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MONOSYNAPTIC CHEMICAL AND ELECTRICAL CONNEXIONS BETWEEN SENSORY AND MOTOR CELLS IN THE CENTRAL NERVOUS SYSTEM OF THE LEECH

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SUMMARY

The synaptic connexions that underlie three different segmental shortening reflexes have been traced by recording intracellularly from individual sensory and motor nerve cells in the C.N.S. of the leech. The fourteen sensory cells involved in these reflexes respond specifically to one of three modalities: touch, pressure, or noxious stimuli applied to the skin. All three types of sensory neurone give rise to excitatory synaptic potentials in two large motoneurones. Each of these motor cells provides excitatory innervation to the longitudinal muscle fibres of the opposite side of the segment. The mechanism of synaptic transmission is, however, different for each type of sensory cell.

1. An impulse in a sensory cell that responds to touch gives rise to a short-latency depolarizing potential in the large longitudinal motoneurones by way of an electrical synapse. This junction rectifies so that excitation can spread in only one direction (from the sensory to the motor cell), whereas a hyperpolarizing potential can pass only in the opposite direction.

2. The synaptic potential evoked in the motoneurone by an action potential in a sensory cell responding to noxious stimuli can be attributed to the action of a chemical transmitter agent and has different properties: the post-synaptic potential arises after a delay of about 2–4 msec, is abolished by high concentrations of Mg, and enhanced by high concentrations of Ca. Several lines of evidence show that this connexion is monosynaptic.

3. The synaptic potential following an impulse in a pressure cell is produced by both chemical and electrical synaptic mechanisms. Recti-

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fication, similar to that described for the touch cell, also occurs at this electrical synapse.

4. One or more impulses in any one of the fourteen mechanoreceptor cells in the ganglion can initiate impulses in the large longitudinal motoneurones to produce a shortening of the segment. The contraction is abolished by blocking impulse initiation in the motoneurones.

5. The arborizations of the sensory cells and the motoneurone within the neuropile have been studied histologically after injecting a fluorescent dye. Their processes are intertwined in a highly complex manner so that the sites of the synaptic junctions cannot be determined with the resolutions so far achieved. Nevertheless, taken together the histological and the electrical results support the idea that individual cells are connected in a stereotyped pattern and operate by distinctive mechanisms.

6. These findings provide a basis for studying the functional role of chemical and electrical synaptic mechanisms in these pathways.

INTRODUCTION

Recent experiments have provided a number of criteria for the unambiguous recognition of individual sensory and motor nerve cells in the C.N.S. of the leech (Nicholls & Baylor, 1968; Stuart, 1970). In each of the segmental ganglia there are fourteen mechanoreceptor cells that respond to specific stimuli applied to discrete areas of the skin; the motoneurones provide excitatory or inhibitory innervation to defined groups of muscle fibres in the body wall. With this information it is possible to say which sensory cells will fire when different mechanical stimuli are applied to a particular area of skin, and which motoneurones are active when the animal is performing its limited repertoire of movements (including shortening, bending or flattening).

A relatively simple nervous system such as this constitutes a manageable ensemble of neurones in which one can study integrative mechanisms to an exceptionally full extent. Natural stimuli can be used to initiate normal movement patterns and these reflexes can be analysed in terms of the topographical connexions of individual neurones and the mechanism of synaptic transmission. A further advantage of leech ganglia is that, although all the synapses are made within a complex neuropile, the distances are short enough for synaptic potentials to be recorded intracellularly from the cell body of a neurone and for currents injected through the micro-electrode to spread to centrally located nerve terminals (Baylor & Nicholls, 1969*c*).

The experiments reported in this paper are concerned with the central connexions between the mechanoreceptor cells and a pair of large motoneurones that supply the longitudinal musculature of a segment. It will be

shown that all three types of sensory neurones (responding to touch, pressure or noxious stimuli) make distinctive chemical or electrical excitatory synapses on the motoneurones, and that these connexions account for the reflex shortening of a segment that is observed when mechanical stimuli are applied to the skin.

These findings provide a basis for investigating directly more general problems, such as the functional role of electrical and chemical synaptic mechanisms in these reflex pathways and the effects of repetitive firing by the presynaptic sensory cells on transmission through the reflex arc (Baylor & Nicholls, 1969*a*, *b*).

METHODS

The methods have, for the most part, been fully described elsewhere (Nicholls & Baylor, 1968; Baylor & Nicholls, 1969*c*; Stuart, 1970). An isolated segmental ganglion, or a ganglion attached to a portion of body wall muscle and skin by its peripheral roots, was pinned in a small bath. Individual sensory and motor nerve cells were impaled by intracellular micro-electrodes for recording and stimulating. These micro-electrodes, which had resistances of 50–120 MΩ, were filled by boiling in an open flask for 6 min in 3 M-KCl; the flask was then sealed and cooled rapidly so as to produce a vacuum. This technique produced sharper electrodes than the usual procedure of boiling for longer periods under reduced pressure. In some experiments synaptic potentials were recorded with electrodes filled with 4 M potassium acetate. These electodes, unlike those filled with KCl, do not cause reversal of inhibitory potentials by leakage of Cl and are more effective for injecting depolarizing currents.

To activate touch cells, natural stimuli were applied by indenting the skin of the receptive field of the cell with a stylus driven by a piezo-electric element. Pressing on the skin or squeezing it with forceps also makes the touch cells fire and in addition recruits the cells responding to pressure or to noxious stimuli, respectively (Nicholls & Baylor, 1968). Contractions of the longitudinal musculature were recorded by a strain gauge. Many of the experiments required simultaneous recording from both sensory and motor cells; in order to see the large motoneurones ganglia were generally mounted with the dorsal surface uppermost. From this side, however, one can see directly only the most laterally located of the sensory cells responding to touch, pressure, and noxious stimuli (Fig. 1). Consequently, these three cells were used for most of the experiments in which a sensory cell and a motoneurone were impaled simultaneously. To study the reflex connexions of the other more medially situated sensory cells (two touch cells, one pressure cell, and one cell responding to noxious stimuli on each side of the ganglion) the ganglion was turned so that either its anterior or posterior surface was uppermost. In these positions it is possible with experience to impale the remaining sensory cells together with the large longitudinal motoneurones.

Solutions. Leech Ringer solution with the following composition was used for most of the experiments (mM): NaCl, 115; KCl, 4·0; $CaCl_2$, 1·8; Tris-maleate (neutralized with NaOH) 10; glucose, 11. For some experiments solutions containing increased concentrations of Ca or Mg (10–20 mM) with an appropriate reduction of Na concentration were used. A high concentration of Ca increases the membrane resistance of leech neurones; it also raises the threshold and thereby reduces 'spontaneous' synaptic activity. Mg was used to block chemical synaptic transmission.

To study the effects of different ions a continuous flow system with a tap was used so that the composition of the bath could be changed without dislodging the intracellular electrodes.

Histological techniques. The morphology of individual cells was studied by injecting them with a fluorescent dye, Procion yellow M-4RS (Stretton & Kravitz, 1968). Glass micro-electrodes were filled with distilled water by boiling, and then refilled with an aqueous solution of the dye (4–10 g/100 ml.). This was allowed to diffuse to the tip for a period of 3–4 days while the electrodes were stored at 4° C. The resistances ranged from 150 to 500 MΩ. After an individual cell had been penetrated and identified unambiguously by its electrical properties, hyperpolarizing pulses of 0·5 sec duration and about 10^{-8} A were applied to the electrode at 1/sec, carrying the negatively charged dye molecules into the cell. A yellow colour appeared within 1–5 min if the penetration and the electrode were satisfactory; the injection was continued until the colour became moderate to intense (5 min–1 hr). The ganglion was usually fixed after keeping it from 1 to 4 hr at room temperature to allow diffusion to occur throughout the neurone. Additional diffusion times of up to 48 hr at 4° C failed to show a more extensive branching pattern in the neuropile. Ganglia were fixed for 1 hr in 1% glutaraldehyde, 4% paraformaldehyde (buffered at pH 4), dehydrated with methanol, equilibrated with propylene oxide, and embedded in plastic (either Epon 812 resin or Maraglas). The intact ganglion could be photographed on a cover slip in a drop of the embedding medium, using a caesium iodide ultra-violet source with appropriate emitter and barrier filters. After curing, serial 5 or 10 μ sections were cut and photographed. These photographs were used for two-dimensional tracings on paper or three-dimesional reconstructions of the cells on glass plates.

RESULTS

Morphology and physiological properties of sensory and motor nerve cells

Fig. 1 shows examples of the reconstructions of each of the three sensory cells and one of the two motoneurones used for most experiments in this study. The major branching patterns of the axons of these neurones were already known from methylene blue-stained preparations (Retzius, 1891) and electrical mapping (Nicholls & Baylor, 1968; Stuart, 1970); however, the arborizations within the ganglion could be seen in far greater detail after the intracellular injection of Procion yellow. Like the other neurones in the ganglion, they are unipolar and make their central synaptic connexions within a neuropile. The laterally situated touch cell (T) (see also Figs. 5 and 14 in Nicholls & Baylor, 1968) sends a single large process out through the posterior root. High-frequency bursts of impulses (up to 200/sec) are initiated in this cell by a light touch applied to the skin within its receptive field (on the dorsum of the animal). The laterally situated neurones responding to pressure (P), or to noxious mechanical stimulation (N), fire at lower maximum frequencies, adapt slowly, and send axons out through both roots on the same side of the ganglion to innervate ventrolateral skin. In contrast, the large longitudinal motoneurone (L) sends its

process across the ganglion where it divides and enters the two contralateral roots. Branches of this cell supply the longitudinal muscle fibres on one side of the animal from mid line to mid line over a length of slightly more than one segment. A single action potential in this cell can give rise to a contraction (Fig. 5 in Stuart, 1970), but the tension which the muscle actually develops in response to a single efferent impulse is variable. For an

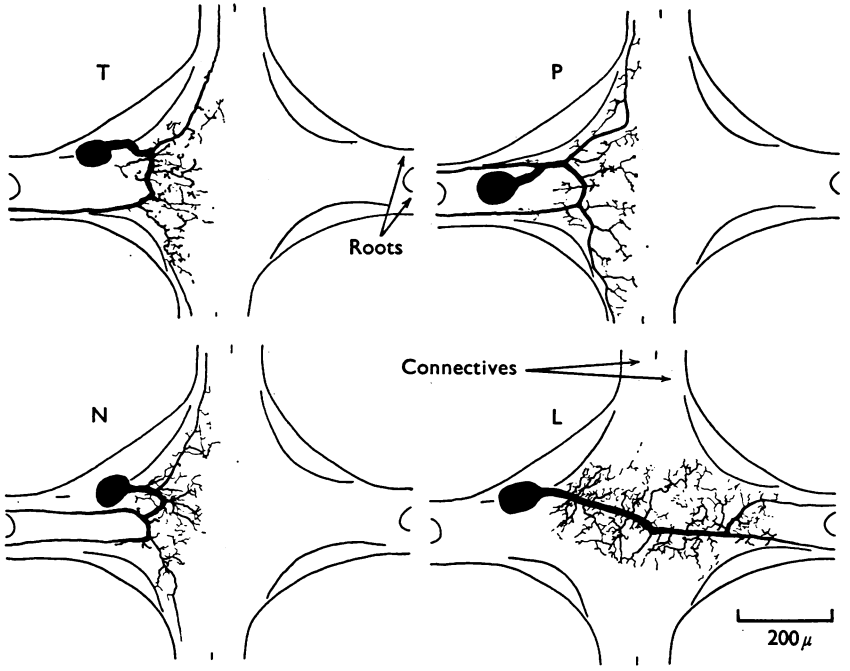

Fig. 1. Drawings to show the characteristic arborizations of the laterally situated sensory cells responding to touch (T), pressure (P), or noxious stimuli (N) and the large motoneurone that innervates longitudinal muscle fibres (L). Photographs were made of serial sections of ganglia in which a single cell had been stained by injecting the fluorescent dye Procion yellow. Reconstructions were made by superimposing tracings of photographs of the serial sections. The sensory cells can be seen to ramify primarily in the ipsilateral neuropile; this does not preclude the possibility of synaptic contacts with the contralateral motoneurone which branches extensively throughout the neuropile. Since the two motoneurones act as a unit, the sites of the junctions cannot be determined (see text). Anterior is above.

individual muscle fibre it depends on the initial length and the level of depolarization produced by the excitatory and inhibitory junctional potentials (EJPs and IJPs). The motoneurone is tightly coupled by a non-rectifying electrical synapse to its contralateral homologue, so that action potentials in the two cells usually occur in synchrony (Stuart, 1970). This

electrical connexion also allows synaptic currents to spread passively between the two neurones (see below). Acting together, the two large longitudinal motoneurones cause a powerful shortening of the segment.

The arborizations of the cells shown in Fig. 1 are so elaborate that we have not been able, at the level of light microscopy, to demonstrate the exact sites of synaptic contact. It is clear, however, that the three sensory cells have many branches in close association with processes of both motoneurones. This has been shown by injecting a sensory cell and either the ipsilateral or contralateral motoneurone in the same ganglion; in such preparations the fine branches of the injected cells are so intertwined that they cannot be distinguished.

As in the lobster, each type of cell has a characteristic 'fingerprint' that is similar in its principal features from ganglion to ganglion in the same and different leeches. The sensory cells always have a profusion of fine branches on the same side of the ganglion as the cell body without obvious extensions across the mid line. These branches presumably make synapses with many cells other than the large longitudinal motoneurones; some connexions, such as those the sensory cells make with their homologues, are already known (Baylor & Nicholls, 1969*c*). Furthermore, there are morphological differences between the three types of sensory cells; for example the P cell shown in Fig. 1 has more widely spaced branches than the T or the N cell, and many of these can be followed to the midline region of the neuropile. In contrast, the large longitudinal motoneurone has branches which ramify throughout most of the neuropile. We are at present studying the extent to which the finer branching pattern of a particular cell varies from ganglion to ganglion.

In the next section experiments are presented which demonstrate sensory-motor connexions, and show that the synaptic mechanism is different for each type of sensory cell.

Synaptic connexions between sensory cells and the large longitudinal motoneurone

Characteristic features of the synaptic potentials. Fig. 2 shows the results of stimulating the sensory cells directly by injecting brief depolarizing pulses while recording from the ipsilateral motoneurone. In each instance a depolarizing, excitatory post-synaptic potential occurred. Potentials of this kind unfailingly followed the presynaptic impulses in the sensory cell; they were never accompanied by hyperpolarizing potentials, even when the recordings were made with electrodes filled with potassium acetate instead of KCl (see Methods). The post-synaptic potential following a single action potential in the P or the N cell almost always exceeded threshold and initiated an impulse in the motoneurone (Fig. 2). The synaptic poten-

tial evoked by a single action potential in the touch cell was often less effective, so that summation of two or more synaptic potentials was necessary for impulse initiation (see for instance Fig. 10*C*). The membrane potential of the motoneurone fluctuated from moment to moment due to continuous bombardment by other 'spontaneous' excitatory and inhibitory inputs; the level of the background activity determined whether or not the motoneurone actually fired.

Fig. 2. Excitatory synaptic potentials in the large longitudinal motoneurone following single impulses in the sensory cells responding to noxious stimuli (N), pressure (P) or touch (T). The upper trace shows the action potential in the sensory cell produced by direct stimulation through the micro-electrode; the middle trace is the intracellular recording from the motoneurone (L). In these records the synaptic potentials reached threshold and caused an impulse. The action potential in the motoneurone is small because it fails to invade the cell body. Lower trace monitors current injected into presynaptic cell.

Smaller depolarizing synaptic potentials with similar properties were also produced in the large longitudinal motoneurone on the other side of the ganglion after each impulse in the three sensory cells. This is expected since the two motoneurones are connected by a non-rectifying electrical synapse and act as a unit. One result of this is that it is not possible by recording synaptic potentials to determine whether a sensory cell makes contact with one or both motoneurones.

Although the three sensory cells were invariably connected to the large longitudinal motoneurones, no synaptic potentials could be recorded in many other cells in the same region of the ganglion following single impulses in the sensory cells (see Discussion).

The following sections provide evidence that the connexions between the three sensory cells and the two motoneurones are monosynaptic and work by different mechanisms.

Chemical and electrical synaptic mechanisms. The mechanisms of synaptic

transmission between the three lateral sensory cells and the large longitudinal motoneurone were investigated by (1) observing the latency of the synaptic potential in the motoneurone; (2) bathing the ganglion in high concentrations of Mg ions, which block chemical synaptic transmission in leech ganglia (Baylor & Nicholls, 1969*c*; Stuart, 1970); and (3) changing the membrane potentials of the pre- and post-synaptic neurones. The results are shown in Figs. 3 to 7.

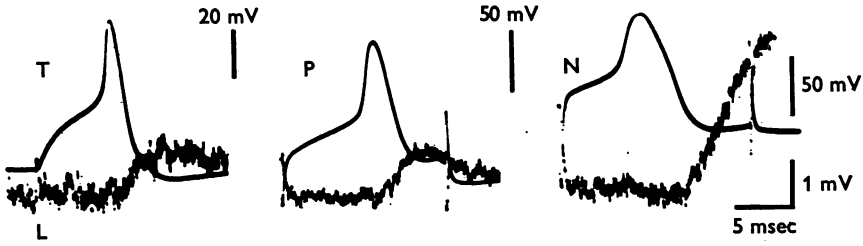

Fig. 3. Intracellular recordings from sensory cells (upper traces) and the large longitudinal motoneurone (lower traces) to show the characteristic latencies of the post-synaptic potentials. There is no appreciable delay between the presynaptic impulses in touch (T) or pressure (P) cells and the onset of the coupling potential recorded in the motoneurone (L); the bathing fluid in these experiments contained 20 mM-Mg to block chemical synapses and reduce the spontaneous bombardment of the motoneurone. The synaptic potential produced by an impulse in the noxious stimulus cell (N) arose only after a latency of about 4 msec. A more accurate measurement of the delay between the arrival of the action potential at the presynaptic nerve terminals and the initiation of the synaptic potential has not been possible because of the distance of the micro-electrodes from the synapse. This experiment was made with the ganglion bathed in Ringer fluid containing 20 mM-Ca. The results suggest that the sensory cells responding to touch and pressure are electrically coupled to the motoneurone.

Depolarizing post-synaptic potential changes in the motoneurone elicited by impulses in the touch cell had the characteristics of coupling potentials mediated by electrical synapses. They arose with no appreciable delay after the action potential (Fig. 3, T) and were unchanged when the Mg concentration in the bathing fluid was increased to 20 mM. Furthermore, current spread directly between the touch cell and the motoneurone (Fig. 4). Unlike the electrical coupling between the two large motoneurones, this junction rectified; no appreciable current spread occurred when the touch cell was hyperpolarized or the motoneurone depolarized. There was no evidence for a chemical component of transmission at this synapse since Mg did not reduce the size or alter the time course of the post-synaptic potential (compare with Fig. 8 in which the synaptic potential evoked by a pressure cell was changed). In one respect,

however, the behaviour resembled that of an excitatory chemical synapse: the size of the coupling potential was increased by hyperpolarizing the motoneurone (Fig. 5), an effect that can probably be explained by the rectifying properties of the junction (Furshpan & Potter, 1959; Auerbach & Bennett, 1969). The functional significance of a junction that allows excitatory potentials to spread in one direction only and inhibitory potentials in the other is considered below.

Synaptic potentials with markedly different properties followed impulses in the N sensory cell. These post-synaptic potentials arose with a delay of 2–4 msec after the peak of the presynaptic action potential (Fig. 3, N) and were reversibly abolished by bathing the preparation in 20 mM-Mg, returning when Ca was added so that its concentration was increased to

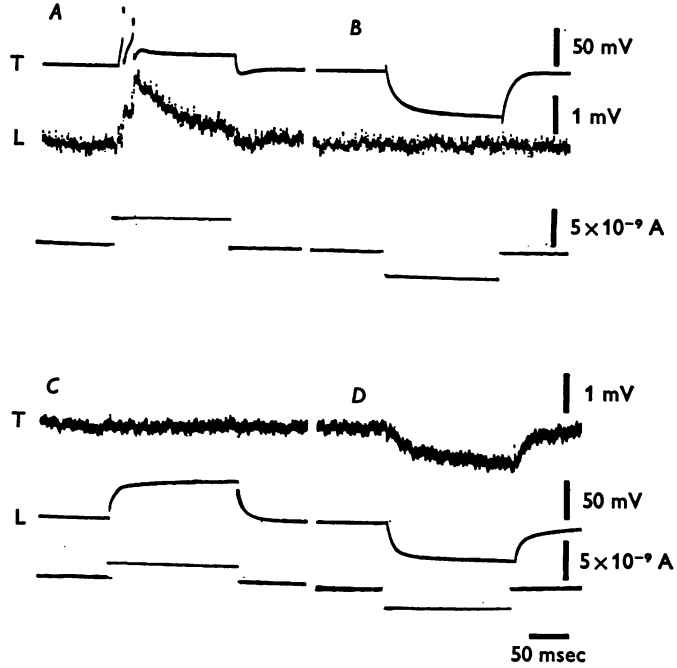

Fig. 4. Intracellular recordings from a touch cell (upper traces) and a large longitudinal motoneurone (middle traces) to demonstrate the presence of a rectifying electrical synapse; currents injected through the micro-electrodes are monitored in the bottom traces. The ganglion was bathed in Ringer fluid containing 20 mM-Mg. *A* and *B* show that depolarizing currents injected into the touch cell (T) spread to the motoneurone (L), while hyperpolarizing currents do not. Note the coupling potentials evoked by each presynaptic impulse. In *C* and *D* depolarizing and hyperpolarizing currents were injected into the motoneurone; only hyperpolarization spread to the sensory cell. At a synapse of this type excitatory influences spread from the sensory to the motor cell, whereas only hyperpolarizing inhibitory potentials can spread in the reverse direction.

15 mM (Fig. 6). The antagonistic action of the two ions is similar to that observed at other chemical synapses where Mg reduces and Ca increases the number of quanta liberated by the presynaptic terminals in response to an impulse (Katz, 1962). The response to changing the potential of the post-synaptic cell was also that expected for a chemical synapse: hyperpolarization of the motoneurone increased the amplitude of the EPSP (excitatory post-synaptic potential), while depolarization reduced it

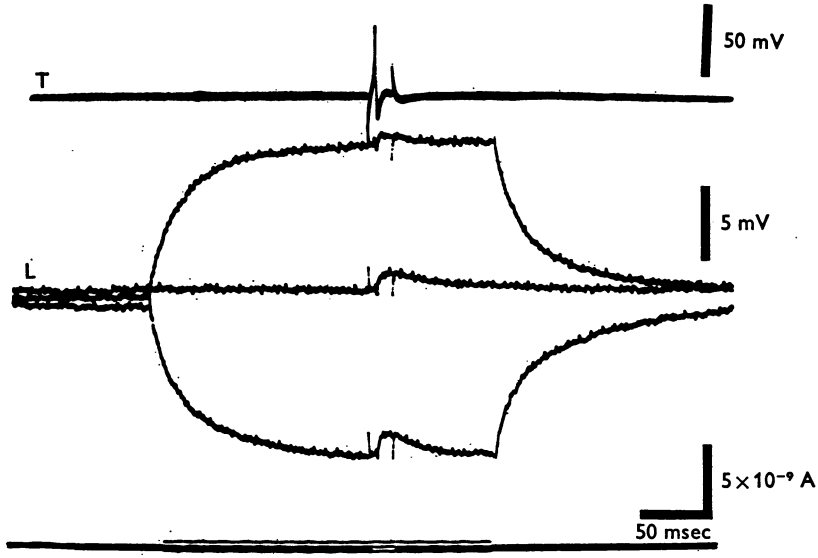

Fig. 5. Effect of varying the membrane potential of the large longitudinal motoneurone (L, middle traces) on the coupling potentials following impulses in the touch cell (T, upper trace). The lower trace is the current monitor. The post-synaptic potential increased in amplitude as the membrane was hyperpolarized, presumably because of the rectifying properties of the junction (see text). Ringer fluid containing 20 mM-Mg was used in this experiment.

(Fig. 7). No current spread could be detected between the N cells and the motoneurone even with large current injections of both polarities.

The third type of sensory neurone, the pressure cell, gave rise to synaptic potentials in the motoneurone with characteristics of both chemical and electrical mechanisms. Thus (1) the synaptic delay was negligible, (2) 20 mM-Mg reversibly reduced the amplitude of the peak without influencing the initial rise (Fig. 8) and (3) current flowed directly between the two cells but, again, in only one direction: depolarization spread from the pressure cell to the motoneurone and hyperpolarization from the motoneurone to the sensory cell. Potential changes in the opposite direction did not cause current flow.

For technical reasons, the results described above were obtained by impaling the laterally situated T, P or N cells in the ganglion (see Methods). In other experiments it was found that the other, more medial, sensory cells also gave rise to distinctive synaptic potentials in the large longitudinal motoneurones. In each case the mechanism was specific for a particular modality: all the touch cells were electrically coupled to this motoneurone by rectifying synapses, the N cells worked by chemical synapses and the P cells by a combination of both mechanisms. The reflex

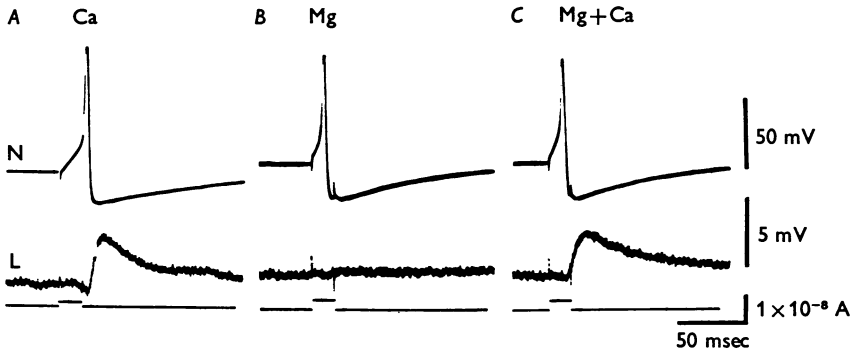

Fig. 6. Antagonistic effects of Mg and Ca on synaptic transmission between the N cells and the large longitudinal motoneurone. The N cell was stimulated to give single impulses (upper traces) by currents injected through the micro-electrode (monitored in bottom traces), while synaptic potentials were recorded intracellularly from the motoneurone (L, middle traces). *A* shows the synaptic potential recorded when Ringer fluid containing no Mg flowed past the ganglion. The Ca concentration was 20 mM to reduce the incidence of spontaneous synaptic potentials on the motoneurone; in this solution the synaptic potentials are often slightly larger than normal. (See Fig. 2 for a record in normal Ringer). *B*, taken 8 min after changing solutions, shows that the synaptic potential was abolished in Ringer fluid containing 18 mM-Mg and 1·8 mM-Ca. Almost complete recovery occurred when the Ca concentration was increased to 15 mM, even though the Mg concentration remained 18 mM (*C*, 3 min after changing fluids). This is the result expected for a chemical synapse.

pathways mediated through electrical synapses can be assumed to be monosynaptic, but it remains to be shown that the chemical synapses between the cells that respond to noxious stimuli or pressure and the motoneurone do not involve an interneurone. This information is essential if an analysis is to be made of the mechanisms involved during facilitation or depression in this reflex pathway.

Evidence for monosynaptic chemical connexions. Chemical synaptic potentials initiated by impulses in N and P cells had the following characteristics, some of which have been described above: (1) the delay was constant (see Fig. 3, 6 and 8), (2) there were no failures (every presynaptic

action potential gave rise to a synaptic potential in the motoneurone), (3) the amplitude of the chemical EPSP was constant ($\pm 20\%$) over long periods provided that the frequency of stimulation was low enough not to give rise to facilitation or depression (D. Purves & J. G. Nicholls, unpublished) and (4) increasing the concentration of Ca reversed the block caused by Mg (Fig. 6) in a continuously graded manner, as one would expect at a monosynaptic chemical synapse. It is unlikely that a high concentration of Ca could act in this way to relieve the transmission block caused by Mg in a polysynaptic pathway.

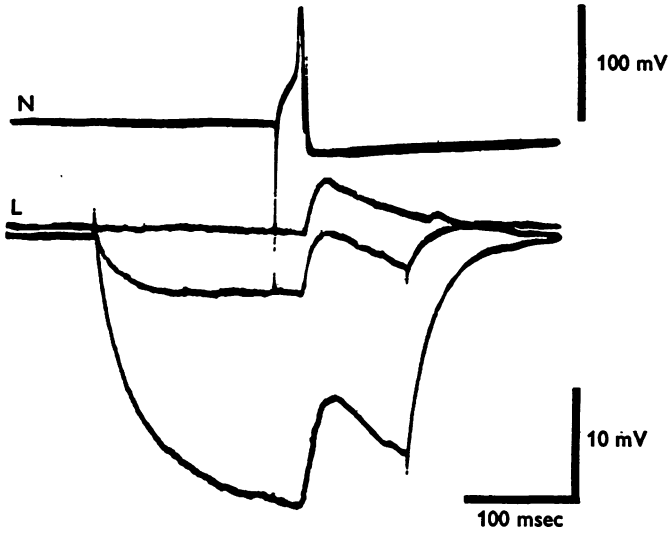

Fig. 7. Effect of hyperpolarization of the longitudinal motoneurone on the synaptic potentials (L, lower traces) evoked by stimulation of an N cell (upper traces). The ganglion was bathed in Ringer solution containing 20 mM-Ca. The current injected to produce maximum hyperpolarization was $1{\cdot}5 \times 10^{-9}$ A.

Fig. 9 provides additional and more direct evidence for a monosynaptic chemical connexion between the N cell and the motoneurone. The principle of this experiment was to observe the effect on the epsp in the motoneurone of shortening the *duration* of the presynaptic action potential by means of a hyperpolarizing current pulse injected through an intracellular electrode. At the frog neuromuscular junction it has been shown that, after the peak of the action potential, the declining depolarization of its falling phase continues to cause transmitter release by the nerve terminals (Katz & Miledi, 1967). In a monosynaptic pathway one might therefore expect a presynaptic action potential of shorter duration to release less transmitter and give rise to smaller and briefer EPSPs. On the

other hand, in a di- or polysynaptic pathway, the EPSP recorded in the motoneurone should be either present or absent depending on whether the interneurone had fired. In Fig. 9 the upper trace shows the presynaptic action potential recorded in the N cell, and the middle trace the EPSP in the motoneurone. The amplitude and the time course of the EPSP were reduced when a large hyperpolarizing current pulse was injected into the sensory cell just before the peak of its action potential (at the arrow in

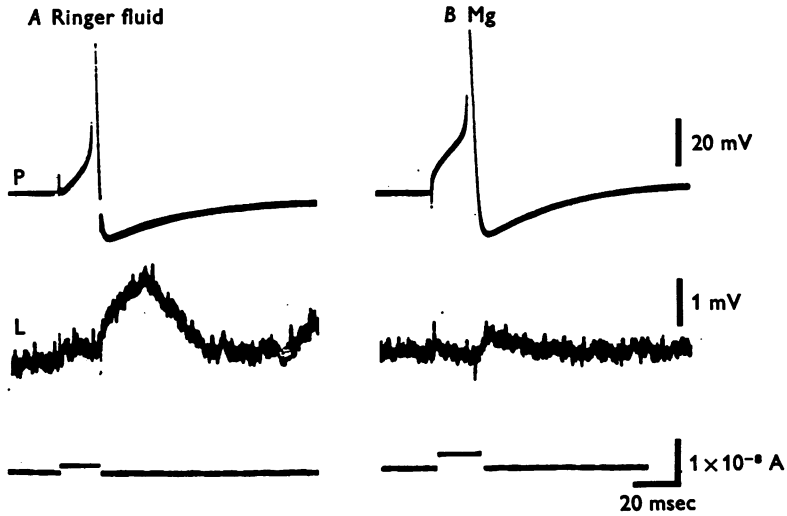

Fig. 8. Effect of increased Mg on transmission between a pressure cell (P, upper trace) and a large longitudinal motoneurone (L, middle trace). Presynaptic action potentials were initiated by stimulating through the micro-electrode (current monitored in bottom trace). In *A* normal Ringer fluid flowed past the ganglion. The records shown in *B* were taken after the Mg concentration had been increased to 20 mM. A small post-synaptic potential consistently appeared in response to stimulation of the P cell: its initial rising phase was unchanged, but a marked reduction occurred in the peak, as if Mg had blocked the chemical but not the electrical component of the synapse. As with the N cell, adding Ca (15 mM) to the solution containing Mg restored the amplitude of the synaptic potential (see Fig. 6). Peaks of the action potentials are cut off.

Fig. 9*B*); varying the instant at which the hyperpolarizing pulse was applied in the action potential cycle led to a continuous gradation of the EPSP from complete abolition to its full amplitude. This experiment suggests that the pathway does not involve an interneurone. It is possible that the effect of the hyperpolarizing current pulse is to impair conduction into the fine presynaptic terminals of the N cell (rather than to reduce transmitter release) but, in this case too, the simplest interpretation would be that the pathway is monosynaptic.

Reflex contractions of longitudinal muscle

The experiments reported above show that sensory cells of three different modalities make specific and direct excitatory connexions on one (or both) large longitudinal motoneurones. It is known that each impulse in this motoneurone gives rise to an EJP in a large population of longitudinal muscle fibres distributed over the contralateral side of the segment (Stuart, 1970). A single impulse, under appropriate initial conditions, can cause a marked shortening of the body wall although the actual degree of tension attained is variable. Accordingly, it was expected that natural stimuli

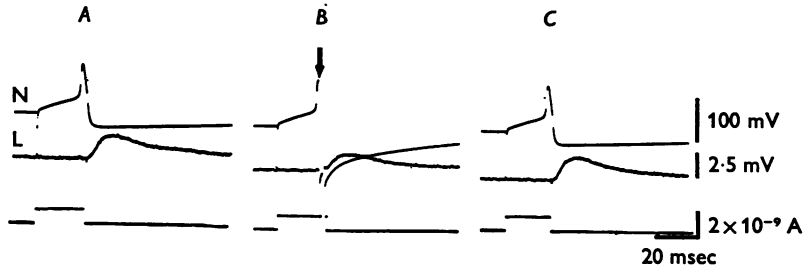

Fig. 9. Demonstration of the monosynaptic connexion between the N cell and the large motoneurone (L) by curtailment of presynaptic action potentials. *A* is a control record in which the upper trace is an intracellular recording from an N cell showing the characteristic action potential elicited by injecting a brief pulse of depolarizing current; the middle trace shows the post-synaptic potential produced in the motor cell, and the lower trace is the current monitor. In *B* the presynaptic spike was curtailed by injecting a strong hyperpolarizing pulse of approximately 10^{-7} A and 3 msec duration at the peak of the action potential (arrow). (The peak of this hyperpolarizing current pulse was not photographed in the monitor trace since the beam was displaced beyond the screen). Note the reduction in the amplitude and duration of the post-synaptic response. Varying the instant at which the action potential was interrupted produced a continuous gradation of the post-synaptic potential recorded in the motoneurone. Record *C* was taken a few sec later and shows complete recovery. The bathing solution contained 20 mM-Ca.

applied to the skin would cause contractions of the segment through these pathways. Nevertheless a number of questions arise concerning the operation of these reflexes in the intact segment. For example, in isolated ganglia a single impulse initiated by direct stimulation of the cell body of a sensory neurone did not always give rise to an efferent impulse in the motoneurone. With the ganglion still connected to the body wall, central and peripheral inhibitory influences might prevent impulses or contractions from occurring. Furthermore, the depolarizing potentials elicited by sensory stimuli might only have stimulated the motoneurone because its membrane

potential had been reduced by penetration with a micro-electrode. For these reasons, experiments were made to determine whether reflex contractions of longitudinal muscle could be evoked by sensory impulses when ganglia were still attached to a portion of the body wall. Some of these tests were made by applying natural stimuli to the skin without impaling the motoneurone until the end of the experiment.

Reflex contractions elicited by touch. The simplest reflex to investigate was that initiated by light touch, because this stimulus can be used to activate a single touch cell without simultaneously causing a discharge in the other touch cells (which have different receptive fields), or in the P and N cells (which have higher thresholds). The experimental arrangement is shown in Fig. 10. The preparation consisted of a length of body wall still connected to a ganglion by the nerve that supplies its dorsal part (see Figs. 1 and 14, Nicholls & Baylor, 1968). The rest of the skin and muscle were denervated by cutting the main anterior and posterior nerve roots (Fig. 10). This dissection preserves the receptive field of the most laterally situated of the three touch cells in the ganglion (the one used in earlier experiments, see Fig. 1), while cutting the peripheral processes of the other two. Sensory and motor impulses travelling in the nerve to and from the ganglion were monitored by external electrodes, and the tension generated by the longitudinal muscle was recorded with the strain gauge. Fig. 10 *A* shows the characteristic, unitary afferent action potential recorded from the nerve branch in response to a touch applied to the dorsal skin. Such impulses were initiated in the peripheral processes of the lateral touch neurone and propagated into its cell body within the ganglion where they could be recorded intracellularly. A single sensory impulse usually caused little or no tension increase in the longitudinal muscle fibres. Light repetitive indentations of the dorsal skin gave rise to bursts of impulses in the lateral touch cell that consistently caused a reflex contraction (Fig. 10*B*). In control experiments the same stimulus applied outside the receptive field of the lateral touch cell failed to cause sensory impulses or contractions (since these areas were denervated).

In recordings such as the one shown in Fig. 10*B* the reflex contraction evoked by the sensory stimulus was immediately preceded by one or more smaller, distinctive, unitary action potentials of opposite polarity in the nerve, travelling outwards from the ganglion towards the muscle (arrows). The efferent action potentials were subsequently shown to originate in the large longitudinal motoneurone by impaling its cell body with a micro-electrode (Fig. 10*C*); the characteristic, small impulse in the root was then seen to be preceded by an action potential in the cell body. When the motoneurone was hyperpolarized with currents passed through the micro-electrode, the synaptic potentials failed to reach threshold, and the

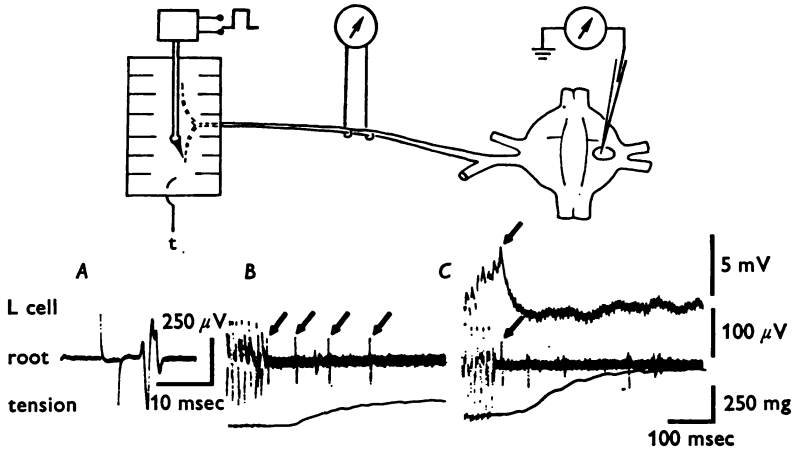

Fig. 10. Demonstration of the shortening reflex elicited by natural stimuli applied to the skin. The experimental arrangement is shown in the diagram. A portion of skin from the dorsum of the animal was touched with a stylus driven by a stimulator through a piezo-electric element. The tension in the underlying longitudinal muscle was monitored with a strain gauge (t) attached to the body wall by a fine thread. External electrodes on the nerve supplying dorsal body wall monitored the afferent sensory discharge and the efferent motor impulses. The micro-electrode shown on the right over the ganglion could be introduced into the large longitudinal motoneurone to establish the origin of the action potentials recorded in the root and to show that the reflex arc could be broken by injecting hyperpolarizing currents into the cell. Record *A* shows the unitary centripetal action potential in a touch axon, recorded extracellularly from the root at a fast sweep speed and low gain, in response to a single indentation of the skin. Note the artifacts due to current flow through the mechanical stimulator. In *B* the upper trace is a similar record from the nerve at a slower sweep and higher gain. A burst of sensory impulses (80/sec) was initiated by touching the skin repetitively: at this gain and sweep speed their peaks are not photographed and they tend to merge with the artifacts. This sensory discharge was followed by unitary efferent impulses (middle trace-arrows) and an increase in tension (lower trace). When the large longitudinal motoneurone was impaled by a micro-electrode (upper trace, *C*) it could be seen that synaptic potentials evoked by touch summed to produce an action potential (arrow) which gave rise to the characteristic unitary potential in the root (middle trace, arrow), and an increase in tension (lower trace). In the experiment illustrated in *C*, a small maintained hyperpolarizing current (not shown) was injected into the motor cell to reduce its 'spontaneous' firing. No increase in tension occurred if the synaptic potentials failed to reach threshold. Considerable variations occur in the amount of tension generated by leech longitudinal muscles in response to a single motor impulse (see text). Occasional action potentials from some other cell can be seen in the root recording.

longitudinal muscle relaxed (Stuart, 1970). These experiments indicate that sensory impulses in a single touch cell, initiated by natural stimuli, give rise to synaptic potentials in the large longitudinal motoneurones; these can reach threshold and cause action potentials that in turn lead to contractions in longitudinal muscle fibres. In several experiments, EJPs instead of tension were recorded intracellularly from longitudinal muscle fibres. The results were similar; usually a burst of two or three sensory impulses was required for an action potential in the motoneurone and an EJP in the longitudinal muscle fibre.

So far, the experiments described on touch neurones have been concerned mainly with the laterally situated cell that is visible when the ganglion is displayed with the dorsal surface uppermost. There are, however, two other, more medial touch cells on each side of the ganglion that also make rectifying electrical synapses on the motoneurone; their processes pass out through both the anterior and posterior roots, but not through the dorsal nerve branch. One supplies ventral and the other lateral skin. Tests were made to determine whether similar shortening reflexes would be initiated by stroking the skin of their receptive fields. The experimental procedure was similar to that illustrated in Fig. 10, except that the ganglion was mounted so that the medial cells could be impaled and the dorsal nerve was cut (denervating the dorsal skin and muscle), while the anterior and posterior nerve roots were left intact. Now stroking dorsal skin was without effect, but light touches applied to the lateral or the ventral fields gave rise to unitary impulses that propagated towards the ganglion and invaded one or the other of the medially situated touch cells. Once again, a short afferent burst was followed first by an efferent discharge, and then by EJPs and contractions of the longitudinal muscle. The efferent impulses were subsequently shown to originate in the large longitudinal motoneurone by recording from its cell body. In other experiments, brief trains of impulses were initiated by intracellular stimulation in each of the three touch cells on the opposite side of the ganglion. These impulses also caused excitation of the longitudinal motoneurone and contractions, as would be expected. We conclude that impulses in any of the six touch cells in a segmental ganglion can give rise to a bilateral reflex shortening by way of the large dorsal motoneurones. However, we cannot at present say whether some touch cells are more effective than others.

Reflex contractions elicited by impulses in sensory cells responding to pressure or noxious stimuli. Experiments designed to study reflexes initiated by the N and P cells were similar to those described above for touch cells. Strong contractions of the longitudinal muscle fibres occurred after an area of skin was pressed to activate the P cell or squeezed to recruit the N cell

in addition. However, as both types of stimuli inevitably cause a brief discharge in the touch cells innervating the same field, the results do not, on their own, demonstrate the role of P or N cells in initiating reflex contractions. Other experiments were therefore made in which individual P or N cells were stimulated with intracellular micro-electrodes. Under these conditions, a single impulse in any one of the P or N cells consistently gave rise to an EJP and a reflex contraction of the longitudinal muscle fibres. The large longitudinal motoneurone was shown to be responsible for the contractions as before, by recording its efferent action potentials in the root and subsequently impaling the cell body. When hyperpolarizing currents were injected into the cell body the synaptic potentials failed to reach threshold and the reflex contraction was abolished. In some experiments impulses in sensory cells still caused a smaller reflex contraction by way of other motoneurones when the large longitudinal cell was not active.

DISCUSSION

Specificity of connexions between sensory and motor cells

Leech neurones have morphological and physiological properties that can be considered 'specific' in that they are characteristic for cells of known function and invariant from animal to animal. In a sensory cell, for example, these properties include the shape and position of its cell body, the distribution of its processes, its modality and receptive field, and the electrical characteristics of its membranes. An individual sensory nerve cell of a given modality also exhibits specificity with respect to its synaptic connexions: intracellular recordings show that it makes synapses on some cells but not others, and that the mechanism of transmission is characteristic for a particular junction (i.e. excitatory or inhibitory; chemical, electrical, or a combination of both). It is possible that a greater degree of specificity also exists in the topography of individual synapses, as in the vertebrate C.N.S., where certain inputs end on characteristic regions of the post-synaptic cell.

Problems of this type could not be resolved by the technique of intracellular dye injection and light microscopy. For example, the anatomical evidence cannot, on its own, demonstrate that the connexions between sensory cells and motoneurones are monosynaptic. Neither do the injected cells show exactly where the synapses occur; thus we do not know whether the sensory cells are connected to the ipsilateral, the contralateral, or to both of the motoneurones. In spite of these limitations, the anatomical reconstructions do provide evidence about the distribution of the synapses. They suggest that the chemical and the electrical connexions of sensory and motor cells occur between multiple small processes rather than at a

single large junction; thus, a considerable portion of the motoneurone membrane within the neuropile must have been depolarized by the action of numerous presynaptic terminals for depolarizing potentials of several millivolts to be recorded in the cell body. The histological studies also show that the fine branches of an individual cell are distributed in a characteristic pattern to definite regions of the neuropile. Although we have not yet studied this 'fingerprint' in detail, the results emphasize that the neuropile, though complex, is highly organized. This is consistent with the electrical recordings which showed that the three types of sensory cell evoked distinctive synaptic potentials in the large longitudinal motoneurone, but not in numerous other cells in its vicinity.

Electron microscopic studies of the neuropile of leech ganglia (Coggeshall & Fawcett, 1964; Gray & Guillery, 1963) indicate that there are many junctions with the appearance of chemical synapses which are beyond the resolving power of the light microscope. It is likely then that reconstructions of individual cells, such as those shown in Fig. 1, represent an oversimplification of the actual arborization. The morphological counterparts of electrical synapses (gap and tight junctions) have not yet been observed.

Possible functions of electrical synapses in the shortening reflexes

Electrical synapses in leech ganglia perform different roles in relation to signalling. For example, in the shortening reflex initiated by touch there are three different types of electrical synapse. The simplest of these to consider from a functional viewpoint is the non-rectifying junction between the two longitudinal motoneurones that allows signals to spread with little attenuation (see Fig. 7 in Stuart, 1970). A change in the membrane potential in one cell always influences its homologue, so that both neurones tend to fire synchronously. This synapse therefore serves to ensure that the shortening of the two sides of the animal is symmetrical.

The electrical junction between the touch cell and the motoneurone has a different function. The rectification at this synapse allows excitation to spread in only one direction, as at a chemical synapse, from the sensory cell to the motoneurone. In addition, however, it presumably makes it possible for hyperpolarizing inhibitory potentials to spread in the opposite direction, from the motoneurone to the presynaptic terminals. This in turn could influence the integrative activity of the sensory cell by changing the amplitude of post-synaptic potentials arising in its processes (see Baylor & Nicholls, 1969*c*). Another possible function for this electrical synapse could be to increase the speed of the reflex by eliminating the delay of 2–4 msec that occurs at chemical synapses in this ganglion. From our experiments this seems unlikely, since a single impulse in a touch cell was usually inadequate to excite the motoneurone; instead, two or three synaptic

potentials had to sum, introducing a prolonged and variable delay. Alternatively, the electrical characteristics of this synapse might have a function related to the after-effects of repetitive stimulation. A prolonged hyperpolarization occurs in touch cells after bursts of impulses initiated by natural stimuli. The rectification would prevent this increase in membrane potential from influencing the motoneurone.

A third electrical synapse in the reflex pathway occurs at an even earlier stage, between the six touch sensory cells in a ganglion. These junctions rectify in a peculiar manner: a depolarization can spread in both directions (i.e. between any two touch cells), but a hyperpolarization cannot spread in either. We have no indication of how these synapses influence signalling by the sensory cells (see Baylor & Nicholls, 1969*c*, for a discussion).

A striking feature of these three different types of electrical synapse is that the type of rectification at each corresponds to the rectifying properties of the non-junctional membranes of the cells involved. Thus the motoneurones are coupled by a non-rectifying synapse and their cell body membranes do not rectify (Fig. 7, Stuart, 1970). Similarly, the rectifying properties of the junctions made by touch cells are consistent with the rectification of the cell body membranes: when two cells whose membranes rectify come into contact, as at sensory–sensory junctions, double rectification occurs (i.e. hyperpolarizing currents spread in neither direction). In contrast, when the same sensory cell makes contact with the motoneurone, only single rectification occurs.

Shortening reflexes

The excitatory connexions that have been traced from the sensory to the motor cells produced the effects that one would expect: touching, pressing or pinching the skin of an isolated segment of the animal consistently gave rise to a contraction of the longitudinal muscle. The position of the stimulus on the body surface was not critical, provided that it initiated a sensory discharge in one or more of the known mechanoreceptor cells in the ganglion. These results show that any one of the fourteen sensory cells can cause excitation in the large longitudinal motoneurones. Individual cells might, however, vary in their effectiveness. Such differences, if they exist, would be difficult to detect because the tension generated by these muscles in response to one or two impulses in the large longitudinal motoneurone is variable, depending on a number of factors we could not control (e.g. the 'spontaneous' bombardment by other motoneurones and the initial tension of the muscle).

In the intact animal, mechanical stimuli also produce a localized shortening, which can be superseded by more complex behaviour such as writhing or swimming. In recent experiments we have found that the sensory cells

also make characteristic excitatory connexions with the large longitudinal motoneurones in adjacent ganglia. These connexions form the basis, in part, for reflexes involving more than one segment (D. Purves and J. G. Nicholls, unpublished).

Naturally occurring stimuli in the normal environment of the leech produce bursts of impulses rather than single action potentials in sensory neurones. Experiments are now in progress to study the effects of trains of presynaptic impulses on chemical and electrical synapses in these monosynaptic reflex pathways.

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REFERENCES

AUERBACH, A. A. & BENNETT, M. V. L. (1969). A rectifying electrotonic synapse in the central nervous system of a vertebrate. *J. gen. Physiol.* **53**, 183–237.

BAYLOR, D. A. & NICHOLLS, J. G. (1969*a*). Changes in extracellular potassium concentration produced by neuronal activity in the central nervous system of the leech. *J. Physiol.* **203**, 555–569.

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

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

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

FURSHPAN, E. J. & POTTER, D. D. (1959). Transmission at the giant motor synapses of the crayfish. *J. Physiol.* **145**, 289–325.

GRAY, E. G. & GUILLERY, R. W. (1963). An electron microscopical study of the ventral nerve cord of the leech. *Z. Zellforsch. mikrosk. Anat.* **60**, 826–849.

KATZ, B. (1962). The transmission of impulses from nerve to muscle, and the subcellular unit of synaptic action. *Proc. R. Soc.* B **155**, 455–477.

KATZ, B. & MILEDI, R. (1967). Modification of transmitter release by electrical interference with motor nerve endings. *Proc. R. Soc.* B **167**, 1–7.

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

RETZIUS, G. (1891). *Biologische Untersuchungen, Neue Folge II*. Stockholm: Sampson and Wallin.

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

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