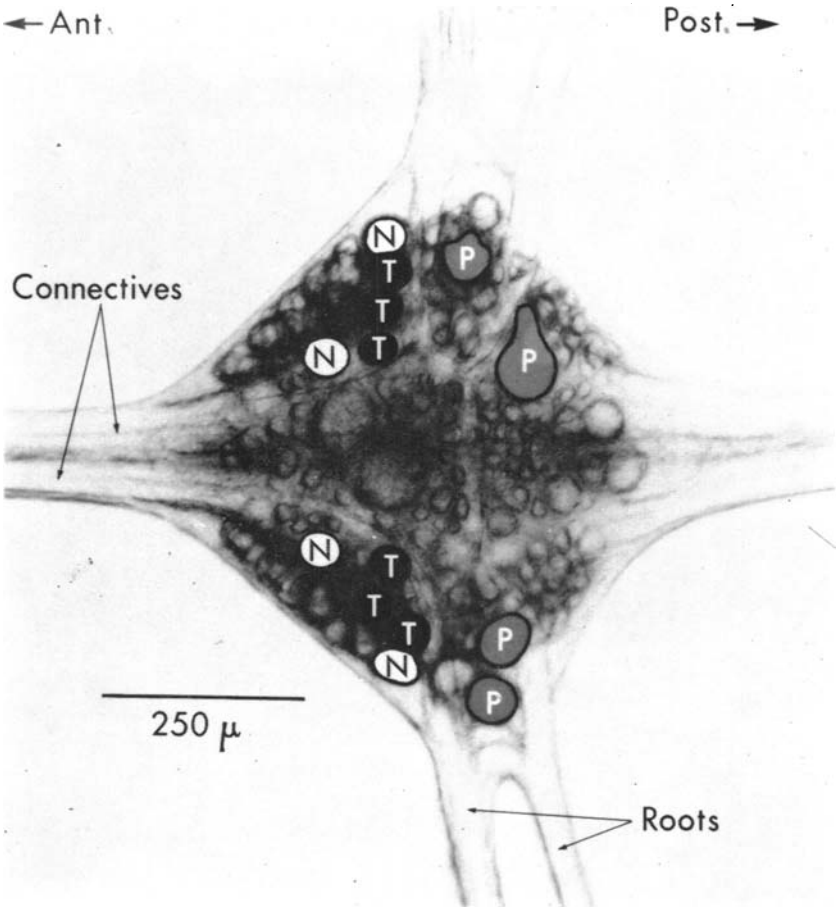
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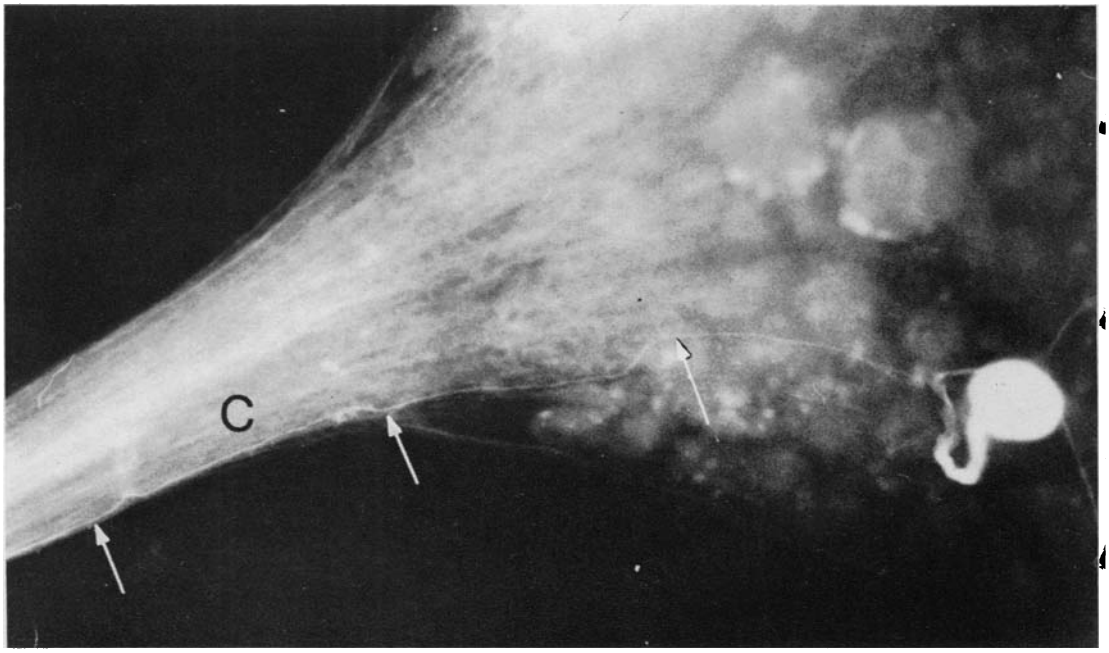
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EXPLANATION OF PLATES

PLATE 1

Photograph of the ventral aspect of a segmental ganglion of a leech. The three cells on each side labelled T are 'touch' sensory cells, each of which responds to light touch applied to a discrete area of the ipsilateral skin. The two P cells respond to pressure and the two N cells to noxious stimuli.

PLATE 2

Photomicrograph of a touch cell which had been previously injected with the fluorescent dye Procion Yellow (see Stretton & Kravitz, 1968). The preparation is a whole mount and some parts are therefore out of focus. One axon, marked by arrows, can be seen to enter the anterior ipsilateral connective (C). By focusing up and down this axon could be traced towards the neighbouring ganglion. In the lower right corner two axons approach the ipsilateral roots (see Plate 1). The axon of a contralateral touch cell which had also been injected can be seen in the other connective.