J. exp. Biol. **143**, 509–527 (1989)
Printed in Great Britain © *The Company of Biologists Limited 1989*

DEPRESSION OF EXCITATORY MOTONEURONES BY A SINGLE NEURONE IN THE LEECH CENTRAL NERVOUS SYSTEM

BY MARTIN WADEPUHL*

Universität Konstanz, Postfach 5560, D-7750 Konstanz, FRG

Accepted 27 January 1989

Summary

Intracellular staining techniques have been used to characterize the morphology of a newly identified neurone, cell 151, in the segmental ganglia of the leech. This neurone ramifies extensively within the neuropile and sends multiple extensions into roots and connectives.

Strong dye coupling and non-rectifying electrical coupling were observed between the contralateral homologues. No action potentials were recorded from the cell body, but postsynaptic potentials and slow potential changes (>1 s, >15 mV) were observed. Upon injection of hyperpolarizing currents, the efferent spike activity, recorded extracellularly, was depressed in both the ipsi- and the contralateral roots of the ganglion. The depression was gradual and non-adapting and occurred reliably only within the ganglion where cell 151 is situated. Depolarization of cell 151 was without consequence for the tonic firing of isolated ganglia.

Many identified excitatory motoneurones follow the hyperpolarization of cell 151. Currents can be exchanged between cell 151 and motoneurones *via* rectifying electrical synapses. Spontaneous hyperpolarizations of cell 151 were correlated with depression of spike frequencies, recorded in whole nerves as well as in identified motoneurones. The membrane potential of cell 151 was drastically altered by bursts from mechanosensory cells.

The ability of cell 151 to distribute inhibition onto a great number of motoneurones and to curtail excessive neuronal activity is discussed.

Introduction

The regulation of overall electrical activity within a nervous system is a prerequisite for its functioning and is of importance for arousal and for stable responses to excitatory peripheral input. Cells with widespread extensions, such as those in the reticular formation of vertebrates, are proposed to be involved in such

* Present address: Vergleichende Neurobiologie, Universität Ulm, Postfach 4066, D-7900 Ulm, FRG.

Key words: leech, identified neurone, dye coupling, electrical coupling, motor control, modulation.

regulation (reviewed by Hobson & Brazier, 1980; Jacobs, 1986). It would be interesting to study these aspects in a nervous system with few, readily identifiable cells, as in the leech central nervous system (CNS). Of the approximately 400 cells contained in a segmental ganglion (Macagno, 1980) about 120 have been identified (Sawyer, 1986).

This paper describes the morphology, intracellular properties and coupling of a newly identified cell which may function as described above. It has extensive branches in the neuropile and in all nerves leaving the ganglion, and a widespread influence on the excitation of other neurones. The cell differs from common leech neurones (for a review see Muller *et al.* 1981) in showing a conspicuous absence of action potentials and having an enormous dendritic field which extends into the roots.

Some aspects of this work have been reported in preliminary form (Wadepuhl, 1986, 1987; Wadepuhl & Schäffner, 1987).

Materials and methods

Leeches (*Hirudo medicinalis L.*) were obtained from a commercial supplier and kept at 15°C.

Preparation

The ventral nerve cord was dissected from the animal in ice-cold leech saline (Kristan *et al.* 1974*a*; Nicholls & Baylor, 1968). Ganglia of segments 9–11 were pinned, ventral side uppermost, over a ridge of Sylgard. The middle ganglion, ganglion 10, was desheathed (Friesen, 1985). All connectives were pinched, unless stated otherwise (Fig. 1). To facilitate simultaneous recordings of ventral cell 151 and dorsal motoneurones, ganglion 10 was folded around a diagonal axis so that the ventral side of the anterior lateral packet became visible. In some cases, cell 151 was impaled from the dorsal side. The preparation was continuously superfused with leech saline. The contents of the bath (3 ml) could be exchanged in less than 1 min. Bath temperature ranged from 19 to 22°C. Cell 151 was identified by its position, the absence of action potentials, the abundance of synaptic potentials and shifts in the resting potential (see Results for details). Motoneurones were identified by size and position, in the case of CV and AE (Muller *et al.* 1981), and according to their action potential in peripheral nerves (Stuart, 1970; Kristan *et al.* 1974*b*). Nomenclature is that of Ort *et al.* (1974).

Electrophysiology

For intracellular recordings, electrodes were filled with $4\,\mathrm{mol\,l^{-1}}$ potassium acetate and had resistances between 20 and 40 MΩ. A bridge balance circuit was used for stimulus compensation during intracellular recordings (List L/M-1). Alternatively, a sample-and-hold circuit using amplifiers designed at the Univer

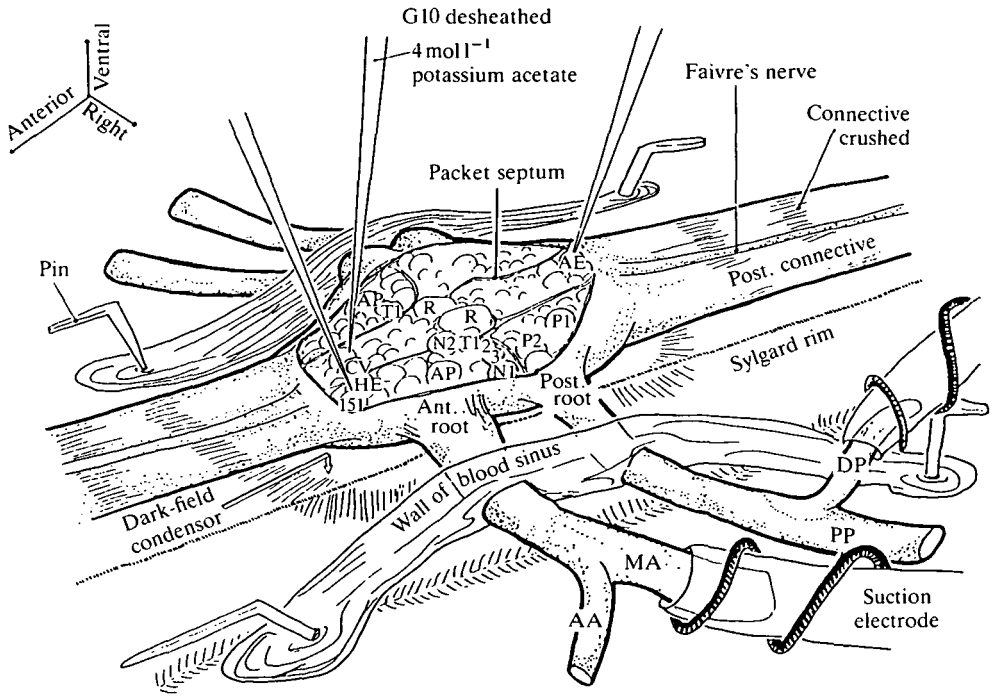

Fig. 1. Drawing of the preparation. The desheathed ganglion 10 lies ventral side up on a Sylgard ridge, pinned through the wall of the blood sinus which is penetrated by nerves. The anterior (Ant.) and posterior (Post.) roots leave the ganglion laterally. AP, anterior pagoda cell; N1, 2, nociceptive cells 1 and 2; P1, 2, pressure-receptive cells 1, 2; T1, 2, 3, touch-receptive cell 1, 2, 3; DP, PP, dorsal and posterior branches of the posterior root; MA, AA, medial and anterior branches of the anterior root: AE, annulus erector motoneurone; CV, excitor of ventrolateral circular muscles; HE, heart excitor motoneurone; R, Retzius cell.

sity of Konstanz was employed to compensate for electrode resistance during current injection. Sampling was automatically triggered by the rising and falling phases of the current pulse. Sample times were set at 150 μs. During this time the voltage drop over the electrode is complete, but changes in membrane potential are still minimal. The sampled voltage was subtracted from the signal and was set to zero after the end of the stimulus. Current was checked against virtual ground. Glass-tipped suction electrodes and Isleworth amplifiers were used for extracellular recordings. The output of the amplifiers was displayed on a Gould ES 1000 electrostatic printer with a frequency response faster than 1 kHz and either stored on an FM tape recorder (Hewlett Packard 3968A) or directly displayed on a storage oscilloscope.

Zero-calcium-high-magnesium (0-Ca^{2+}-high-Mg^{2+}) saline was obtained by omitting $CaCl_2$ from standard artificial leech saline, reducing NaCl to either 96·8 or 57·5 mmol l^{-1} and adding 20 or 40 mmol l^{-1} $MgCl_2$, respectively.

Neuroanatomy

Cobalt injection electrodes were filled with 6% hexammine cobaltic chloride (Cohex) (50 MΩ). Depolarizing current (2–4 nA, for 10 min) was used for ionophoretic injection (Brogan & Pitman, 1981). Diffusion time varied from 20 min for intraganglionic to 4 h for interganglionic stains. To facilitate interganglionic stains, the two ganglia were electrically isolated with a glycerin gap. A d.c. current of a few microamps was applied between the different compartments, with positive current polarity at the injection site. The cobalt was precipitated and intensified (Bacon & Altman, 1977) and the preparation was dehydrated in alcohol and cleared in methyl salicylate.

For horseradish peroxidase (HRP) injection, glass electrodes were filled with 2% horseradish peroxidase (Sigma Type VI in 1% Fast Green in 250 mmol l^{-1} KCl; Muller *et al.* 1981). Electrodes were bevelled to 15–20 MΩ by sliding the tips along a fine Degussit grindstone. The dye was pressure injected (69–207 kPa). After 1 h diffusion time the ganglia were fixed in 2·5% glutaraldehyde, treated with saponin, diaminobenzidine and $CoCl_2$, and developed with H_2O_2. After dehydration they were transferred to methyl salicylate and viewed as wholemounts. For serial sections, ganglia were embedded in Durcupan.

The data presented here were obtained from 68 penetrations of cell 151.

Results

Morphology

The cell body of the newly identified cell lies in the anterior tip of the anterolateral packet, anterior to the heart excitor (HE) motoneurone and often below the superficial cell body layer. All other nearby neurones have action potentials and a large single axon in the contralateral axon in the medial branch of the anterior root (MA), similar to motoneurones. The position of this cell corresponds best to that of cell 151 in the map summarized by Muller *et al.* (1981). The cell occurs segmentally and could be identified in all ganglia examined (ganglion 3, 4 and 7–13).

HRP staining (Fig. 2A) revealed a large primary neurite in the longitudinal axis of the ganglion extending into the anterior and posterior connectives. A short secondary neurite originates from the anterior portion of the primary neurite and connects to the cell body. Large secondary neurites branch off at right angles to the ipsi- and contralateral roots. These flatten in the lateral neuropile and project many fine branches into the roots (Fig. 2A,C). Higher-order neurites ramify extensively in the ipsi- and contralateral neuropile. Neurites of cell 151 run in the anterior and posterior connectives and in Faivre's nerve, though with some variation (M. Wadepuhl & K. H. Schäffner, in preparation). Multiple neurites have been found in each connective.

Dye coupling with the contralateral homologue has been seen in all Cohex (Fig. 2B,D) and Lucifer Yellow (LY) fills (Wadepuhl, 1987). Such wholemounts

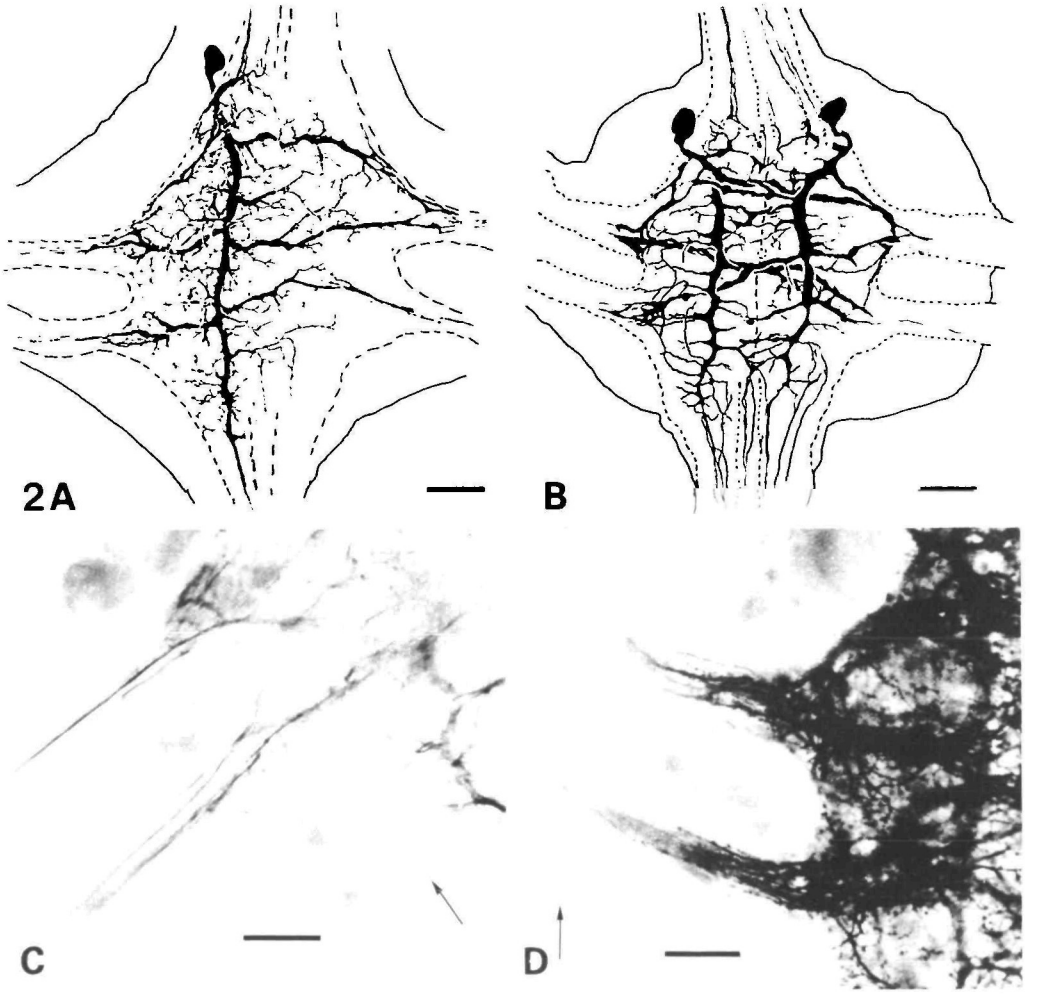

Fig. 2. Morphology of cell 151. (A) HRP stain. (B) Cohex stain. The dye was injected into the left cell body only; because of the dense ramification (see also Fig. 2D), only major branches are drawn. A and B are *camera lucida* drawings of wholemounts. The anterior end is at the top; stippled lines mark the outline of the neuropile. Multiple neurites in roots of cells filled with HRP (C) or Cohex (D). Photographs taken from wholemounts. Arrows point towards the anterior. Scale bars, 50 μm.

show a close association of secondary neurites around the sagittal midline of the ganglion.

Injection of HRP in a single cell 151 revealed a maximum of 16 branches at the base of the roots. This exceeds by far the number of branches known for other leech neurones. Owing to dye coupling, this number roughly doubled in cobalt fills.

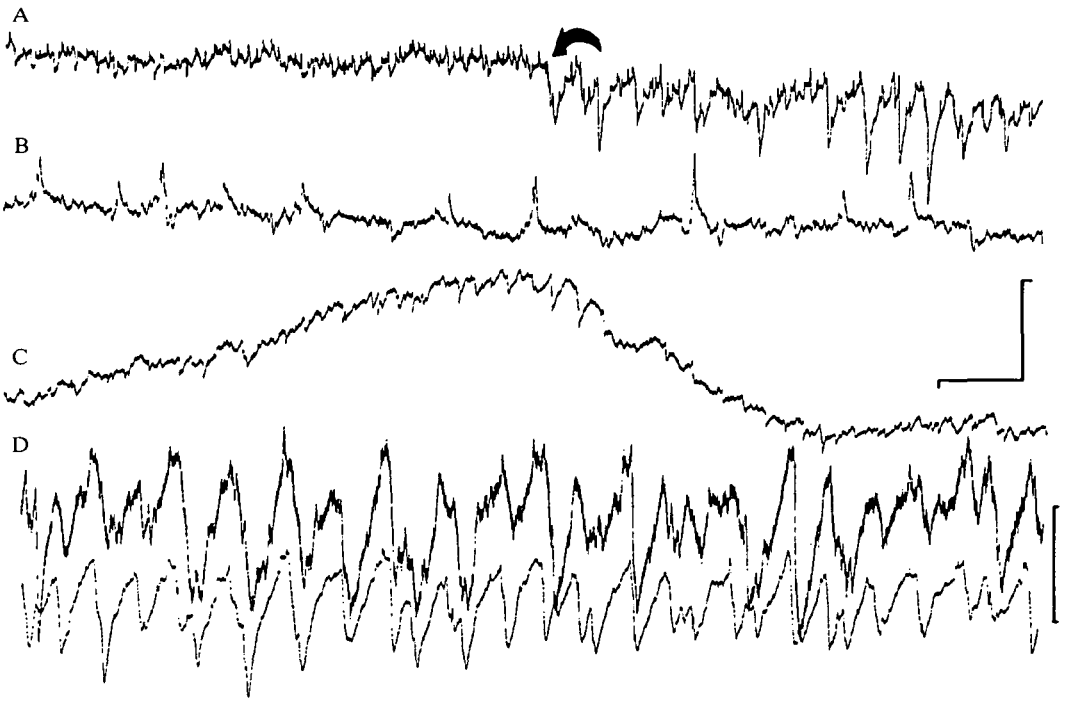

Fig. 3. Spontaneous intracellular signals of cell 151. IPSPs (A), EPSPs (B) and slow potential changes (C) recorded from the soma. Size and type may change abruptly (A, arrow). (D) High degree of synchrony between left (upper trace) and right cell 151 in the same ganglion. Note the absence of action potentials. A and upper trace of D are recordings from the same cell. Calibration: 10 mV, 1 s (A, B, C); 10 mV (upper trace) 5 mV (lower trace), 300 ms (D).

Physiology

The resting potential of cell 151 in the absence of long-lasting depolarizations (see below) was −36 mV (S.D. = ±5·3 mV; 11 cells).

Cell 151 was characterized by the absence of action potentials and the occurrence of IPSPs of about 50 ms duration and 5–10 mV amplitude (Fig. 3A). The latter disappeared in 0-Ca^{2+}-saline, increased in amplitude upon depolarization and decreased with hyperpolarization. In most cases, IPSPs occurred synchronously in both contralateral homologues (Fig. 3D). Sometimes, however, there were large IPSPs of low frequency in one cell and concomitant small-amplitude high-frequency signals in the homologue, indicating desynchronization of input.

In addition to the frequent hyperpolarizing potentials, spontaneous depolarizations were observed (Fig. 3B). Similar depolarizations were seen in response to bursts of N cell action potentials, whereas P cells responded differently (see below, Fig. 10B). Their time course, variation in amplitude and occurrence at different

membrane potentials indicate that they might have been coupling potentials or EPSPs, rather than action potentials.

About 25 % of the preparations showed slow spontaneous potential changes of several seconds with amplitudes of more than 15 mV (Fig. 3C). Similar potential changes were obtained by stimulating the posterior branch of the posterior root.

Except for minute deflections, depolarizing potentials of constant size were never observed. There was no correlation between signals in extracellular recordings of the DP (dorsal branch of the posterior root) and MA nerves and potential changes in cell 151. No action potentials were elicited in cell 151 by (1) injection of depolarizing current, (2) strong rebounds after prolonged hyperpolarizations, (3) stimulation of the roots or (4) depolarizations through bursts of P and N cells. Action potentials were also absent in preparations with intact connectives in a chain of three segmental ganglia. It is unlikely that the cell was permanently refractory owing to insufficiently high membrane potential (Burrows & Siegler, 1978; Hengstenberg, 1977), since the resting potential was always in the same range or even lower than those of spiking motoneurones. Furthermore, perfusion of saline with increased oxygen content made no difference. Experiments with short hyper- or depolarizing 0·5 nA pulses superimposed on a 500 ms hyperpolarization of 1 or 2 nA did not elicit any active responses.

Electrical coupling was examined by injecting current pulses into one cell and recording the response in the contralateral homologue. Both depolarizing and hyperpolarizing currents were equally well transmitted (Fig. 4A). The coupling factor for small voltage changes had a mean of 0·36 (range 0·21–0·87, $N = 15$, three cells). Currents greater than +3 nA and less than −3 nA consistently caused less potential change in the follower cell, even when the nonlinearities in the I/V curve (Fig. 5A) were taken into account. This was confirmed in cases where stimulating electrodes could be balanced over the entire current range (Fig. 4A). Coupling persisted in 0-Ca^{2+}-high-Mg^{2+} saline (Fig. 4B). The strong coupling might enhance the simultaneous potential changes in both segmental homologues (Fig. 3D).

Based on the I/V relationship of cell 151, an estimation of membrane potential changes induced by current injection can be made even when balancing of the electrodes is made impossible by long or repeated pulses and high current amplitudes. The I/V curve also provides a first test of whether nonlinearities other than the delayed and anomalous rectification described below are present.

At the beginning of an experiment, depolarizing pulses often prompted rapid potential deflections, probably IPSPs elicited by an unknown source. These IPSPs curtailed the depolarization (Fig. 5B) to 25 mV above the resting potential. Complete I/V curves were therefore obtained only from preparations in which membrane potential fluctuations had declined (Fig. 5A,C). Under such conditions the input resistance decreased at high negative and positive currents. This indicates delayed and anomalous rectification. For example, slope resistances with current amplitudes between −2 and +2 nA were 10·5, 7·6 and 4·5 MΩ in three different cells, compared to 14·5, 9·7 and 7·6 MΩ, respectively, at the extreme

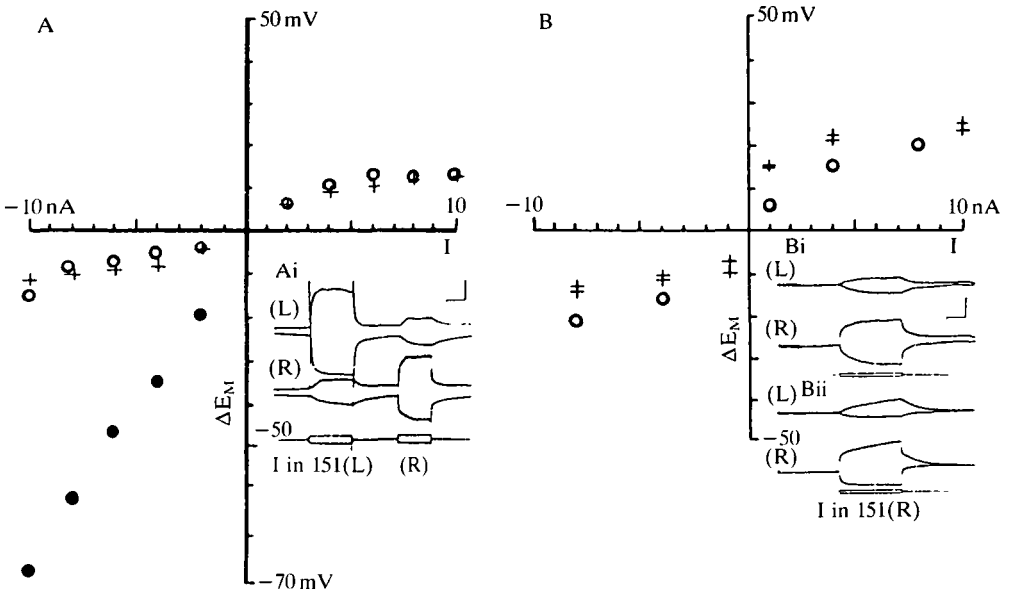

Fig. 4. Ipsi- and contralateral cell 151 are coupled by a non-rectifying electrical synapse. (A) I/V plot shows no rectification dependent on the polarity. (+) Current from right (R) to left (L); (○) current from left to right. The filled circles mark potential changes in right cell 151, into which current was injected. (Ai) Responses of cells 151 to current pulses into contralateral homologue. Calibration 20 mV, 10 nA, 100 ms. (B) I/V plot shows electrical coupling between contralateral homologues of cell 151 in normal (+, also Bi) and in 0-Ca^{2+}-high-Mg^{2+} saline (20 mmol l^{-1} Mg^{2+}; ○, also Bii). Calibration 10 mV, 10 nA, 100 ms. Bi and Bii are from a different preparation from B. Stimulation in A and B was *via* a balanced bridge circuit.

ends of the curves in the same preparations. The membrane potential decreased by only 2–3 mV after penetration by the second electrode, which lends credibility to the above values. Sudden shifts of the I/V curve (Fig. 5A, at +1, 2 and 3 nA) or small nonlinearities between −1 and +1 nA were not studied in detail.

Effects of cell 151 on spiking activity in the roots

To evaluate the functional significance of the extensive branching of cell 151, hyperpolarizing currents were injected into cell 151 while changes in spontaneous activity of the roots were monitored by extracellular recordings. Ganglia not mediating any specific behaviour produced tonic efferent activity. In most recordings the dorsal branch of the posterior root (DP) and the medial branch of the anterior root (MA) (Figs 1, 6Aii) were used. DP carries large efferent signals of identified motoneurones 3 and L (Ort *et al.* 1974). MA contains most of the approximately 2500 axons of the anterior roots (Wilkinson & Coggeshall, 1975). A representative sample of efferent activity can be recorded in these nerves; for example, 16 of the 21 units active in swimming are found here (Ort *et al.* 1974).

Hyperpolarization of any cell 151 resulted in ipsi- and contralateral reduction of

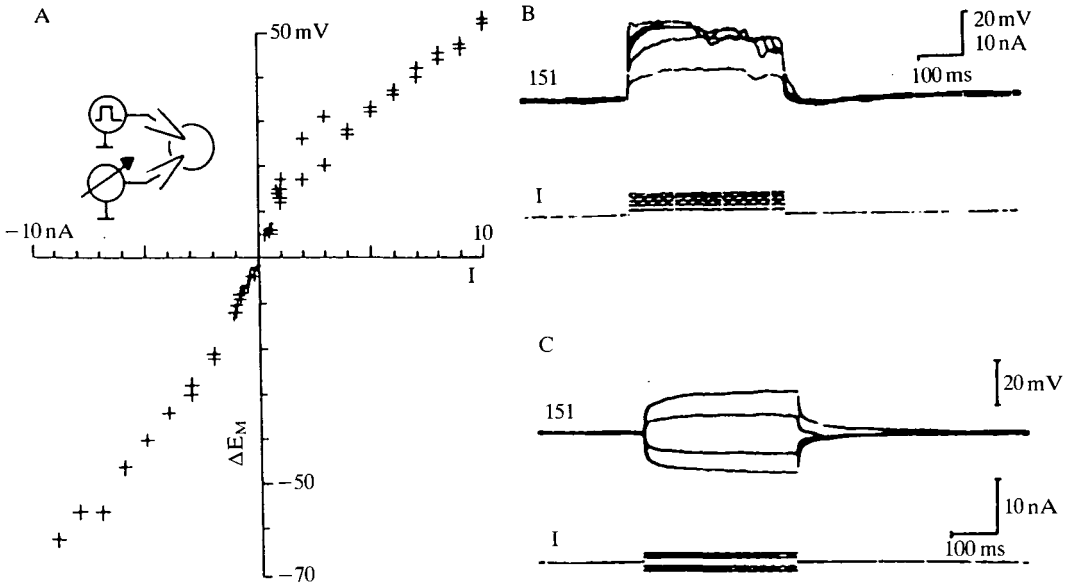

Fig. 5. I/V curve of cell 151. (A) Current–voltage relationships of cell 151 [no postsynaptic potentials during test pulse (see text)]. Recording situation is shown in inset. (B) In fresh preparations depolarizations induce IPSPs. These potentials drastically curtail the effect of current injections. Hyperpolarizing rectangular current pulses injected at about the same time yield membrane responses no different from those in C. (C) Recording used for A. Measurements were taken at the end of test pulses.

activity in DP and MA. The degree depended on the amount of current injected and was the same in all nerves. Especially with strong current injections, previously inactive units sometimes began firing with low frequency [Fig. 6Ai, MA(L), DP(L)]. The average discharge frequency during hyperpolarization of cell 151 was calculated as a percentage of average frequency measured 1 s prior to current injection. These values were plotted against the amount of current injected (Fig. 6Bi,ii). A linear regression line was calculated for every nerve recorded (summarized in Fig. 6Ci–iv). The results clearly show that the depression of spike activity by hyperpolarization of cell 151 affects all nerves in all preparations to a strikingly similar degree. Furthermore, spike frequency reduction is highly correlated with the amount of current injected, with little variation from animal to animal. Injection of 10 nA hyperpolarizing current into cell 151 silenced all nerves (Fig. 6C). Sometimes only −2 nA was enough to reduce spike frequency by more than 50 %. This reduction of spike frequency did not adapt even with test pulses lasting longer than 10 s (Fig. 7), and increased gradually with gradual increase of current. However, passing depolarizing current into cell 151 did not increase the frequency in peripheral nerves (Fig. 7B), even at high current amplitudes.

To study interganglionic effects, the roots of the two ganglia posterior to the injection site were examined (Fig. 7). In this case the connectives were not

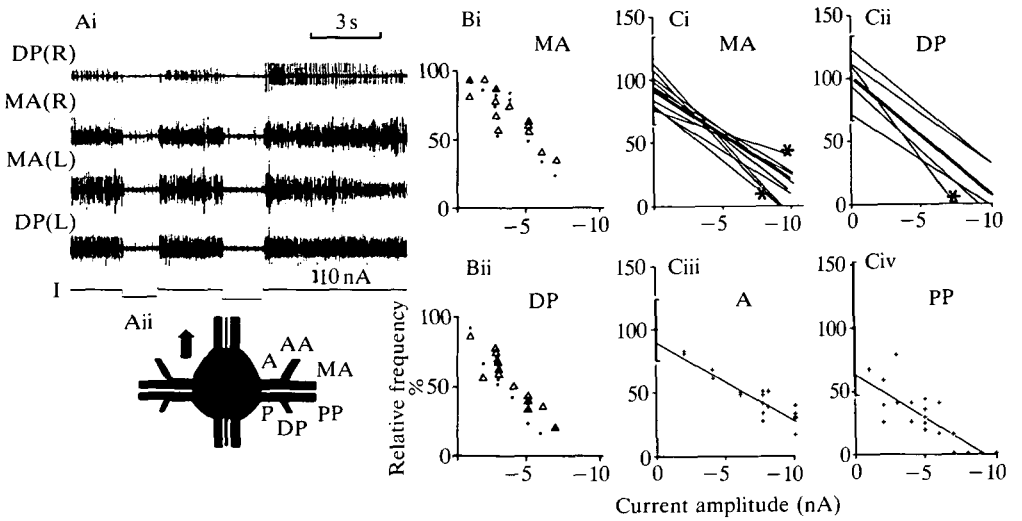

Fig. 6. Reduction of spike frequency by cell 151 in peripheral nerves. (Ai) Reduction in spike frequency in the dorsal branch of the posterior root (DP) and the medial branch of the anterior root (MA), both ipsi- and contralateral to the cell body of cell 151, into which the current was injected. L, left; R, right side. (Aii) Schematic drawing of ganglion with abbreviation of main peripheral nerves as used to identify subfigures. A, anterior root; P, posterior root; AA, anterior branch of A; PP, posterior branch of P. (Bi,ii) Relative reduction of average frequency of recordings in Ai during injection of hyperpolarizing current into cell 151 plotted against current amplitude. 100% corresponds to average frequency during 1 s immediately prior to current injection. Left nerves (●), right nerves (△). (Ci–iv) Summary of reduction of spike frequency plotted against the amplitude of hyperpolarizing current injected into cell 151. MA, five animals, $N = 154$, f_{pre} [= average frequency 1 s prior to current injection (= 100%)] = 22 ± 8 Hz; DP, four animals, $N = 154$, $f_{pre} = 20 \pm 7$ Hz; A, one animal, $N = 18$, $f_{pre} = 27 \pm 6$ Hz; PP, one animal, $N = 26$; $f_{pre} = 10 \pm 5$ Hz. Each cell line was drawn through 12–25 points distributed over 5–9 current steps. Data points of each measurement are reduced to a straight line by least-squares linear regression. Ipsi- and contralateral recording sites are evaluated separately. In Ci and Cii a numerical average of all slopes and intersection points was used to construct a mean regression line (heavy line). Significance level of correlation coefficients were 0·1%, except for lines marked by asterisks. The thick bar on the ordinates gives the standard deviation of spike frequency (in %) just before the test pulse.

pinched. The correlation between current amplitude and reduction in spike frequency was only significant for one of five recordings ($P = 0{\cdot}01$). Current injections into cell 151 caused a non-adapting influence of the same sign in almost all neurones recorded extracellularly.

Effects of cell 151 on identified motoneurones

Efferent action potentials recorded in peripheral nerves of isolated ganglia are produced by motoneurones and neurosecretory cells. Simultaneous recordings of cell 151 and neurosecretory cells (Retzius, Leydig, anterior pagoda) did not yiel

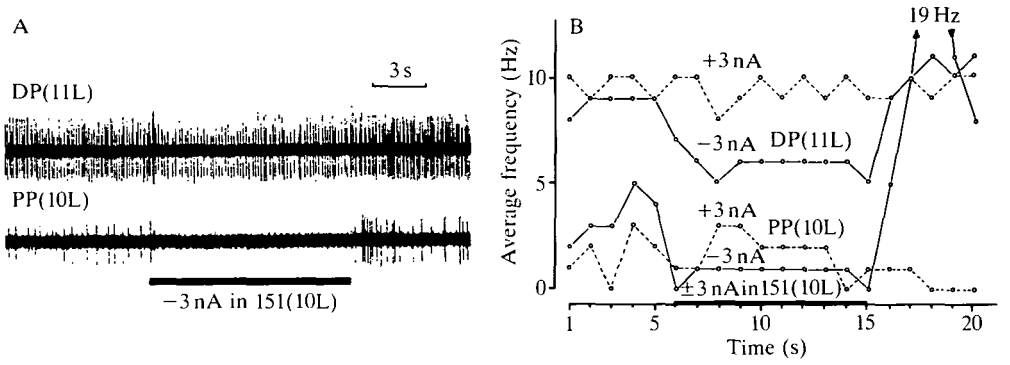

Fig. 7. Effect of long-lasting current injections. (A) Frequencies in nerves of the same (nerve PP) and the next posterior ganglion (nerve DP) are reduced as long as cell 151 is hyperpolarized. (B) Time course of average frequency (sample time 1 s) prior to, during and after long depolarizing (+3 nA, continuous line) and hyperpolarizing (−3 nA, dotted line) current injections in cell 151 recorded from nerves leaving the same [PP(10L)] or the adjacent [DP(11L)] ganglion with respect to the location of the cell body of cell 151.

evidence of interaction and were not followed up in this study. Hyperpolarization of cell 151 induced simultaneous hyperpolarization in identified excitatory motoneurones and thus depressed action potentials in the roots (Fig. 8A) ($N = 22$, two exceptions). It took about 100 ms for the effect to develop fully. Motoneurones on the dorsal and the ventral surfaces of the ganglion (AE, annulus erector, and CV, excitor of ventrolateral circular muscles, in Fig. 8D) as well as many unidentified cells of the anterolateral packet of the ganglion were tested and showed the same response. No changes were found in the heart excitor motoneurones.

The relative reduction in frequency of spikes recorded intracellularly was plotted against the amount of current injected into cell 151 (Fig. 8E). The resulting regression line is comparable to those obtained from nerve recordings (Fig. 6C). Current amplitudes larger than 3 nA were necessary to produce distinct effects on membrane potentials. As expected, reduction of spike frequency recorded intracellularly varied more than in extracellular measurements. In inhibitory motoneurones, hyperpolarization of cell 151 caused an increase in spike frequency (Fig. 8C). The response to depolarization of cell 151 was a slight increase of spike frequency in excitatory motoneurones (Fig. 8Bii). These phenomena may account for the occasional occurrence of additional units in extracellular records.

Thus, all aspects of the extracellular effect, i.e. number of cells affected, polarity, time course and exceptions, were demonstrated with identified cells.

Synapses between cell 151 and motoneurones

To distinguish between chemical and electrical transmission of currents between

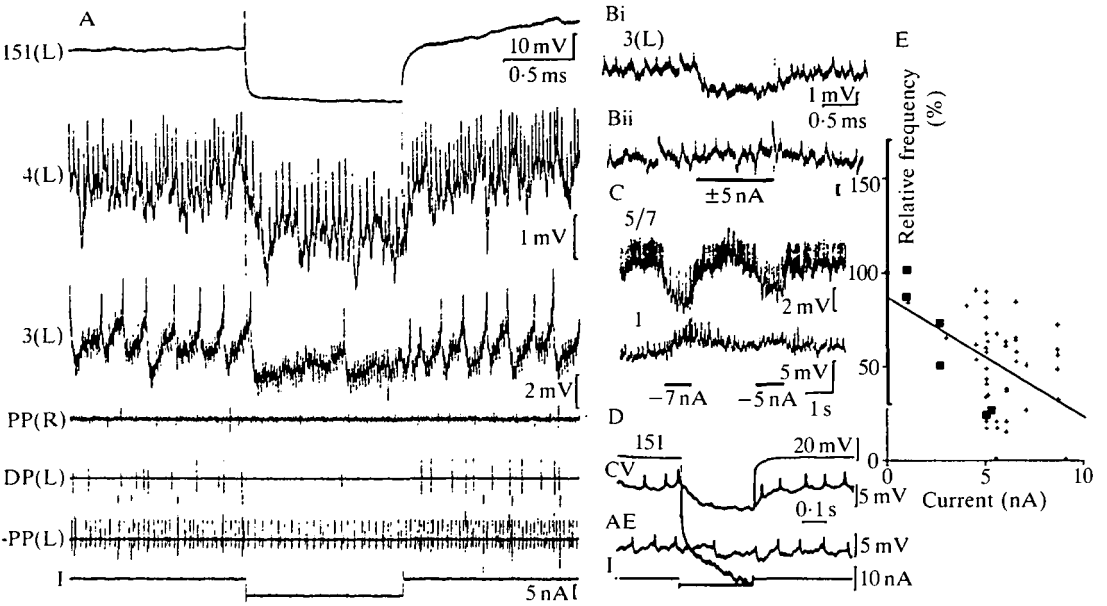

Fig. 8. Effect of current injection into cell 151 on motoneurones. (A) Injection of hyperpolarizing current (I) into cell 151 hyperpolarizes intracellularly recorded excitors of ventral (4) and dorsal (3) longitudinal muscles and reduces spike frequency in extracellularly recorded branches of the posterior root (DP, PP) (Voltage changes in cell 151 were recorded using a sample-and-hold circuit.) Note the slow onset of hyperpolarization in motoneurones and the absence of adaptation in this and the following traces. (B) Cell 3 shows hyperpolarization (Bi), but no depolarization (Bii) upon respective current injection into cell 151. (C) Inhibitory neurones (i) may be activated by hyperpolarization of cell 151, whereas excitatory neurones (here 5/7) are inhibited. (D) Motoneurones with cell bodies lying on the ventral side of the ganglion (such as the excitor of the ventral circular muscles, CV, or the annulus erector motoneurone, AE) also follow hyperpolarization of cell 151 to varying degrees. (Stimulation electrode balanced by bridge circuit.) (E) Effect of hyperpolarizing currents injected into cell 151 (abscissa) on motoneurones 3, 4, 5/7 and L (+). (■) Variability of frequency reduction in cell L within a single preparation. Data points show reduction of frequency as a percentage of average motoneurone frequency 1 s prior to current injection. Correlation significant at $P = 0{\cdot}01$ (see Fig. 6). $N = 58$, nine animals, mean frequency 1 s prior to current injection (= 100 %), 15 ± 1 Hz.

cell 151 and motoneurone 3 or L, current was injected into both cells in standard artificial leech saline and in 0-Ca^{2+}-high-Mg^{2+} saline.

In normal or 0-Ca^{2+}-high-Mg^{2+} saline, hyperpolarization (Figs 8B, 9) passed from cell 151 to motoneurone 3 or L and depolarizations passed from the motoneurones to cell 151. In normal saline (Fig. 9Ai), this depolarization of cell 151 was sometimes masked by IPSPs. Neither hyperpolarization in motoneurones nor depolarization in cell 151 affected the postsynaptic cell (Fig. 9Biii,iv). Therefore, a rectifying electrical synapse exists between cell 151 and motoneurones L and 3. This would explain the ineffectiveness of depolarizing potentials

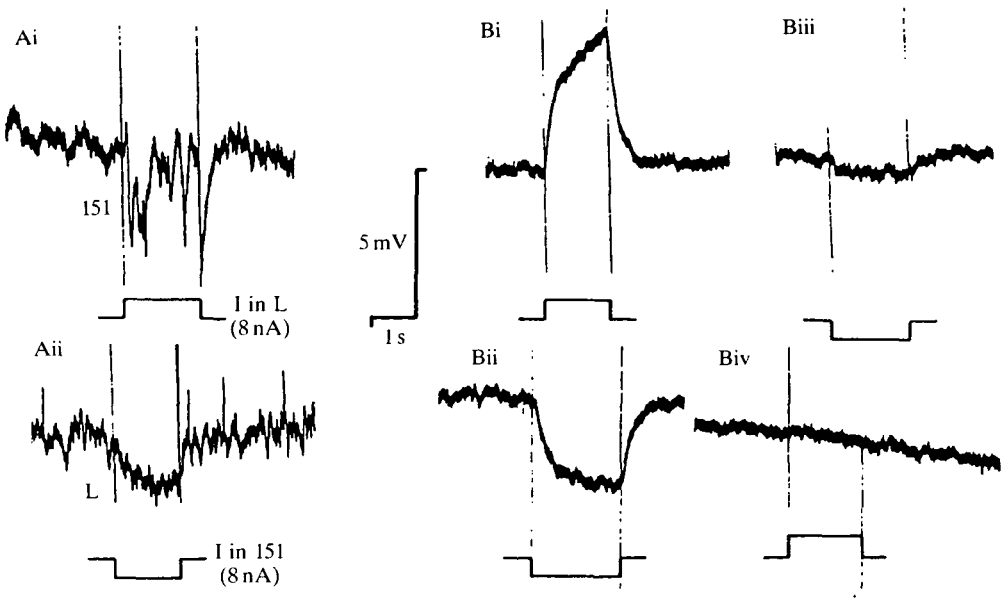

Fig. 9. A rectifying electrical synapse between cell 151 and motoneurone L. (Ai) In standard artificial leech saline, injection of depolarizing current in cell L elicits IPSPs in cell 151. (Aii) Hyperpolarizing currents injected into cell 151 hyperpolarize the motoneurone. (B) In leech saline without calcium but with 40 mmol l^{-1} magnesium, depolarizations (Bi) but not hyperpolarizations (Biii) are transmitted from cell L to cell 151. Hyperpolarizations (Bii) but not depolarizations (Biv) are transmitted from cell 151 to cell L. Only time and direction of current injection into the presynaptic cells are indicated.

and the widespread depression of all efferent activity which occurred when cell 151 was hyperpolarized. The gradual onset of the effect of cell 151 is in keeping with the weakness of the electrical coupling.

Membrane potential of cell 151 correlates with spike frequency in efferent neurones

Hyperpolarizing currents injected into cell 151 cause a reduction of efferent spike activity. Do spontaneous changes in the membrane potential of cell 151 correlate with changes in frequency of efferent spikes? As seen from a sequence of large IPSPs in cell 151 (Fig. 10Ai,ii), such IPSPs reduced extracellular recorded spike frequency by 18%. This also holds true for identified motoneurones (Fig. 11). Almost every IPSP in cell 151 coincided with a spike interval in motoneurone 3 (Fig. 11Ai). Upon increase of IPSP amplitude the interval was widened. In addition, slow potential shifts of both cells were strongly correlated (Fig. 11Aii,iii).

Fig. 11B allows a direct comparison of the effect of current-induced hyperpolarizations of cell 151 with those occurring spontaneously. IPSPs of about 10 mV produced about half the potential change in the postsynaptic cells that was

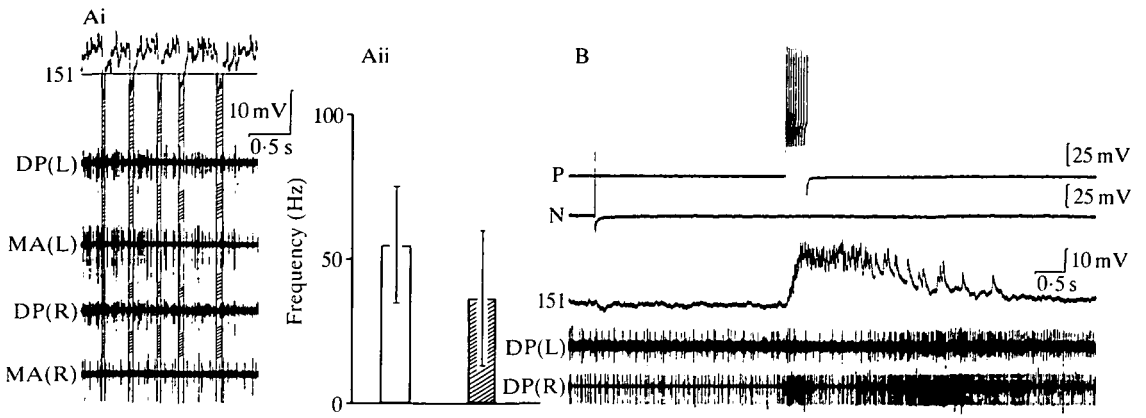

Fig. 10. Spontaneous membrane potential changes in cell 151 and spike activity in the ganglionic roots. (Ai) Large IPSPs in cell 151 often correlate with reduction of efferent spike activity in the roots. A threshold divides the recording of cell 151 (upper trace) into two types of time segments, one during which cell 151 is strongly hyperpolarized (hatched segments), and one during which it is relatively depolarized. Lower traces are extracellular recordings from the roots. (Aii) Mean frequency and standard deviation of action potentials in all nerves shown in Ai recorded while cell 151 was relatively depolarized (open bar) or strongly hyperpolarized (hatched bar). The difference is significant according to the one-sided paired t-test at $P = 0{\cdot}05$. (B) A sensory cell changes the membrane potential of cell 151. A burst of nine action potentials induced by current injection into a pressure-receptive cell (P) causes a strong depolarization of cell 151 during the initial bending reflex. During the slow decay, short PSPs of unknown origin can also be seen. Note that their hyperpolarizing slopes correlate with reduction of spike frequency in the extracellular recordings (two bottom traces).

produced by 4 nA hyperpolarizing current. In both instances hyperpolarizations in AE were smaller than in CV.

Mechanoreceptive cells can drastically alter the membrane potential of cell 151. In the example shown in Fig. 10B, a burst in the P cell caused cell 151 to depolarize at exactly the time when the motor activity of the bending reflex (Kristan, 1982) would be expected. In the second phase of increased spike activity, after termination of the bending reflex, extracellularly recorded signals were grouped in bursts, especially obvious in DP(L). The bursts stopped with hyperpolarizing potential changes of cell 151. A single action potential of an N cell hardly changed the membrane potential of cell 151 (Fig. 10B). A second spike within 150 ms, however, elicited a depolarizing response of up to 45 mV. Thus, strong input is necessary to bring cell 151 into action. Modulation of the membrane potential of cell 151 by sensory cells was of a duration (about 1 s) comparable to spontaneous changes (Fig. 3C). This time course is not a movement artefact, since P and N cell (second trace in Fig. 10B) recordings remained stable.

In conclusion, spontaneous hyperpolarization of cell 151 coincides with drops in extra- and intracellularly recorded neural activity of the ganglion.

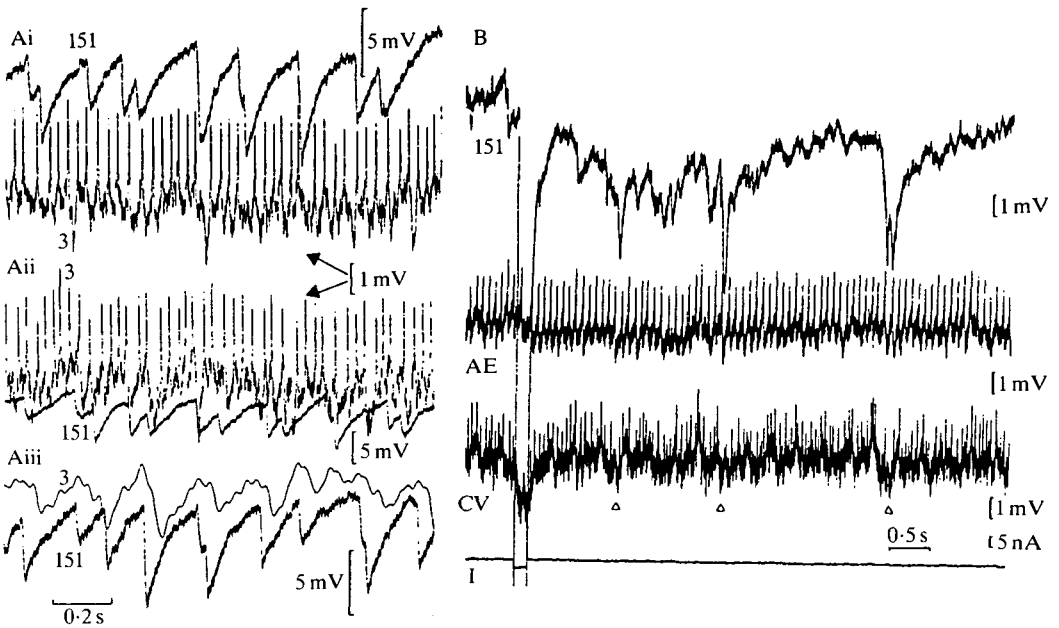

Fig. 11. IPSPs in cell 151 and membrane potential changes in motoneurones. (Ai) IPSPs in cell 151 correlate with gaps between spikes of motoneurone 3. Larger IPSPs correlate with longer gaps. (Aii) Slow potential shifts in cell 151 correlate with and generally precede low-frequency voltage fluctuations in cell 3. (Aiii) Correlation of Aii becomes even more obvious when action potentials of the motoneurone are filtered out (bandpass 0·02–0·17 Hz). (B) Comparison of hyperpolarizations in motoneurones induced by current injection and by spontaneous IPSPs in cell 151. IPSPs of several millivolts occurring spontaneously in cell 151 (151, △) correlate with hyperpolarizations in the CV motoneurone. Their amplitudes are comparable with those in response to a −4 nA current pulse in cell 151 (beginning of recording, electrode not balanced). The hyperpolarization of motoneurone AE is smaller, but marked by gaps between action potentials. I, current trace.

Discussion

The extent of the branching of cell 151 surpasses that of other identified cells in the leech CNS (Retzius, 1891; Muller *et al.* 1981). The two segmental homologues invade the entire neuropile area of a ganglion and send neurites into all roots and connectives. The multiple extensions into the roots and the connectives is a feature unusual in other identified neurones; the Br cell in molluscs (Elekes *et al.* 1983) is the only other example. No cell with such a combination of properties has been described in invertebrates (Bullock & Horridge, 1965; Strausfeld, 1975; Mill, 1975, 1982).

LY or $CoCl_2$ pass through electrical (Stewart, 1981) or chemical (Strausfeld & Bassemir, 1983) synapses, with some restrictions for $CoCl_2$ (Politoff *et al.* 1974; Strausfeld & Obermayer, 1976). In this preparation Cohex and LY crossed non-rectifying electrical junctions equally well. This observation should be considered

for Cohex fills in other preparations. Possible sites of electrical synapses are the anterior and medial commissures, where secondary neurites of both cells 151 intermingle. Thus, Cohex has proved to be useful for intracellular staining in the leech.

The multitude of intracellularly recorded electrical signals and the absence of action potentials is rarely observed in other leech neurones. Action potentials would seem to be necessary to transmit signals along the interganglionic processes. There is a slight possibility that cell 151 produces action potentials in its primary neurite in the connectives, or in fine processes in the roots. However, the signals recorded in the cell bodies would be even smaller than action potentials recorded from motoneurones (1–5 mV, Stuart, 1970). Action potentials generated in the large neurites within the ganglion, however, would have been detected. Therefore, no action potentials are necessary for the intraganglionic effects on motoneurones.

The I/V plot for cell 151 (Fig. 5A) shows a slope resistance half that of other leech neurones with comparable cell body diameter (e.g. Friesen, 1985), but equal to those of T, P, N and Retzius cells (Kleinhaus & Prichard, 1977). The combination of a small cell body with a short connection to the massive primary neurite, the well-developed secondary neurites and the electrical coupling readily account for the values measured in cell 151.

Cell 151 is an example of a single neurone which determines the level of output activity of all main peripheral nerves of an invertebrate ganglion. Action potentials of excitatory motoneurones are depressed by hyperpolarizing currents passing through a rectifying electrical synapse. This type of synapse also explains the rather small effects observed when cell 151 is depolarized. The absence of dye coupling in such a case is compatible with the findings of Friesen (1985).

The effects of cell 151 on motoneurones are not strong compared with membrane potential changes induced by their primary inhibitors. For example, only 5 % of the current is necessary to produce a similar inhibition in motoneurone 3 *via* its inhibitory motoneurone 1 (Granzow *et al.* 1985). Still, inhibition through cell 151 seems to be physiologically meaningful for the following reasons. (1) The diameter and profusion of the branches of cell 151 as well as the tight electrical coupling to its contralateral homologue account for the small input resistance and the need to inject larger amount of currents. (2) Large current injections (3–10 nA) are necessary to reset the swim circuit by small interneurones (Weeks, 1982; Friesen, 1985). (3) Spontaneous membrane potential changes of −30 mV in cell 151 can be mimicked by current injections of −3 nA. This clearly reduces spike frequency in efferent nerves (50 %) and would alter muscle tension (Mason & Kristan, 1982). (4) A strong interaction between cell 151 and many motoneurones would constantly resynchronize their activity and interfere with the production of different motor coordinations.

The extensive ramification of this cell complemented by strong electrical coupling to its contralateral homologue provide the morphological basis for the physiological properties. The massive primary neurite of cell 151 might carry

enough channels for the production of large currents, and should show large length constants. The neuronal surface is likely to exceed that of an isolated Retzius cell body, which produces currents of several hundred nanoamps (Stewart *et al.* 1986). Large primary neurites are also common among nonspiking neurones in the crayfish swimmeret system (Paul & Mulloney, 1985). The uncommon branching pattern of cell 151 in the transitional area between neuropile and roots is also of functional interest, since action potentials of motoneurones are initiated in this region (Granzow *et al.* 1985).

Furthermore, this cell is under the influence of sensory cells which are known to take part in the initiation of several motor patterns (Fig. 10; Kristan, 1982; Debski & Friesen, 1987). IPSPs, EPSPs and slow potential changes are triggered not only by sensory input (Fig. 10) but also by depolarizing currents into cell 151 and by motoneurone spikes.

Thus, two of the three criteria necessary to establish a function of a neurone, as stated by Kupferman & Weiss (1979), are fulfilled: (1) current injection into cell 151 *causes* the depressing effect on many motoneurones, and (2) spontaneous hyperpolarizations in cell 151 *correlate* with reduction in efferent spike frequency. That this cell is indeed *necessary* (3) for the functioning of the ganglion must be proved by photoinactivation experiments.

Cell 151 might collect and relay signals representing the activity state of the motoneurones, as shown for the nonspiking cell C (Muller & Scott, 1981). In addition, it might distribute and even amplify inhibition of motoneurones, minimizing excessive activity. Depolarization of motoneurones (Fig. 9Ai) and of cell 151 itself (Fig. 5B) are prompted by large IPSPs in cell 151, which, in turn, reduce excitation in motoneurones (Fig. 11). For one cell to exert such widespread and uniform influence on so many neurones is unique. It occurs during normal functioning of the ganglion and might be part of a negative feedback loop.

The author thanks Professor W. Rathmayer for essential support in his laboratory and for detailed comments on the manuscript. Drs A. Wenning-Erxleben and C. Erxleben were of great help in revising the manuscript. Drs A. Mason and J. Gloever introduced me to their version of the HRP procedures. Ms M. A. Cahill improved the English and Ms R. Eberle assisted in all histological procedures and in the preparation of the figures.

References

BACON, J. & ALTMAN, J. S. (1977). A silver intensification method for cobalt-filled neurons in whole mount preparations. *Brain Res.* **138**, 359–363.

BROGAN, R. T. & PITMAN, R. M. (1981). Axonal regeneration in an identified insect motoneuron. *J. Physiol., Lond.* **319**, 34–35P.

BULLOCK, T. H. & HORRIDGE, G. A. (1965). *Structure and Function in the Nervous System of Invertebrates*. Two vols. San Francisco: W. H. Freeman.

BURROWS, M. & SIEGLER, M. V. S. (1978). Graded synaptic transmission between local interneurones and motoneurones in the metathoracic ganglion of the locust. *J. Physiol., Lond.* **285**, 231–255.

DEBSKI, E. A. & FRIESEN, W. O. (1987). Intracellular stimulation of sensory cells elicits swimming activity in the medicinal leech. *J. comp. Physiol* A **160**, 447–457.

ELEKES, K., VEHOVSZKY, Á. & SALÀNKI, J. (1983). Ultrastructure of synaptic connections of a bimodal pacemaker giant neuron in the central nervous system of *Helix pomatia* L. *Neurosci. Letts* **8**, 617–629.

FRIESEN, O. (1985). Neuronal control of leech swimming movements: interactions between cell 60 and previously described oscillator neurons. *J. comp. Physiol.* A **156**, 231–242.

GRANZOW, B., FRIESEN, O. & KRISTAN, W. B., JR (1985). Physiological and morphological analysis of synaptic transmission between leech motor neurons. *J. Neurosci.* **5**, 2035–2050.

HOBSON, J. A. & BRAZIER, M. A. B. (1980). *The Reticular Formation Revisited: Specifying Function for a Nonspecific System.* International brain research organization monograph series, **6**. New York: Raven Press.

HENGSTENBERG, R. (1977). Spike response of "nonspiking" visual interneurones. *Nature, Lond.* **270**, 338–340.

JACOBS, B. L. (1986). Single unit activity of locus coeruleus neurons in behaving animals. *Progr. Neurobiol.* **27**, 183–194.

KLEINHAUS, A. L. & PRICHARD, J. W. (1977). Close relation between TEA responses and Ca-dependent membrane phenomena of four identified leech neurones. *J. Physiol., Lond.* **270**, 181–194.

KRISTAN, B. W., JR (1982). Sensory and motor neurones responsible for the local bending response in leeches. *J. exp. Biol.* **96**, 161–180.

KRISTAN, W. B., JR, STENT, G. S. & ORT, C. A. (1974*a*). Neuronal control of swimming in the medicinal leech. I. Dynamics of the swimming rhythm. *J. comp. Physiol.* A **94**, 97–119.

KRISTAN, B. W., JR, STENT, G. S. & ORT, C. A. (1974*b*). Neuronal control of swimming in the medicinal leech. III. Impulse pattern of motor neurons. *J. comp. Physiol.* A **94**, 155–176.

KUPFERMAN, I. & WEISS, K. R. (1979). The command neuron concept. *Behav. Brain. Sci.* **1**, 3–39.

MACAGNO, E. R. (1980). Number and distribution of neurons in leech segmental ganglia. *J. comp. Neurol.* **190**, 283–302.

MASON, A. & KRISTAN, B. W., JR (1982). Neuronal excitation, inhibition and modulation of leech longitudinal muscle. *J. comp. Physiol.* A **146**, 527–536.

MILL, P. J. (1975). The organisation of the nervous system in annelids. In *"Simple" Nervous Systems* (ed. P. N. R. Usherwood & D. R. Newth), pp. 211–264. London: Edward Arnold Publ.

MILL, P. J. (1982). Recent developments in earthworm neurobiology. *Comp. Biochem. Physiol.* **73**A, 641–661.

MULLER, K. J., NICHOLLS, J. G. & STENT, G. S. (1981). *Neurobiology of the Leech.* Cold Spring Harbor: Cold Spring Harbor Laboratory.

MULLER, K. J. & SCOTT, S. A. (1981). Transmission at a direct electrical connection mediated by an interneuron in the leech. *J. Physiol., Lond.* **311**, 565–583.

NICHOLLS, J. G. & BAYLOR, D. A. (1968). Specific modalities and receptive fields of sensory neurons in the CNS of the leech. *J. Neurophysiol.* **31**, 704–756.

ORT, C. A., KRISTAN, W. B., JR & STENT, G. S. (1974). Neuronal control of swimming in the medicinal leech. II. Identification and connections of motoneurons. *J. comp. Physiol.* A **94**, 121–154.

PAUL, D. H. & MULLONEY, B. (1985). Nonspiking local interneuron in the motor pattern generator for the crayfish swimmeret. *J. Neurophysiol.* **54**, 28–39.

POLITOFF, A., PAPPAS, G. D. & BENNETT, M. V. L. (1974). Cobalt ions cross an electrotonic synapse if cytoplasmatic concentration is low. *Brain Res.* **76**, 343–346.

RETZIUS, G. (1891). Zur Kenntnis des centralen Nervensystems der Würmer. *Biologische Untersuchungen, Neue Folge* II, 1–28.

SAWYER, R. T. (1986). *Leech Biology and Behaviour*, vol. 1. Cambridge: Clarendon Press.

STEWART, R. R., ADAMS, W. B. & NICHOLLS, J. G. (1986). Na-, K- and Ca-currents in identified leech neurones in culture. *Soc. Neurosci. Abstr.* **12**, 1350.

STEWART, W. W. (1981). Lucifer dyes – Highly fluorescent dyes for biological tracing. *Nature, Lond.* **292**, 17–21.

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

STRAUSFELD, N. J. (1975). *Atlas of an Insect Brain.* Berlin-Heidelberg-New York: Springer.

STRAUSFELD, N. J. & BASSEMIR, U. K. (1983). Cobalt-coupled neurons of a giant fibre system in Diptera. *J. Neurocytol.* **12**, 971–991.

STRAUSFELD, N. J. & OBERMAYER, M. (1976). Resolution of interneuronal and transsynaptic migration of cobalt in the insect visual and central nervous systems. *J. Comp. Physiol.* A **110**, 1–12.

WADEPUHL, M. (1986). Reduktion von motorischer Spontanaktivität beim Blutegel durch segmentale Zellpaare. Neurobiologen Tagung Göttingen. In *Sensomotorik/Identifizierte Neurone: Beiträge zur 14. Göttinger Neurobiologentagung* (ed. N. Elsner & W. Rathmayer), pp. 54. Stuttgart, New York: Thieme.

WADEPUHL, M. (1987). A morpho- and physiologically uncommon neuron in the leech CNS. *Naturwissenschaften* **74**, 43–45.

WADEPUHL, M. & SCHÄFFNER, K. H. (1987). An unusual cell in the CNS of the leech: Correlation with motoneuron activity and its fine structure. In *New Frontiers in Brain Research: Proceedings of the 15th Göttingen Neurobiology Conference* (ed. N. Elsner & O. Creutzfeld), p. 68. Stuttgart, New York: Thieme.

WEEKS, J. C. (1982). Synaptic basis of swim initiation in the leech. II. A pattern-generating neuron (cell 208) which mediates motor effects of swim-initiating neurons. *J. comp. Physiol.* A **148**, 265–279.

WILKINSON, M. & COGGESHALL, R. E. (1975). Axonal number and sizes in the connectives and peripheral nerves of the leech. *J. comp. Neurol.* **162**, 387–396.