CONDUCTION BLOCK SILENCES PARTS OF A CHEMICAL SYNAPSE IN THE LEECH CENTRAL NERVOUS SYSTEM

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SUMMARY

1. The pressure (P) sensory neurones innvervating the ventral skin of the medicinal leech have receptive fields comprising a central region of skin innervated by two thicker axons and two neighbouring regions innervated by two thinner axons. Impulses originating in the thinner axons may fail to propagate through the central ganglion, apparently blocked at the branch point of large and small axons.

2. The P neurone excites the longitudinal (L) motoneurone, and blocked impulses originating in the anterior fine axon produce e.p.s.p.s that are less than one-half normal amplitude. Blocked impulses in the posterior fine axon are typically ineffective.

3. The branches of P and L neurones, marked with intracellularly injected horseradish peroxidase or with Lucifer Yellow, make synaptic contact at up to sixty-six sites within the neuropile. Of P neurone branches emerging from the two fine axons, those from the posterior axon make fewer contacts, usually one or two at most, while branches from the anterior axon represent no more than half the total contacts. From cell to cell there is some variation in the total number of contacts, the distribution of branches, and the strength of transmission.

4. The locations of contacts measured morphologically correlate well with their distributions as predicted from reductions in e.p.s.p. amplitude during conduction block.

INTRODUCTION

An important feature of many synapses is that the strength of transmission changes with activity. Those changes that last only seconds or minutes are usually considered short term and can involve presynaptic release. Classic examples are facilitation, post-tetanic potentiation and depression, in which successive impulses release more transmitter or less transmitter in an activity-dependent fashion (del Castillo & Katz,

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1954; Hubbard, 1974). Another short-term change, which like the others was first understood at the neuromuscular junction, results from the failure of nerve impulses during repetitive firing to invade some of the neurone's terminal branches, thereby blocking release and reducing the size of the motor unit (Krnjević & Miledi, 1959). The phenomenon can be accounted for in terms of the branched structure of the neurone, the increased electrical load at some branch points, and the neurone's distributed contacts upon the muscle cell; it has now been observed for motor axons in various animals (Bittner, 1968; Parnas, 1972; Hatt & Smith, 1976). Branch-point failure can apparently also modulate chemical transmission between neurones, but a direct demonstration has been difficult (Edwards, Redman & Walmsley, 1976; Jack, Redman, & Wong, 1981; Lüscher, Ruenzel & Henneman, 1983a,b).

Conduction block occurs in active sensory neurones in the leech at central branch points, where fine axons from the periphery meet thicker axons (Yau, 1976). Activity hyperpolarizes the cell, depending upon the type of neurone, either by activation of an electrogenic sodium pump or by a calcium-dependent increase in potassium conductance or both (Baylor & Nicholls, 1969; Jansen & Nicholls, 1973; Van Essen, 1973), making it more difficult for the finer axons to excite the thicker (Parnas & Segev, 1979). The resulting conduction block prevents impulses from reaching those synaptic terminals beyond the blocked branch point, even within the ganglion (Grossman, Parnas, & Spira, 1979; Muller & Scott, 1981). Thus, the geometrical pattern of contacts between a given sensory cell and its synaptic partners could in principle critically determine the effectiveness of transmission during conduction block. Consequently, block could provide a means for examining transmission at selected groups of contacts. In fact, electrical synaptic potentials that the touch sensory neurones elicit in certain interneurones are reduced in amplitude during block (Muller & Scott, 1981). In addition the chemical synaptic potential that another mechanosensory neurone, the pressure or P cell, evokes in the longitudinal (L) motoneurone may be eliminated when conduction along one particular axon is blocked (Yau, 1975).

The present experiments were performed on the P and L neurones in the leech to test whether conduction block can under normal conditions reduce the strength of the synapse between two neurones by activating only a circumscribed subset of contacts. The P sensory cell was stimulated focally at the skin and the contacts between sensory and motor cells examined with electrophysiological recording, light microscopy and electron microscopy, permitting the identification of monosynaptic connexions between individual cells. The results (1) confirm Yau's (1975) finding that synaptic potentials can be eliminated with conduction block and (2) they extend to central chemical synapses the observation that branch-point failure can modulate transmission without entirely eliminating it. The reductions in synaptic transmission are accounted for by the geometry of cell–cell contacts.

<div align="center">METHODS</div>

Animals. Leeches *Hirudo medicinalis* were obtained from suppliers in France (Ricarimpex, 33980 Audenge, Bordeaux) and, for a few early experiments, in the F.R.G. (Blutegel Import und Versand, Röntgenstrasse, Recklinghausen-Sud), and maintained at 15 °C in artificial spring water (0·5 g solid

Forty Fathoms artificial sea water (Marine Enterprises, Towson, MD, U.S.A.) per litre of water; Muller & Scott, 1979).

Physiology. Chains of ganglia and attached skin were dissected from the animal and pinned to silicone rubber (Sylgard 184, Dow-Corning) in dishes containing saline of the following composition (mM): NaCl, 115; KCl, 4; $CaCl_2$, 1·8; and Tris maleate, 10, pH 7·4. In some cases, to enhance the magnitude of the e.p.s.p.s, the saline used for electrophysiological recording contained 8 mM-$CaCl_2$, replacing sodium mole for mole with calcium. This was done for the first six cell pairs in Table 1, for example. Intracellular recordings were made with conventional techniques (e.g. Appendix C, Muller, Nicholls & Stent, 1981). In brief, thin-walled glass micropipettes containing 4 M-potassium acetate were used; they had resistances of 20–50 MΩ, measured in saline before penetrating neurones. A bridge circuit was used to facilitate recording while stimulating through the same micro-electrode. P cells were also excited at their sensory terminals in the skin with brief (0·5 ms) voltage pulses of various amplitudes (2–15 V) applied through fine polyethylene electrodes filled with physiological saline. Similar electrodes were used for extracellular recording. Signals were recorded on film and with an FM tape recorder having a frequency response to 5 kHz. In many cases, signals were averaged on-line or from tape with a signal averager (Dagan Corp., Minneapolis, MN, U.S.A., or a microcomputer and A/D converter, low-pass filtered at 640 Hz).

Of the two P cells on each side of the ganglion, the one innervating ventral skin (P_v) was for technical reasons easier and more useful to study. To aid the selective stimulation of particular P_v cells, which can be identified by their positions within ganglia (Nicholls & Baylor, 1968), their homologues in ganglia immediately anterior and posterior to the ganglion to be studied were usually destroyed by intracellular injection of protease (Parnas & Bowling, 1977). Additionally, in most experiments, including those in Table 1, the contralateral L motoneurone was killed with protease to ensure that no apparent effects of branch-point failure were mediated by the electrically coupled homologue. These preparations were incubated for several hours or overnight in a modified Leibowitz-15 solution (Ready & Nicholls, 1979). To permit simultaneous access to the skin and dorsum of the central ganglion, and to ensure that only the axons of the P cell's minor receptive fields were stimulated, the segmental nerves containing axons from the major receptive field of the central ganglion typically were severed prior to recording. No physiological differences were seen between cultured and freshly dissected preparations. Not included are the results of more than thirty experiments that were incomplete because of deteriorating recordings that prevented reliable measurement of synaptic potentials during conduction block from either the anterior or posterior minor receptive field after taking recordings from the other. None the less, the partial results were entirely consistent with those obtained with more complete records.

Histology. To prepare tissue for electron microscopy after physiological study, L motoneurones and P sensory cells were injected with horseradish peroxidase (HRP; Sigma, type VI; 2% in 0·2 M-KCl with 0·2% (w/v) Fast Green FCF) under pressure through bevelled micropipettes (Muller & McMahan, 1976; Yau, 1976; Bowling, Nicholls & Parnas, 1978). Tissue was incubated in culture medium for several hours, allowing the enzyme to fill remote processes of the two cells within the ganglion, and then fixed 30 min at room temperature in 1·6% (v/v) glutaraldehyde and 0·8% (w/v) paraformaldehyde in 0·08 M-sodium cacodylate, pH 7·4, containing 5 mM-$CaCl_2$. The reaction of a saturated solution of 3,3'-diaminobenzidine (DAB; Aldrich, technical grade, filtered before use) with a drop or two of 1% (v/v) H_2O_2 was followed visually (Muller & McMahan, 1976).

The tissue was then post-fixed in 1% (w/v) OsO_4 in 0·1 M-sodium cacodylate, pH 7·4, for 2 h at room temperature, stained *en bloc* with ice-cold maleate-buffered uranyl acetate, pH 5·2, dehydrated in graded ethanols, and embedded in Epon 812. In order to see stained processes, the osmicated ganglia were first sectioned for light microscopy. Sections 8 μm thick were cut with a diamond knife after softening the block surface with a tacking iron and were dried onto a large cover-slip. Groups of thick sections were re-embedded in Epon at 60 °C for a day, with the cover-slip and sections inverted on a Sylgard 184 mould the thickness of a microscope slide. Each section was photographed as a through-focus series viewed with Nomarski interference optics. The embedded tissue was released from the cover-slip by putting the cover-slip's edges into liquid N_2 and prying the plastic loose with a scalpel blade. After computer-assisted analysis of the photographs was performed (Macagno, Levinthal & Sobel, 1979), selected thick sections were re-sectioned, placed on Formvar-coated grids and stained with lead citrate for examination in a JEOL JEM-100S electron microscope equipped with an eucentric goniometer.

When light microscopy was to be used without electron microscopy, only the L motoneurone

was injected with HRP after physiological recording, and a few hours later, after the enzyme had diffused throughout the motoneurone, the P sensory cell was injected with 3 % Lucifer Yellow CH dye (Stewart, 1978) under pressure through a micropipette. The living tissue was incubated in a saturated solution of 3,3'-diaminobenzidine in physiological saline, pH 7, and after 5 min a few drops of 3 % H_2O_2 were added to the solution and the reaction allowed to proceed under microscopic inspection for another 2–5 min (Macagno, Muller, Kristan, DeRiemer, Stewart & Granzow, 1981). Following a rinse in saline and fixation for 1 h with 4 % paraformaldehyde in 0·1 M-phosphate buffer, pH 7·4, ganglia were dehydrated in graded ethanol washes and mounted in methyl salicylate under a cover-slip sealed with clear fingernail polish. The HRP- and Lucifer Yellow-filled cells were examined simultaneously by balancing the intensity of the fluorescent epi-illumination and the transmitted light until both cells were clearly visible. Sites of apparent contact were viewed through a 100 × oil-immersion objective.

RESULTS

The pressure cell's receptive fields and its excitation of the L motoneurone

All three modalities of mechanosensory neurone in leech ganglia, touch (T), pressure (P) and nociceptive (N), innervate ipsilateral patches of skin that extend over their own segment and neighbouring segments and are contiguous (Nicholls & Baylor, 1968; Yau, 1975, 1976; Blackshaw, 1981; Blackshaw, Nicholls & Parnas, 1982). The best studied are the T cells, which can be stimulated by lightly touching the skin. The specific stimulus for the P cell is stronger, pressing on the skin, but to deliver a focal, precisely timed stimulus it is useful to apply an electrical shock to the skin through a suction electrode. Maps of innervated areas of skin (receptive-field maps) are the same whether generated with mechanical or with electrical stimuli.

The four P cells in the ganglion, two on each side, have overlapping receptive fields that each cover more than a quarter of the circumference of the animal in the cell's segment (Nicholls & Baylor, 1968) and the two adjacent segments (Fig. 1). Each cell's receptive field is partitioned into 'subfields', one for each axon leaving the C.N.S. along a segmental nerve root. The P cell's axons that project from the ipsilateral roots of the cell's own ganglion are 2–3 μm in diameter. Thinner axons, 0·4–0·7 μm in diameter, branch from the thicker axons within the ganglion, extend anteriorly and posteriorly down the ipsilateral connectives and project from roots of adjacent ganglia to innervate 'minor' receptive fields. Single impulses generated in any region of the P cell's receptive field will typically propagate through the ganglion, invading the soma, and leave via the cell's other axons that exit from the ganglion.

P cells excite the large longitudinal motoneurones, or L cells, located bilaterally on the dorsum of the ganglion (Stuart, 1970). The motoneurones shorten the longitudinal muscles and are active when the animal shortens, which it does, for example, when it is poked. The P cell excites the L cells, evoking in the soma an approximately 2 mV e.p.s.p. having both chemical and weak electrical components (Nicholls & Purves, 1970). A smaller synaptic potential is recorded in the contralateral L cell than in the ipsilateral L cell. The two L cells are electrically coupled to one another, but by destroying single L cells with protease it has been shown that each independently receives input both from ipsilateral and from contralateral sensory cells (Bowling et al. 1978).

Impulses in the L cell apparently arise in the contralateral segmental nerves, do not invade the ganglion, and are therefore small compared to synaptic potentials

recorded in the cell body. Since the cell fires in response to either an e.p.s.p. or a depolarization of the same size caused by current passed through the recording micro-electrode, the cell body is assumed to be a meaningful site to monitor synaptic activity. This is consistent with the geometry of the L motoneurone, discussed below, which indicates that the cell body lies electrically between ipsilateral synapses and the cell's contralateral axons.
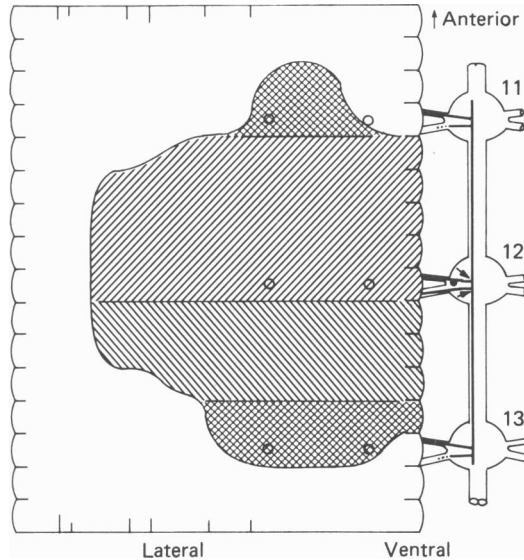

Fig. 1. P sensory neurone receptive field covers portions of three segments. For the ventral pressure cell (P_v) shown here the receptive field consists of a larger region (hatched) in the cell's own segment, the major field, and two smaller patches (cross-hatched) in adjacent segments, the minor fields. The major field is innervated by larger axons, while the minor fields are innervated by finer axons that pass through adjacent ganglia. The major and minor fields are contiguous but do not overlap. Each ganglion contains a bilaterally symmetrical pair of P_v cells, one of which is shown, and a pair of pressure sensory neurones that innervate dorsal skin (P_d cells), which have receptive fields otherwise similar to those of P_v cells. At branch points in the central ganglion (arrows) the fine axon from the minor fields meet the thick axons innervating the major field (see also Pl. 3). Each 1 mm annulus which circumscribes the animal is indicated on the left and right of the skin by a scallop. Five annuli comprise the segment, the centre of which is marked by sensillae, two of which in the ventral skin are indicated with small circles for each of the three segments shown. Marks along top and bottom margins of skin indicate positions of longitudinal bands of pigment. *Ventral* denotes the ventral mid-line, which normally lies beneath the nerve cord; the lateral margin of the skin extends above *lateral*; dorsal mid-line is not shown. Dots extending from axons in ganglia 11 and 13 lie in segmental nerves in which the axons are variably present. Ganglia, normally about ½ mm in diameter, are shown disproportionately large.

The size of the e.p.s.p. evoked in the L motoneurone by an impulse in the P cell is normally independent of where in the P cell the impulse originates, whether arising in the minor receptive fields or the major field upon skin stimulation, or in the cell body by direct stimulation with the intracellular micro-electrode (Yau, 1975; see e.g. Fig. 4). This is, of course, because impulses generated either in the periphery or in

the cell body normally spread to the same extent throughout the cell's processes, as can be shown by suction-electrode recordings from roots and connectives.

The arborizations of P and L neurones and their sites of synapse

The P cell, like other mechanosensory neurones, arborizes primarily in the ipsilateral neuropile of the ganglion but usually sends some branches across the
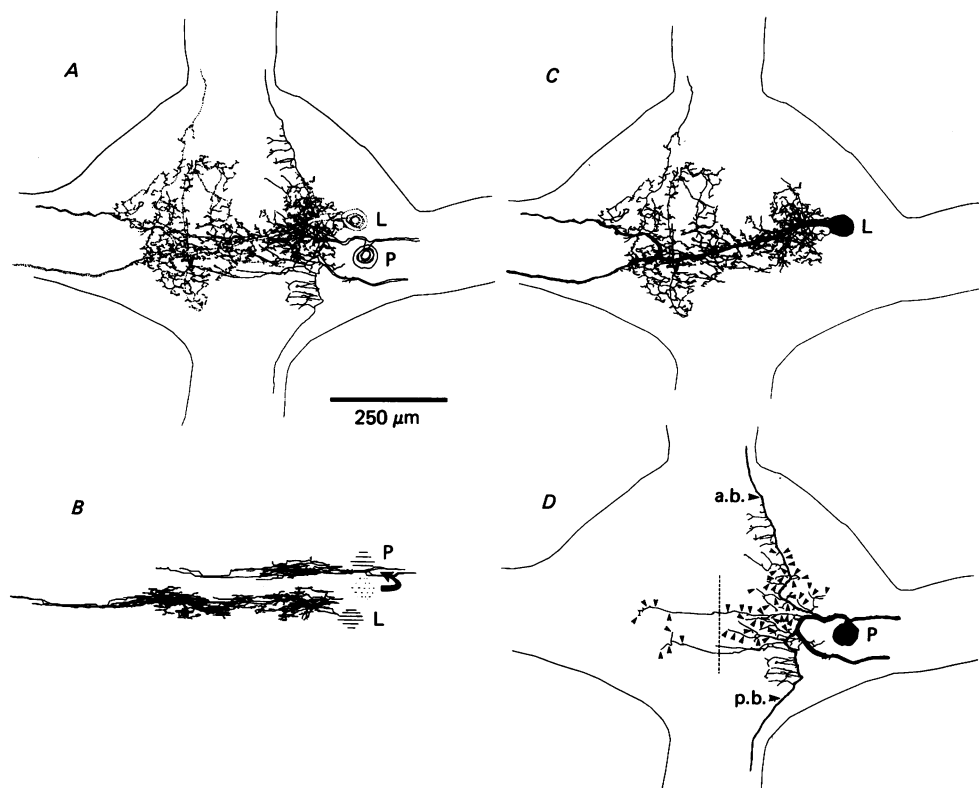

Fig. 2. Computer reconstruction of the P and L neurones from cell pair No. 4 of Table 1. The P cell was injected with Lucifer Yellow and the L cell with horseradish peroxidase. *A* displays both cells and the outline of the ganglion, with the L cell shown in dashed style. The view is on the dorsal aspect of the ganglion; anterior is up. *B* is a view of both cells along the longitudinal axis of the leech; ventral side is up. The P cell has been displaced upward for clarity, as represented by the arrow. The normal position of the P cell body is shown in dashed style. *C* displays the L cell alone. The cell body and primary processes have been darkened to emphasize their diameters. *D* displays the P cell alone, with arrowheads marking sites of putative contact between the P and L cells. The cell body and primary processes were darkened as in *C*. The dashed line indicates the mid line; contact points indicated to its left are considered to be contralateral (see Table 1). a.b., anterior axon branch; p.b., posterior axon branch.

middle of the neuropile to arborize contralaterally (Fig. 2). The cell's main axons exit from the ganglion via the ipsilateral roots, while its smaller axons leave via the ipsilateral connectives and extend through adjacent ganglia, arborizing there on their way to the minor receptive fields in neighbouring segment's skin. Within ganglia, secondary branches emerge from the laterally lying axons like the teeth of a comb,

extending toward the ganglion mid line and sometimes across it. Varicosities along and at the ends of the secondary branches of sensory neurones are sites of synapses; for the P cells the varicosities resemble clustered berries (Muller & McMahan, 1976). The computer reconstructions in Fig. 2 allow one to examine the ganglion from posterior to anterior as well as in the usual ventro-dorsal view. They indicate that the secondary branches arborize principally in a broad layer within the neuropile.
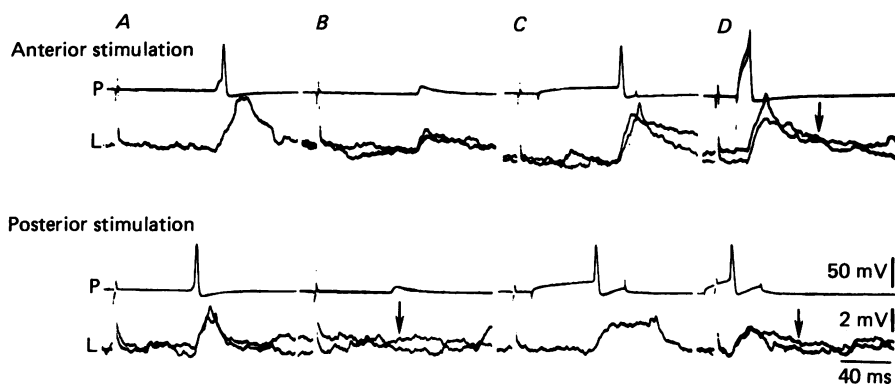

Fig. 3. Conduction block reduces or eliminates synaptic transmission from the P sensory neurone to the L motoneurone. Stimulating the anterior minor field (top pairs of traces) or posterior minor field (bottom pairs of traces) evoked impulses which soon blocked at a frequency of 2 Hz. Unblocked and nearly blocked impulses (*A*) evoked synaptic potentials in the L cell. (These were sometimes suprathreshold.) Only for anteriorly generated impulses was there a residual synaptic potential during conduction block (*B*). However, depolarization of the P cell with a current pulse that was by itself subthreshold relieved the block and restored the synaptic potential to its full size (*C*). A collision (*D*) between an impulse generated in the cell body and the impulse generated in the periphery blocked the synaptic potential that accompanied the impulse invading from the periphery. Arrows indicate the timing of synaptic potentials had there been no collision (*D*) or during block (*B*). Stimulation of the skin, marked by a biphasic artifact in the sensory neurone traces and coincident artifact in the motoneurone traces, occurred approximately 20 ms after the beginning of each sweep. In some records two sweeps are superimposed. Preparation was bathed in saline containing 1.8 mM-$CaCl_2$. Resting potentials of P and L cells were -53 and -47 mV respectively. Same preparation as shown in Pls. 1 and 2.

These regions are also occupied by the other sensory neurones, suggesting the formation of a 'sensory neuropile'.

In contrast to the P neurone, the L motoneurone axon crosses the ganglion, bifurcates, and exits within the contralateral roots. The motoneurone extends highly branched dendrites away from its axon in many directions within the neuropile, and consequently looks bushy (Fig. 2*C*). The L cell does not extend axons into adjacent ganglia, although one fine process typically approaches the contralateral anterior connective. No presynaptic varicosities are evident within the neuropile, thus the L cell has been considered to be largely post-synaptic within the ganglion (Muller & McMahan, 1976), and the present findings confirm this. This contrasts with some other motoneurones in the ganglion (Granzow, Friesen & Kristan, 1985). It is likely, however, that the L cell makes excitatory synapses on the longitudinal musculature of the nerve cord, just as it does upon body-wall muscle, for bilateral contractions

of the neighbouring connectives occur with each impulse in the L cell (E. R. Macagno, K. J. Muller & R. M. Pitman, unpublished observations), and synapses are made on the muscle fibres near the ganglion's dorsal surface (Tulsi & Coggeshall, 1971). Computer reconstruction and image rotation to a posterio-anterior view (Fig. 2) show that the dendritic arbor is flattened dorsoventrally as it crosses the neuropile, intersecting regions occupied by the P cell processes.

Electron microscopy was used to identify synapses between single P and L neurones that had been studied physiologically and then injected with HRP as an electron-dense tracer. In principle, the differences in ultrastructure of the P and L neurones would permit *a priori* the identification of the P cell as the presynaptic element and L cells as post-synaptic, even within a few sections. But to be certain of the cellular identities, 8 μm thick serial sections were cut through ganglia (Pl. 1) and individual branches were traced to their cell bodies. Likely sites of contact were identified for subsequent re-sectioning and viewing in the electron microscope. Consistent with previous results (Muller & McMahan, 1976), the L cell was always post-synaptic, and the P cell was presynaptic and never post-synaptic to itself. The five contacts identified in the light microscope between P and L cells in two preparations that were re-examined after re-sectioning were indeed chemical synapses (Pl. 2). The synapses between the P and L neurones were typical of those previously reported for the presynaptic varicosities of the P neurone, and those made upon the L motoneurone were characteristic of others made upon it and other motoneurones by sensory cells and by unidentified presynaptic cells (Purves & McMahan, 1972; Muller & McMahan, 1976).

For electron microscopy the doses of HRP injected were critical and the preparation and analysis of tissue laborious, therefore light microscopy of whole ganglia was preferable once it had been established that apparent contacts viewed through the light microscope were probably synapses. To localize with assurance the sites of contact it was useful to label the two cells with different markers. Therefore, in later electrophysiological studies HRP was injected into the L motoneurone and Lucifer Yellow into the P neurone (Pl. 3). Analysis of the sites of apparent contact in seven cells that had been studied physiologically showed that contacts occurred where the arbors of the two cells intersected; contacts were distributed in the anterior and middle portion of the neuropile, with one notable exception, and most were ipsilateral (Table 1). The contacts between pairs of P and L cells were between the cells' secondary or higher order branches and varied in number from twenty-six to sixty-six. However, there was less variation in the percentage of apparent contacts involving branches of the P cell's anterior, finer axon; this was $40 \pm 10\%$ of the total. In most cases the remainder were made by secondary branches that emerged from the primary axon coursing in the mediolateral portion of the neuropile. These included a variable fraction of contacts that were in the contralateral neuropile. The results from electron microscopy are in agreement with these, and suggest a consistent, asymmetrical distribution of synaptic contacts between the two cells.

Conduction block attenuates synaptic potentials

P cell impulses generated by stimulating the skin did not necessarily reach the cell body if they arose in the minor fields (Fig. 3), in contrast with the invasion of the

TABLE 1. Contacts and synaptic potentials between particular pairs of P and L neurones

Cell pair no.	Number of apparent contacts					Size of e.p.s.p. (mV)			Percentage contacts from Anterior	Percentage e.p.s.p. from Anterior
	Anterior	Middle	Posterior	Contra-lateral	Total	Anterior	Posterior	Unblocked		
1	14	7	1	12	34	0·5	0·0	2·5	41	20
2	22	17	2	25	66	0·5	0·0	2·0	33	25
3	12	13	1	14	40	1·0	0·0	4·0	30	25
4	23	8	15	8	50	0·8	0·7	2·3	46	35
5	14	12	0	0	26	0·25	0·0	1·0	54	25
6	20	23	2	15	60	0·6	0·0	3·0	33	20
7	14	11	2	0	27	0·5	0·0	1·0	52	50

In all cases except pair No. 4, the contralateral contacts were made by secondary axons arising from the middle, thicker axon of the P cell. Four posterior contacts of pair No. 4 were contralateral and are listed in both categories.

cell body from the major field (Nicholls & Baylor, 1968). Extracellular and intracellular recordings have confirmed that the impulses failed to propagate beyond the junction that the minor field's fine conducting axon made in the ganglion with the thicker primary axons (Yau, 1975), as the morphology of the cells might suggest. In most instances, impulses were blocked during and after periods of activity, but
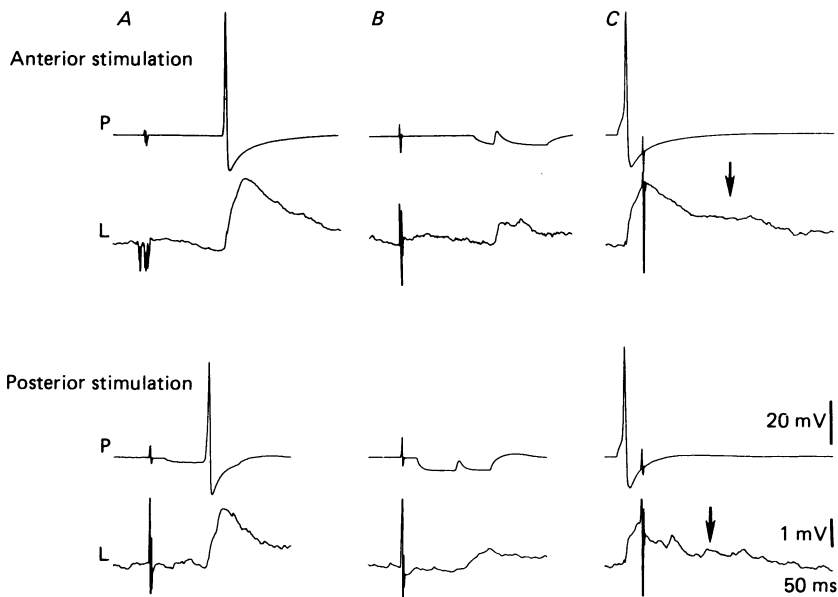

Fig. 4. Preparation in which synaptic potentials in the L cell persisted during conduction block in the posterior axon branch. Stimulating the anterior minor field (top pairs of traces) or posterior minor field (bottom pairs of traces) at 3 Hz evoked impulses (A) that were evidently blocked at the anterior and posterior branch points, as determined with extracellular recording, during hyperpolarization of the P cell soma with extrinsic current (B). Conduction block reduced but did not eliminate the synaptic potentials. Collision with a centrifugal impulse generated in the P cell body (C) eliminated the synaptic effect of skin stimulation. Arrows in C indicate the timing of full synaptic potentials had there been no collision. Stimulation of the skin, marked by a biphasic artifact in the sensory neurone traces, occurred approximately 50 ms after the beginning of each sweep. Traces represent averages of from eight to forty sweeps, accounting for variations in base-line noise. Resting potentials of P and L cells were −49 and −62 mV, respectively. Bathing solution contained 8 mM-$CaCl_2$. Preparation shown in Pl. 3; cell pair No. 4 in Table 1.

sometimes block occurred persistently from the onset of recording and was relieved only when the cell was depolarized with current passed through the recording micro-electrode in the neurone soma. Conversely, it was sometimes necessary to hyperpolarize the P cell body to produce conduction block at low stimulation frequencies.

Simultaneous recordings from the P and L neurones showed that the e.p.s.p. in the L motoneurone was consistently reduced during conduction block (Figs. 3 and 4). In all but one preparation, those blocked impulses that originated in the posterior minor field evoked no detectable synaptic potential, while blocked impulses that arose in the anterior minor field elicited synaptic potentials that reached one-half to

one-fifth the unblocked size (Table 1). Collision experiments were performed several times for each preparation to determine that the synaptic potential was elicited directly by the blocked impulse in the P cell and not via a separate, parallel pathway. A centrifugal impulse was produced with the micro-electrode in the soma so as to collide with one arising a short time later in the periphery (e.g. Fig. 3 D). The resultant elimination of the peripherally elicited e.p.s.p. occurred whether the incoming impulse would have been full-size (unblocked) or blocked within the ganglion, and whether collision occurred in the periphery or in the connectives between ganglia, as judged by extracellular recordings from the roots of the adjacent ganglion carrying the minor-field axons. Synaptic depression may have developed at stimulation frequencies that produced conduction block, since during an experiment later e.p.s.p.s were often smaller than those recorded earlier.

Conduction block in the sensory cells is caused by a hyperpolarization of the neurone after activity (Van Essen, 1973; Yau, 1976). In P cells hyperpolarization is due to an electrogenic sodium pump and to a calcium-dependent increase in potassium conductance (Jansen & Nicholls, 1973). Thus, depolarization of the P cell by injection of positive current into the soma through the recording micro-electrode relieves conduction block (Fig. 3C) and conversely, hyperpolarization can cause block (Fig. 4B). Since the posterior branch-point is further from the cell body, the site of the intracellular micro-electrode, larger injected currents are required to produce a block at the posterior branch point or to relieve block once it is established.

The distribution of contacts between the P and L cells shows that each P cell contacts the contralateral as well as the ipsilateral L cell; in one case fifty points of apparent contact with the contralateral L cell were counted. Under normal conditions, therefore, a component of the synaptic potential recorded in one L cell could arise in the contralateral L cell and pass across the non-rectifying electrical synapse that links the paired motoneurones (Stuart, 1970). To eliminate this possibility in the experiments reported here, single, contralateral L cells were killed with intracellular protease injection and the effects of branch-point failure on synaptic transmission determined. The synaptic potentials in the remaining L cell were, in some cases, smaller than in uninjected ganglia, but the fractional reduction with conduction block was the same whether the contralateral L cell had been killed or not. Subsequent HRP injection into the surviving L cells showed that their morphology was not affected by the protease injections, and indeed no deleterious effects, short- or long-term, have been reported in cells electrically coupled to those destroyed by protease injection (Bowling *et al.* 1978; Scott & Muller, 1980).

Morphology and physiology compared

During conduction block, the strength of the synaptic connexion was reduced in approximate proportion to the numbers of apparent contacts that were evidently no longer reached by nerve impulses arising in minor receptive fields, but on a strength per contact basis, the anterior contacts were weaker, as recorded in the L cell soma. Thus from Table 1 we can calculate voltage per contact for anterior branches, which ranged from 20 to 80 μV/contact, but were typically 30 μV/contact. In contrast, the over-all strength of contacts was consistently higher, ranging from 30 to 100 μV, and was typically 40 or 50 μV. For each cell the pattern was consistent, but it is not clear

whether the weaker, anterior contacts can be accounted for simply by their greater distance from the recording site at the L cell soma.

The receptive field of each mechanosensory neurone in leech ganglia is a patchwork of subfields, each innervated by either a large or a small axon. During activity the receptive field apparently may shrink as impulses travelling along finer axons from outlying subfields fail to propagate through the central ganglion. The apparent shrinkage, as measured in the cell body, is accompanied by reduced synaptic transmission. For the P cell synapse with the L cell, during conduction block from the posterior minor field, transmission typically fails entirely and during block from the anterior, transmission is reduced by more than one-half.

Transmission failure and attenuated transmission correlate with the predicted drop out of activated synaptic contacts during conduction block. Synapses are situated within the ganglion's neuropile along and at the ends of the P cell's secondary processes, which emerge from the thick and thin axons innervating the receptive subfields. Conduction block probably occurs where the thick and thin axons meet (Pl. 3). The distribution of apparent contacts between P and L neurones can be most easily mapped using light microscopy, but to test whether the contacts are synapses, the tissue must be re-sectioned and examined in the electron microscope. All five such contacts that have been sectioned in this work are chemical synapses of the type described previously for P and for L neurones (Purves & McMahan, 1972; Muller & McMahan, 1976), thus it seems likely that apparent contacts detected in the light microscope are indeed sites of synapses.

Several quantitative aspects of the measured synaptic potentials merit addressing. First, although one or two contacts were often seen between secondary processes emerging from the posterior sensory axon and branches of the L motoneurone, in these cases no e.p.s.p.s were measured in the L cell during posterior block. This was not surprising, for even if two contacts had been transmitting they would have been expected to produce a combined synaptic potential of at most 40–100 μV, which was exactly the estimated level of noise in the averaged records. Only once were more than two posterior contacts seen (Fig. 4; Pl. 3), and that L cell had a substantial synaptic potential during posterior block in the P sensory neurone. Secondly, the number of contacts of the anterior axon's secondary processes was for each cell larger than expected from the size of the residual synaptic potential during anterior conduction block. Although the apparently reduced contribution to e.p.s.p.s made by contacts on the anterior axonal branches can be easily explained by the distance from the L cell soma, it has not been demonstrated that all contacts are equally effective or active at the site of synapse.

Thirdly, for the P cell there was considerable variability in the number of contralaterally projecting secondary processes and, therefore, variability in the number of contralateral contacts with the L cell. The contralaterally projecting processes of the P cell emerged chiefly from the main axon; when the number of their contacts was added to the number of others from the main axon, the total correlated with the fraction of the e.p.s.p. lost during anterior conduction block. This suggested

that the contralateral contacts provided a significant input to the L cell, and was evidence beyond that gained from T cells (Muller & Scott, 1981) that long secondary branches are normally actively invaded by impulses.

What role does conduction block play in sensory integration?

Van Essen (1973) found that conduction block in leech sensory neurones occurs under conditions that also produce sensory adaptation, which is a decrease in excitability that occurs in the periphery, and he showed that activity-dependent hyperpolarization contributes to both phenomena. Yau (1976) discovered the minor receptive fields of leech sensory neurones and proposed that conduction block sharpens spatial discrimination by reducing the overlap of fields. Thus leech mechano-sensory neurones function much as do retinal ganglion cells in mammals, where receptive fields shrink with light adaptation (Kuffler, 1953), although evidently the underlying mechanisms are different between the two phyla.

Another similarity shared by vertebrate and leech systems is that both exhibit considerable overlap of parallel pathways. Each small region of skin is likely to be innervated by thick axons of one or two sensory cells of each modality, T, P, or N, and by one or more finer axons of sensory cells in the two neighbouring ganglia. Except at unusually high firing frequencies, all these axons are expected to conduct without fail as they enter the ganglion along segmental peripheral nerves, and there the axon terminals should transmit normally to post-synaptic targets. Among those targets is the L motoneurone, for sensory neurones excite L motoneurones in adjacent ganglia as well as in their own ganglion (Jansen, Muller & Nicholls, 1974). At firing frequencies of a few impulses per second, when P cell impulses originating in the periphery in fine axons evidently fail at central branch points, L motoneurones will still be excited by other P cells whose large axons innervate the periphery. Thus, conduction block should only diminish and not eliminate synaptic excitation of motoneurones when pressure stimuli are delivered to skin in adjacent segments. Presynaptic inhibition might also influence conduction block (Van Essen, 1973) and thus indirectly alter the strength of synaptic transmission.

In the C.N.S. of vertebrates conduction block has been proposed to have various functions. For example, it may operate in feline sensory afferents to reduce transmission (Edwards *et al.* 1976), with block relieved during post-tetanic potentiation (for review see Lüscher *et al.* 1983*a*), and branch-point failure evidently limits the spread of impulses in the dendritic arborization of alligator Purkinje cells (Llinás & Nicholson, 1971). Because branched, excitable processes are characteristic of many neurones, branch-point failure may operate widely in neurones that have been less favourable for analysis, in a fashion analogous to that in the leech (Barron & Matthews, 1935; Chung, Raymond & Lettvin, 1970).

Conduction block as a tool for locating synapses

Conduction block can provide a means for comparing synapse structure and function. In the leech, where the distribution of contacts between P and L neurones correlates with the strength of transmission during conduction block, it should be possible to determine functionally which groups of secondary branches contact particular post-synaptic targets. For example, do the posterior secondary branches

of the P cell, which in most cases do not contact the L motoneurone, synapse instead with the annulus erector motoneurone, which is also post-synaptic to the P cell (Muller & Nicholls, 1974) and has branches in the posterior region of neuropile (Muller & McMahan, 1976)? Other physiological evidence (Muller & Nicholls, 1974) indicates that separate sets of P cell synapses contact the L and annulus erector motoneurones. Thus for the P cell and other leech sensory neurones (DeRiemer & Macagno, 1981; Muller & Scott, 1981), branch-point failure may act as a switch to turn off transmission to only a subset of post-synaptic targets.

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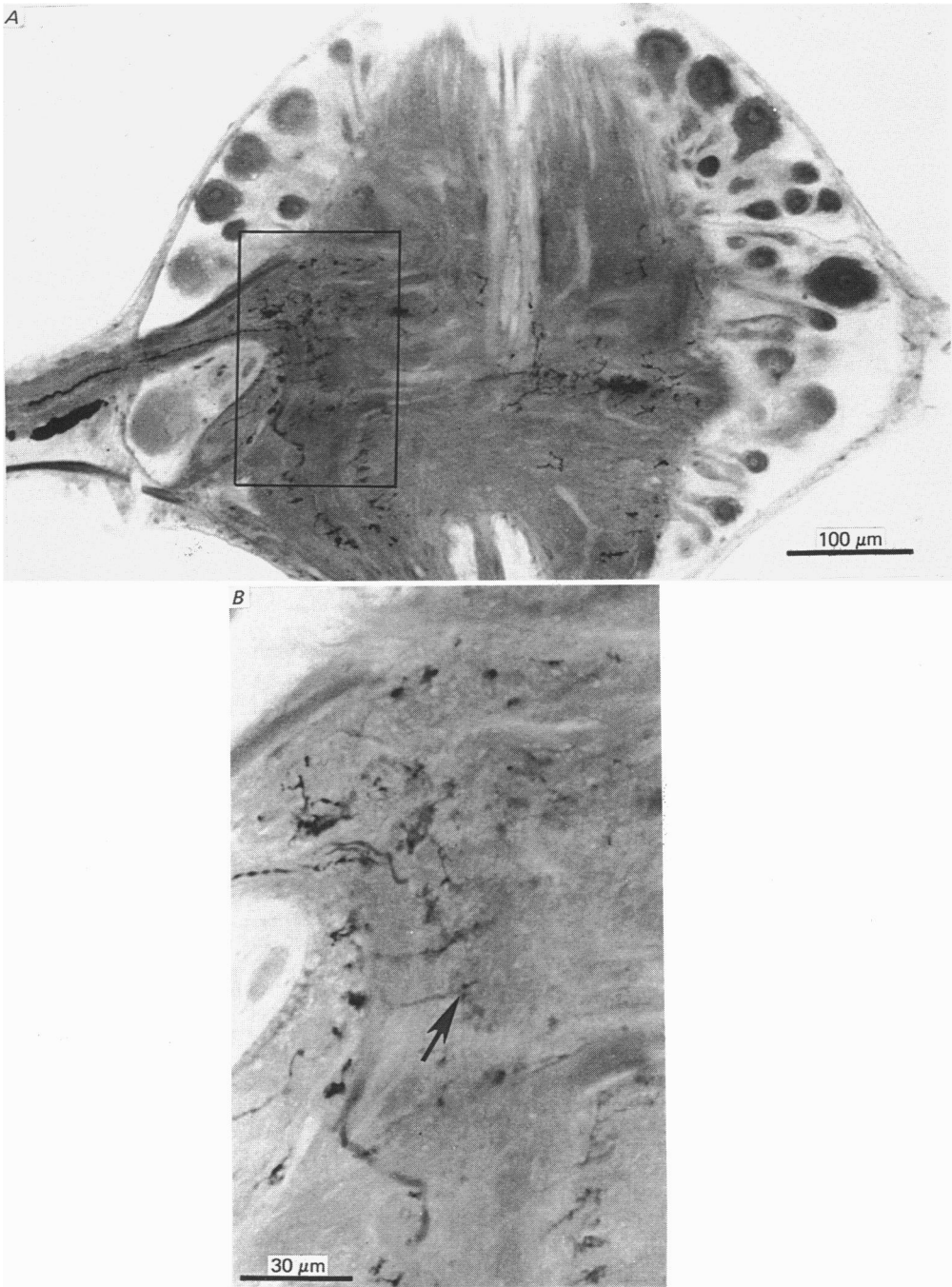
PLATE 1

Section through the ganglion neuropile includes contacts between the P sensory neurone and the ipsilateral L motoneurone. *A* shows a section, 8 μm thick, cut through the ganglion and viewed from the dorsum. It contains darkly stained axons and branches in the left segmental nerves and neuropile. Cell bodies darkened with osmium but unstained are visible in a layer surrounding the shaded neuropile. In other sections, not shown, the injected P and L cells are on the left. The identities of particular axons, sensory or motor, were determined by tracing them in successive sections to their cell bodies. At higher power (*B*, as outlined in box in *A*) sites of apparent contact are visible (e.g. at arrow, shown to be a synapse in Pl. 2). Dorsal view; anterior is toward the top.
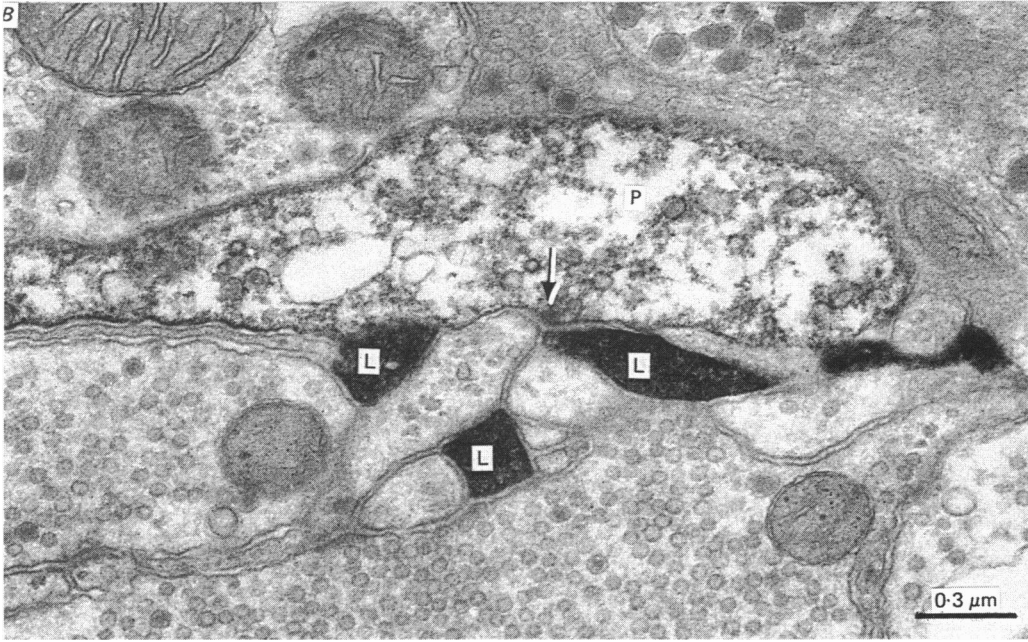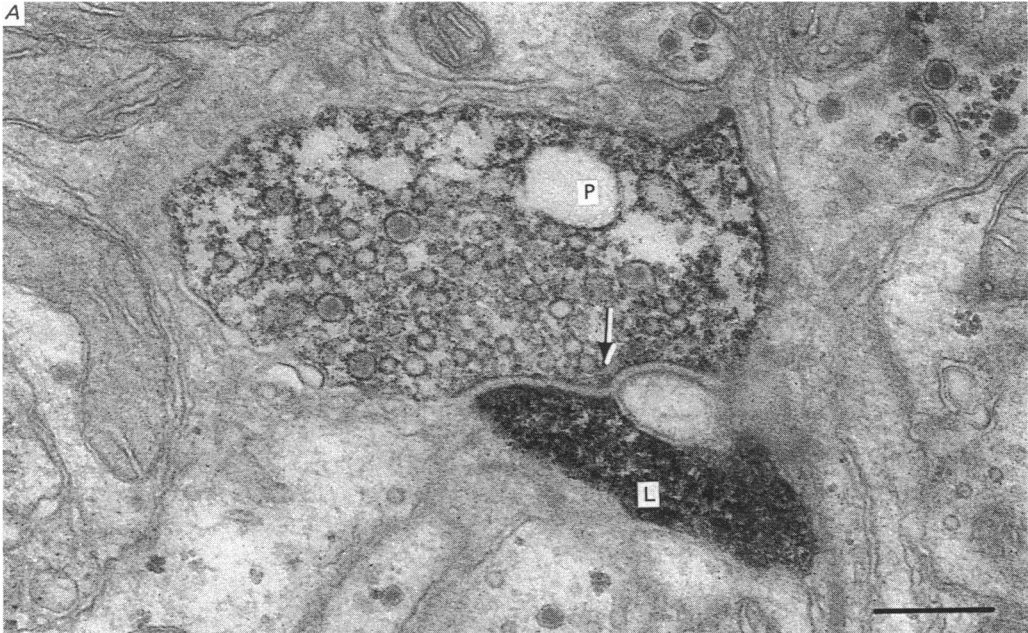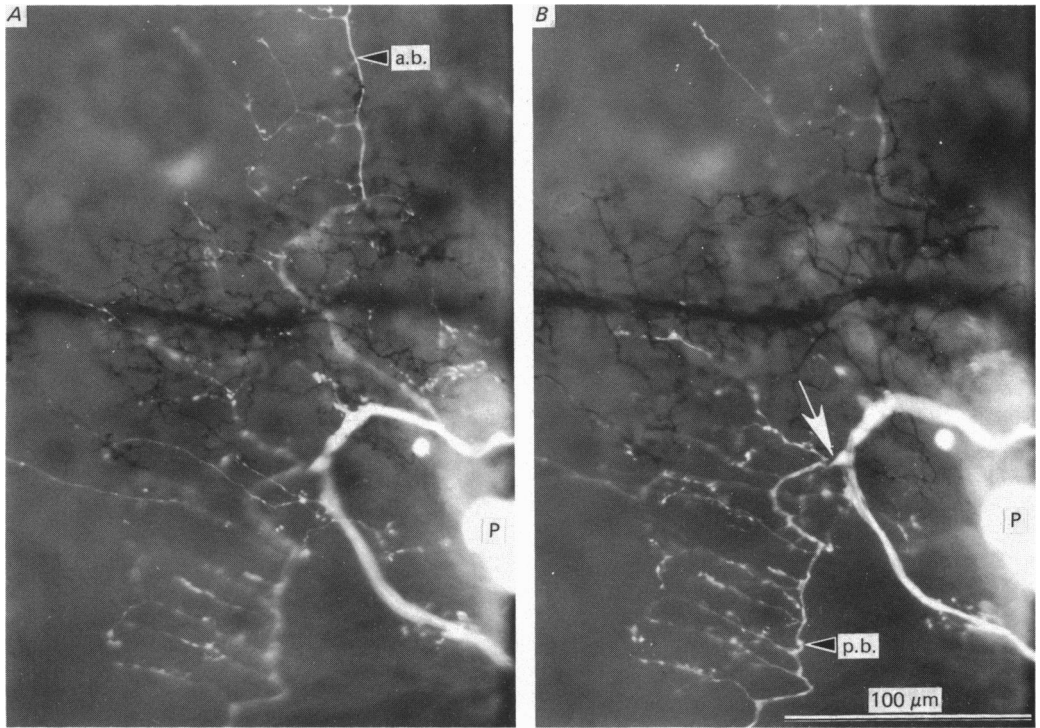
PLATE 2

Synapses at contacts between P and L cells. In *A* and *B* are examples of two different synapses (arrows) between the P and L neurones in the section depicted in Pl. 1 and an adjacent section recut for electron microscopy. As shown, the P cell is presynaptic and the L cell is post-synaptic to other, unidentified neurones. Thin sections were tilted to sharpen the view of the plasma membranes at the synapse.

PLATE 3

Micrographs of the preparation shown in Fig. 2 (cell pair No. 4 in Table 1). The P cell (perikaryon labelled P) was filled with the fluorescent dye Lucifer Yellow. Its processes appear white; processes of the L motoneurone were filled with horseradish peroxidase and appear dark. Two focal planes are shown: in *A*, the anterior axon branch (a.b.) is in better focus, whereas in *B*, the posterior axon branch (p.b.) is in focus. Secondary branches of the anterior and central axons of the P cell overlap more extensively and make more putative contacts with the L cell than do secondary processes emerging from the posterior branch (see Table 1). Arrow in *B* indicates point where the posterior axon branch emerges from the main axon.

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