MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES OF A STRETCH RECEPTOR NEURONE IN THE LEECH, *Hirudo medicinalis*

by

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Blackshaw,S.E., Parnas,I. and Thompson,S.W.N. (1984b) Changes in the central arbourisation of primary afferent neurones during development of the leech nervous system.
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SUMMARY

This study gives the first description of an identified stretch receptor neurone in a soft bodied invertebrate.

Intracellular recordings were made from the stretch receptor neurone (SRN), whose peripheral cell body and terminals lie in the ventral longitudinal muscle of the body wall of the medicinal leech, Hirudo medicinalis. Injection of depolarising current (3-4 nA) into the cell body elicited a large (40-70 mV) overshooting action potential. Paired intracellular recordings made from the cell body and its from its axon approximately 3-4 mm away as it enters the segmental ganglion showed that the action potential elicited in the soma is not actively propagated. Rather, imposed voltage changes are transmitted decrementally to the central nervous system, the axon being unable to support regenerative activity. The large length constant of the SRN (4.1 mm) and high specific membrane resistance (22.0 Kohm cm^2) make the cell well adapted to conduct voltage changes over large distances with minimal decay of signal amplitude.

Intracellular injection of the enzyme horseradish peroxidase (HRP) and the fluorescent dye Lucifer Yellow (LY) was used to study the morphology of the SRN. Two flat fan-shaped sensory terminals, approximately 70 µm wide but only 2-3 microns deep are separated by a large (70 µm times 30 µm) cell body. A 10 µm diameter axon runs centrally from the proximal fan shaped dendrite towards the CNS and at a distance of approximately 3-4 mm arborises within the ipsilateral half of the segmental ganglion in a characteristic branching pattern.

The response of the neurone to changes in body wall length was studied by applying single ramp stretches or ramp releases to a thin strip of the ventral longitudinal muscle with which the SRN was associated whilst recording intracellularly from its axon as it enters the segmental ganglion. The cell responded to a ramp stretch stimulus with an electrotonically conducted hyperpolarising potential, maintained for the duration of the stretch. The neurone responded to a ramp release of the longitudinal muscle with an electrotonic depolarising potential. This consisted of an initial dynamic component followed by a lower amplitude static response which was maintained for the duration of the ramp stimulus.

The responses of the cell to both a ramp release and ramp stretch were graded and proportional to the final amplitude of the stimulus. Stepped changes of body wall length resulted in concomitant stepped hyperpolarisation or depolarisation of the SRN membrane potential. Evidence is presented that the SRN responds to changes in longitudinal muscle tension rather than its absolute length. From observations on the tension changes which accompany natural changes in body wall posture and which occur during locomotory behaviours, it is apparent that tension changes during active contraction and relaxation of the dorsal and ventral longitudinal muscle are not those which are predicted from observation of the passive behaviour of the muscle during imposed stretches and ramp releases.

The SRN is innervated by sensory neurones whose cell bodies are situated within the CNS. Paired intracellular recordings have shown

that the SRN is post synaptic to three different modalities of primary sensory neurone. Action potentials arising in one of these mechanosensory neurones, the ipsilateral lateral nociceptive neurone, are followed on a 1:1 basis and with a constant latency by an excitatory post synaptic potential (epsp) in the peripheral cell body of the SRN. Action potentials elicited by direct depolarisation of all centrally situated ipsilateral touch and pressure mechanosensory neurone cell bodies also elicited epsp's in the SRN soma. No synaptic connectivity was recorded either between the ipsilateral medial nociceptive neurone and the SRN or any contralaterally sited mechanosensory neurones and the SRN.

The central connections of the SRN with identified motoneurones to the longitudinal muscle of the body wall was investigated. Dual intracellular HRP injections showed that close apposition of SRN axon branches and dendrites from the large longitudinal motoneurone (L cell) occured within the ganglionic neuropile. Considerable overlap of the dendritic trees of motoneurones involved in the swimming rhythm and the SRN were also shown within the neuropile.

Paired electrical recordings were made to investigate the effect of SRN activity upon motoneurone discharge. Imposed DC changes of the membrane potential of the ventral stretch receptor neurone did not produce any consistent modulation of the activity of individual identified motoneurones recorded either intracellularly from their centrally situated somata or extracellularly from their projecting axons. Preliminary experiments were carried out to determine the

degree of coherence of motoneurone activity in the presence and absence of SRN input. When this method was applied to three simultaneous extracellular motoneurone spike trains the coherence of activity between any two motoneurones was only significant in the presence of the SRN input.

INTRODUCTION

GENERAL INTRODUCTION

The need for feedback

In order that a muscle be a useful servant under the control of the nervous system, it must be susceptible to precise control over a wide range of muscle lengths, tensions, speeds and loads. Even relatively simple movements may involve the coordination of many separate muscles with the corresponding need for precision of control of their individual lengths and tensions. For vertebrate and invertebrate alike the problem is the same, that control of body position be accomplished in the face of such variables as muscle fatigue, contraction of antagonists and synergists (including the homonymous muscle)μ and externally applied disturbances as might be encountered.

For the majority of free living animals therefore it is important that correct sensory information is presented to the central nervous system, which is responsible not only for the initiation of motor sequences but also their subsequent realisation and the monitoring of external distubances effecting such activity. In such a way modifications may be made to a continuing motor output, or to initiate new patterns of activity.

Stretch receptors, highly specialized sensory organs, relay proprioceptive information arising as a result of stretch or contraction of the muscle with which they are intimately associated. They occur across a number of animal groups and function in a

basically similar manner. Their terminals are deformed by stretch and are often inserted into specialized muscle fibres. A comparison of vertebrate stretch receptors with various types of invertebrate receptors show interesting parallels in their construction and arrangement, each designed to perform the same proprioceptive function, but variously adapted to their own particular requirements.

REVIEW OF THE LITERATURE

CHAPTER 1.0 VERTEBRATE AND INVERTEBRATE STRETCH RECEPTORS

1.1 The Vertebrate Muscle Spindle

The literature concerning the structure and function of stretch receptors is legion. This is particularly true of the vertebrates where the study of muscle sensory end organs has made considerable advances since the first description in 1863 of "muskelspindeln" in mammals by Kuhne. Subsequent controversy over their function was resolved by the curiously overlooked work of Onanoff in 1890 and in 1894 by Sherrington. After sectioning of the ventral spinal roots, Onanoff observed atrophy of only a few of the nerve fibres to the spindle, whilst destruction of the dorsal root ganglia caused nearly all of them to degenerate. He wisely conluded that the muscle spindle was a sense organ under motor control. Sherrington (1894) described the effects of simultaneous section within the dura of the dorsal and ventral spinal roots in the cat and the monkey. This causes degeneration of muscle motor fibres whilst leaving the gross morphology of the afferent fibre terminals unaffected. He concluded that "the muscle spindle proves therefore to be a sensorial organ". From his investigation of the structure and innervation of the muscle spindle and his observations regarding the knee-jerk reflex in vastus medialis before and after spindle organ ablation, the tentative inference was made that "the stimulus to which the organs are specially adapted is mechanical in quality"

It was not until the early 1930's that B.H.C Matthews made the first single unit recordings from muscle afferent nerve fibres to

give the first general outline of the functioning of the muscle spindle and some aspect of its control. On the basis of the different responses of a number of afferent units to steady stretch or contraction of the appropriate muscle in the cat, he described 3 types of sensory discharge named A, B and C. He suggested that the A discharge was derived from muscle spindle endings, B activity derived from Golgi tendon organs and C activity originated from free nerve endings in the muscle fascia. He further subdivided the A sensory discharge into into two characterisyic types, one of which adapted rapidly and the other which adapted slowly to a muscle contraction elicited by a supramaximal excitation of the muscle nerve. This was perhaps a natural consequence of Ruffini's earlier histological description of distinct primary and secondary afferent endings from the spindle (Ruffini 1898). Such a dual structural and functional approach has since been widely applied to the mechanism of action of the mammalian muscle spindle.

It was not until the work of Hunt (1954) and later Cooper (1959, 1961) that a suitable functional criterium for distinguishing between the primary and secondary afferent discharge was agreed upon. Hunt identified primary and secondary fibres by means of their different conduction velocities and suggested that spindle fibres of group I diameter which terminated as the primary sensory endings had a lower threshold to stretch than the smaller diameter group II fibres which terminated as the secondary sensory endings. It was Cooper however, again using the criteria of conduction velocity to identify the primary and secondary afferent fibres, who demonstrated the real physiological differences of the two endings ; namely the greater

dynamic sensitivity of the primary ending to an imposed stretch of the muscle than that of the secondary endings. Coopers' work, later confirmed by Harvey and Matthews (1961) and Matthews (1963) showed that the response of primary and secondary endings to a maintained stretch are approximately equal whilst the primary ending is much more sensitive to the dynamic part of the stimulus. Hence the existence of two characteristic types of muscle afferent types was established. Both types show approximately an equal position sensitivity, whilst one is distinguished by its greater velocity sensitivity. stimulusμ the other which is slowly adapting. A feature common with other, invertebrate, stretch receptors.

A further feature in common with other stretch receptor systems is the existence of an independent motor supply to the intrafusal muscle fibres. In 1930 Eccles and Sherrington demonstrated the division of motoneurone axon diameters in muscle nerves into two distinct populations. In 1945 Leksell succeeded in recording the compound "gamma" wave produced by activation of the smaller axons. Subsequently in an important experiment he used a pressure block to selectively block impulse transmission in the larger, lower threshold alpha motoneurones and demonstrated that stimulation of the sciatic nerve at a stimulus intensity sufficient to excite the higher threshold gamma fibres produced no significant tension within the gastrocnemius muscle. He further showed that selective activation of the smaller diameter motor fibres increased the afferent discharge from the muscle provided the muscle was subject to some initial degree of stretch. The function of the gamma or "fusimotor" fibres as they were called, was rapidly confirmed by the

elegant work of Kuffler, Hunt and Quilliam (1951). They showed that stimulation of a single gamma axon to a muscle spindle could increase the frequency of discharge of a single spindle afferent fibre from that muscle without producing any measurable tension increase.

The intrafusal muscle fibres have now been divided into three distinct groups, each distinguished by its response characteristics its histological profile, the type of contraction it undergoes and the specific type of gamma axon it receives. Thus many years of painstaking work by Barker (1970), Boyd (1981) and colleagues has established the division of fusimotor axons supplying the intrafusal muscle fibres into two groups. (i), the dynamic gamma axons, terminating exclusively on bag 1 fibres the activity of which leads to increased sensitivity of the Ia afferent endings to the velocity of muscle stretch, and (ii), the static gamma axons which end on both bag 2 and chain fibres and are responsible for the static component of the spindle discharge.

1.2 The Crustacean Muscle Receptor organ.

In contrast to the now well documented mammalian muscle spindle, no corresponding structures were known to exist in any invertebrate group until the description by Alexandrovicz in 1951 of sensory structures in the dorsal abdominal muscle of the crustaceans Homarus vulgaris and Palinurus vulgaris. Assuming that these organs responded to stimuli resulting from muscular activity, he adopted the name muscle receptor organs (MRO's) for them. Most species of decapod crustacea now examined are known to possess MRO's associated with the dorsal musculature of the abdomen (Alexandrovicz, 1951, 1952a, 1952b, 1956; Pilgrim, 1960.) all of which are similar in their structure and response to stretch.

Alexandrovicz (1951), using Methylene Blue staining showed that the majority of the abdominal segments of the lobster had two pairs of MRO's, one either side of the mid-line and closely associated with the superficial dorsal extensor musculature. They bridge the joint between successive segments and hence are extended when the abdomen is flexed. Each MRO is composed of a receptor muscle (RM) and the closely associated cell body of the sensory neurone plus its afferent axon, along with efferent axons varying in number depending upon the species.

The two RM's on a given side of a segment differ from each other in size, histological appearance, pattern of innervation and points of attachment. The shorter of the pair (RM1) usually lies more laterally and is often the thinner of the two. The histological profiles of RM1 and RM2 reflects the respective fast and slow categories into which these muscles fall. The lateral RM1 is a tonic muscle, undergoes slow graded contractions and is analagous to the superficial tonic extensors of the abdomen, and indeed is innervated by branches of the motoneurones supplying this muscle. The medial RM2 is a phasic muscle which undergoes rapid twitchlike contractions and is innervated by branches of the motoneurones supplying the deep phasic abdominal musculature.

Unlike the mammalian muscle spindle afferents, the sensory neurones associated with the crustacean RM's are conspic ously large. The cell bodies lie in close association with the RM and are often over 100 microns in diameter. The terminal dendritic tree is embedded in a region of connective tissue in the centre of the respective receptor muscle. Its shape varies according to species and the RM with which it is associated (Alexandrovicz 1951, 1952 ; Florey and Florey 1955). Sensory neurone 1 (SN1) is associated with the lateral RM1 and has an obliquely arranged dendritic field. Together with RM1 it forms the tonic, slowly adapting MRO. Sensory nuerone 2 (SN2) is associated with the medial RM2 and has bushy short dendrites arranged more perpendicular to the RM. SN2 and RM2 together form the phasic, rapidly adapting MRO.

In addition to the large afferent neurones, three types of efferent axons supply each MRO (Alexandrovicz, 1951, 1952 Florey and Florey, 1955.). The actual arrangement of the three elements differs from species to species. In the lobster, Alexandrovicz reported two large diameter motor axons, one to each RM. In addition each receptor muscle is often innervated at its polar region by branches of the motor axons supplying the adjacent dorsal abdominal muscles, (analogous to the shared alpha-motoneurone supply of the frog extra and intrafusal fibres (Katz, 1949) and the dynamic and static beta supply of the mammalian muscle spindle (Laporte, 1980). Also described was a number of "accessory" fibres innervating the afferent neurones directly. Three such neurones have been shown in the lobster (Alexandrovicz, 1967) and in the crayfish (Jansen,Nja, Ormstad and Walloe, 1971). Alexandrovicz's idea that these accessory nerves may exert "a suppressing action on the excitability of the MRO" was confirmed by Kuffler (1954) who showed that activating the accessory axon to the SN1 blocked impulse initiation in that neurone.

Alexandrovicz's hypothesis (Alexandrovicz, 1951) based upon anatomical considerations, that the response properties of the two MRO's on any one side were different, was confirmed by the first physiological study of the receptors by Wiersma, Furshpan and Florey (1953). They found that trains of impulses of two distinct amplitudes could be recorded from the dorsal nerve trunk of a stretched segment and that the two spike trains showed different properties. The most noticeable difference in the behaviour was in the response to a maintained muscle stretch. One of the spike trains

adapted very slowly to the stimulus whilst the other responded in a phasic, rapidly adapting manner. By selective ablation of one or other of the sensory neurone cell bodies, Wiersma et.al. showed that the tonic activity arose from RM1 and that of the phasic activity was from RM2.

Unlike the vertebrate spindle, which lies hidden in the body of the muscle, the Crustacean MRO is a separate and external organ, easy to dissect, isolate and maintain as an experimental preparation for several hours. It has then several attributes for the study of the process of sensory transduction. The process of excitation and inhibition in the receptor neurones was studied in a definitive series of papers in the early fifties by Carlos Eyzaguire and Stephen Kuffler (Kuffler, 1954 Eyzaguire and Kuffler, 1955a,b; Kuffler and Eyzaguire, 1955.) the results of which had great bearing upon the understanding of the functioning of nervous elements in general.

Recording intracellularly from the cell body of the neurone they showed that stretch applied to the receptor muscle resulted in depolarisation which followed precisely the time course of the distortion. The depolarisation was graded, depending upon the rate and degree of stretch, and that this graded depolarisation or "generator potential" spread electrotonically from the dendrites to the soma, later confirmed by Terzuolo and Washizu (1960, 1962). Both SN1 and SN2 are, as we have seen, also innervated by efferent inhibitory fibres which correspond to the thick and thin accessory

fibres of Alexandrovicz (1951). Activity within these fibres reduces the amplitude of the generator potential hence preventing threshold depolarisation at the spike initiating zone of the cell. (Kuffler, 1954). It is the balance of such inhibitory and stretch-induced excitatory action which determines wether the membrane potential is set above or below the threshold firing level.

Hence the crustacean MRO contains many features essentialy similar to the mammalian muscle spindle. In both systems sensory neurones, deformed by stretch, are associated with specialized receptor muscles arranged in parallel with the main force-producing fibres. Each system has one or more rapidly adapting afferent neurones associated with a receptor muscle of "fast" histological profile and one or more afferent whose discharge adapts slowly and which is associated with receptor muscles of "slow" histological profile. Indeed the basis of the excitatory afferent discharge in both crustacean and mammalian system is the same in the existance of the underlying depolarising receptor potential (Katz, 1950 Kuffler, 1954). Both systems are under independant motor control and both have two means for eliciting afferent discharge, (i) stretch of the muscle in which the receptor terminals are embedded, and (ii) active contraction of the receptor muscles themselves, producing deformation of the receptor terminals. Moreover the crustacean stretch receptor is capable of even finer control via the efferent inhibitory fibres.

1.3 Insect Muscle Receptor Organs

In contrast to the literature covering stretch receptors in the Vertebrates and the Crustaceans, the electrophysiological investigation of stretch receptors among the Insects is a relatively unexplored area of work. The first description of structures described as stretch receptors was that of Finlayson and Lowenstein (1955, 1958). Using Methylene Blue staining techniques they described sensory structures in representatives of the insect orders Orthoptera, Odonata, Hymenoptera, and Lepidoptera. These studies were further extended by Osborne and Finlayson (1962). Again using material stained vitally with Methylene Blue, a description of structures within a further seven insect orders was given, Ephemeroptera, Plecoptera, Dictyoptera, Dermaptera, Coleoptera, Neuroptera, and Trichoptera. Only however in insects from the three orders Neuroptera (Lacewings,Antlions), Trichoptera (Caddisflies), and Lepidoptera (Butterflies and Moths) is there a special receptor muscle which is independent from the ordinary muscle of the body, with a separate motor innervation and which is hence a true muscle receptor organ.

The muscle receptor organs present in such insect species are generally few in number and attached to the intersegmental folds at each end of the segment by connective tissue fibres. In the final instar larva of Antheraea pernyi, a member of the giant silkmoth family, there is a pair of such receptors in the meso- and

metathorax and in abdominal segments 1 to 9. The modified muscle fibre forming the RM lies just above the dorsal longitudinal muscle. The morphology of individual Lepidopteran MRO's shows remarkable similarities to those of the Crustacean MRO (see Finlayson and Lowenstein, 1958 and Alexandrovicz, 1951). In the moth therefore as in crustacea the peripheral cell body and short terminal dendrites of the receptor neurone are closely associated with the receptor muscle. In the case of the insect MRO however the receptor muscle consists of a single muscle fibre. In the central region of this fibre, generally adjacent to the sensory neurone soma, is a swelling enclosing the giant nucleus of the muscle cell, often measuring over 600 µm in length. In this central region of the receptor, bare terminal tips of the sensory neurone dendrites become closely apposed to the surface of the receptor muscle fibre. The receptor muscle also receives an efferent supply at this central region and at about a dozen other points along the fibre from a separate branch of the tergal nerve which runs to innervate the remainder of the dorsal musculature of the segment.

The response of the Lepidopteran MRO to stretch was first investigated by Finlayson and Lowenstein (1958). They showed that the MRO was a tonic receptor which maintained a regular resting dischage for many hours. The unadapted peak frequency of discharge reached immediately after stretching showed a linear relationship to the displacement up to a maximum length, after which "over stretch" and a fall in the discharge frequency was seen. When an MRO was stretched at any velocity a phasic response was also apparent" that is, the discharge throughout the period of stretching was higher

than would be expected from the position sense at any length alone.

In a later more comprehensive analysis of the complex response of the Lepidopteran MRO, Weevers recognized three components of the receptor response (Weevers, 1966a). i) A position response as previously described by Finlayson and Lowensteinμ ii) a velocity response, and iii) an acceleration response, represented by the overshoot of increased frequency at the begining of a ramp stretch and upon relaxation. Weevers showed that this "change of rate response" is a true acceleration response and hence not to be compared with the initial burst of activity seen in the frequency time curve of the response of the mammalian muscle spindle to a ramp stretch. This has been described in the muscle spindle as a πstarting to moveπ function, the prominance of which varies under different conditions and is most likely dependent upon a static frictional force rather than the absolute value of the acceleration

A striking difference between the insect MRO and either the crustacean abdominal muscle receptor organ or the mammalian muscle spindle is that the insect MRO has only a single type of sensory neurone to the receptor muscle. The receptor organ itself is composed of only three cells, the sensory neurone, the muscle cell and an additional "fibre tract cell". This single sensory neurone is however capable of signalling three components present in the stretch stimulus. Its adaption rate is far lower than that of either the crustacean tonic receptor or the secondary endings of the vertebrate muscle spindle. As an interesting consequence therefore,

only two receptors per segment are necessary in order to rapidly supply exact information about the insects movements.

The insect MRO, like the crustacean MRO and the muscle spindle, shows an increased sensory discharge frequency upon stimulation of the efferent nerve supply to the receptor muscle (Weevers, 1966b). Similar to the crustacean MRO, the changes in RM efferent discharge exert a negative feedback control over MRO afferent discharge. Hence upon MRO extension the tonic RM efferent activity is reflexly inhibited, preventing overstimulation of the afferent terminals. Relaxation of the MRO allows reflex disinhibition of the RM motor activity which takes up the slack from the RM. An important function of the RM therefore is to extend the useful range of lengths and velocities over which the MRO may operate.

CHAPTER 2.0 CONTROL OF CELL FUNCTION BY NON-IMPULSIVE ACTIVITY.

The activity and responses of the three proprioceptors previously described fall neatly within the traditional concept of a nervous system in which the nerve impulse and nervous function are closely bound together. From the earliest work upon nerve excitability the idea was soon established that activity was associated with an impulse which propagated down the axon with finite velocity. From the results of work carried out over the past few decades however, it has become increasingly apparent that there are a number of exceptions to this general rule. It is now clear that the basic functions of neuronesμ the reception, transmission, generation and integration of nerve signals, may be carried out in the absence of all-or-nothing impulses.

At about the same time that Alexandrovicz described the MRO's in the abdomen of the lobster, the first intracellular recordings of graded, non-impulse activity were obtained from the vertebrate neuromuscular junction (Fatt and Katz, 1951). A few years later the first intracellular recordings of post-synaptic excitatory and inhibitory potentials in the mammalian CNS were obtained (Coombs et.al., 1955a,b). The importance of graded electrical activity in the overall functioning of neurones was further reinforced in the papers of Eyzaguire and Kuffler (1955a,b, Kuffler and Eyzaguire, 1955). Realizing the advantage of the crayfish stretch receptor

organs with their accessible cell bodies and the ability to control the state of excitability of the cells' via their physiological response to stretch, the mechanism of excitation between dendrites, cell soma and axon was studied. It was shown that excitation originated in the distal portions of the dendrites which were depolarised by stretch deformation. This depolarising potential, the generator potential, was maintained for the duration of the stretch. Electrotonic spread of the generator potential from the dendrites reduced the membrane potential of the nearby cell body. Thus the resting potential and the level of excitability of the cell body were set by local events occuring within the dendrites.

At this time the distinction between the all-or-nothing impulse and the graded receptor and synaptic potentials seemed quite clear, and both were clearly interlinked. What was uncertain however was the role played by the graded, prolonged and decrementally conducted potentials which spread through the complicated geometry of dendritic trees. To what extent could these non-propagated potentials influence the integrative activity of the neurone ? And could in fact synaptic output be mediated without the involvement of an all-or-nothing impulse ? Closer study of the neuromuscular junction in fact revealed that synapse activation was not critically dependent upon propagation of the impulse into the terminal itself. Rather, it was the degree of depolarisation of the terminal membrane which was important. del Castillo and Katz (1954) showed that at the frog neuromuscular junction the post-synaptic events could be modified by local electrotonic changes in the pre-synaptic nerve endings, without the involvement of nerve impulses. They observed

that the frequency of random minature end-plate potentials and that the subsequent size of the transmitted response, more specifically, the quantal content of the end-plate-potential, were continuosly graded with the degree of pre-synaptic membrane depolarisation.

Although the frog neuromuscular junction had a number of advantages for studying the mechanism of transmitter release, a serious drawback of the preparation was that it was not possible at the time to make intracellular recordings from the fine pre-synaptic terminals and thereby obtain direct measurements of the pre-synaptic membrane potential. This problem was overcome by Katz and Miledi (1967) who used the "giant" synapse of the squid stellate ganglion. Again they demonstrated that a passive potential was no less effective in producing a post-synaptic response than was the action potential. Transmitter release was not due to regenerative sodium entry, but to the level of pre-synaptic depolarisation itself. Of course this work has its own intrinsic importance in the detailed analysis of synaptic mechanisms. For the purposes of the present discussion however, it was also important in demonstrating that synaptic output from any point of a neurone can be effectively controlled by any small, graded depolarisation of the pre-synaptic membrane at or near that point. The idea that synaptic output as well as inputs could occur on cell bodies or dendrites was of course quite revolutionary and totally alien to the "dynamic polarisation" doctrine of the neurone widely held at the time. At this time therefore the distinction between neuronal dendrites with a function based purely on integration of presynaptic input and cell axons with a simple output function was becoming less clear. A revision of the

semantics of cell topography appeared necessary, demonstrated by the description of the non-impulsive integration within the retina.

2.1 Non-impulsive activity and integration within the retina

Among the first cell types to exhibit synaptic output from dendrites (dendro-dendritic synapses) were the retinal neurones of the primate eye (Dowling and Boycott, 1966). The retina is a particularly favourable structure for the analysis of the organization of nervous tissue. It is one of the most readily accessible and simplest parts of the central nervous system, it contains only five types of cells whose somata are distributed into three descrete layers. The synaptic contacts are limited to two layers, each layer of which has just three cell types contributing synapses to it. Finally the flow of information may be regarded as travelling from photoreceptor cells to ganglion cells, resulting in a relatively simple situation when deducing likely synaptic contacts.

Electron microscope studies have revealed several important functional considerations concerning synaptic interaction. The amacrine cells, by definition cells without impulses, have processes which ramify throughout the inner plexiform layer of the retina and make contact primarily with the bipolar cells. These contacts with the bipolar cells are reciprocal, the amacrine cells receive inputs from bipolar cells as well as synapsing back with them. Similarly,

horizontal cells, whose processes are seen to be neither clearly dendritic nor axonic in appearance, are post-synaptic to both types of photoreceptor cell as well as being reciprocally pre-synaptic to the photoreceptor cells.

It was clear from the outset that because of the unusual synaptic organization of the horizontal and amacrine cells , they might be expected to demonstrate some unusual physiological properties. In a fine investigation Werblin and Dowling (1969), recording intracellularly from the retina of Necturus maculosus, described the electrical characteristics of the five cell types present. Only the amacrine and ganglion cells, cells of the inner plexiform layer, responded to illumination with classical regenerative spike activity which were generally superimposed upon a small membrane depolarisation. The cells of the outer plexiform layer, the photoreceptors, horizontal and bipolar cells responded with slow, graded potentials to a light stimulus. The response of the photoreceptor cells (it was not possible to distinguish between rods and cones) consisted of a hyperpolarisation, comprising an initial transient, decaying to give a steady hyperpolarised potential, graded with stimulus intensity. The response of the horizontal cells recorded intracellularly, like that of the photoreceptor cells, was also hyperpolarizing and graded with stimulus intensity. Similar to photoreceptors and horizontals, the bipolar cells generated only slow, graded potentials in response to retinal illumination. Unlike the previous two cells however the bipolar cells either hyperpolarised or depolarised to the light stimulus. Approximately half of the bipolar cell population responded by polarisation in one

direction, the other half polarising in the other direction.

At first sight the hyperpolarising response of the photoreceptor cells may seem a little surprising when compared with what is observed in other first order sensory receptors. Direct measurements have shown that in the dark the cell membrane is equally permeable to both sodium and potassium. This large resting sodium permeability of the photoreceptors generates the "dark current" which has the effect of continuosly depolarising the cell. Consequently the photoreceptors are continuously releasing synaptic transmitter to their post-synaptic cells. Illumination decreases the sodium ion influx and hence reduces the positive inward current in the photoreceptor resulting in a hyperpolarisation of its membrane potential. The result of illumination is therefore a reduction in the rate of transmitter release. The tonic release of transmitter by the photoreceptor cells in the dark is reponsible for the low (depolarised) resting potential of the post-synaptic horizontal cells. The decrease in transmitter release by the photoreceptors upon illumination is consequently responsible for the hyperpolarising response of the horizontal cells.

This early work has led to the identification of synapses arising from "presynaptic" dendrites in a variety of different preparations including the vertebrate spinal cord and thalamus (Ralston, 1968a,b), the leech segmental ganglion (Muller and McMahan, 1976) and in lobster stomatosensory ganglion (King, 1976). The regulation of such synaptic outputs may be controlled by local non-propagated

graded potentials. Evidence from the neuromuscular junction and the squid stellate ganglion giant synapse has revealed that synaptic activation is due to the absolute degree of presynaptic depolarisation and not the nerve impulse itself. A number of cells of the retina, including the photoreceptors themselves, are all the more interesting in that they show a hyperpolarizing receptor potential in response to illumination, postsynaptic activation thus depends on the rate of transmitter release in the light. Other retinal cell types, namely the bipolar cells are distributed into two populations ; those showing a hyperpolarizing response to illumination ; others a depolarizing response. Other primary sensory neurones of different modality have been shown to respond with non-impulsive analogue voltage changes. In particular the cells of the acoustico-lateralis system respond with graded potential changes of either polarity dependant upon the directionality of the stimulus (Hudspeth and Corey, 1977). Subsequent deviations inthe rate of transmitter release effectively control post-synaptic activity.

2.2 Sensory Transduction in Hair Cells

In vertebrates, hair cells are the primary receptors in the auditory, vestibular and lateral-line systems, the so-called acoustico-lateralis system. These cells, similar to the vertebrate retinal photoreceptors, are specialized, axon deficient neuroepithelial cells which synaptically excite second order afferent neurones. The hair cells of the acoustico-lateralis system

are all morphologically similar and are characterized at their apical surface by a bundle of sensory hairs. They are generally cylindrical or flask shaped and range in size from 4 to 10 μm for the mammalian organ of Corti, through the intermediate sized cells of the bullfrog sacculus (15μm diam. , Hudspeth and Corey, 1977) to the large hair cells of the mudpuppy lateral line (20μm diam. Harris et.al. 1970). The hair cells are innervated at their base by afferent endings of second order sensory nerve fibres and by one or more efferent endings.

The adequate stimulus for all hair cells is the sameμ a shearing displacement producing a lateral movement of the sensory hairs. Intracellular recordings of the response of the hair cells to a linear displacement were made by Hudspeth and Corey (1977). Using fine glass electrodes, remarkably bent over the distal 200μm to allow vertical cell penetrations with horizontally placed electrodes, graded potentials usually 5-10mV in amplitude were recorded in response to displacement of the sensory hairs. In response to a static displacement of the hair cell bundle, the direction of polarization of the receptor potential depended upon the direction of the imposed displacement. Furthermore, the receptor potentials were asymmetrical. A 0.5μm movement of the hair bundle in the direction of the largest of the stereocilia gave a large depolarising potential change whilst the same movement in the opposite direction gave a much smaller hyperpolarising potential. The input-output relationship determined for the cells is roughly linear up to displacements approximately plus or minus 0.4μm, at higher amplitudes the response saturates at either extremes of

flexion. The input-output curve is therefore sigmoidal but highly asymmetrical with more rapid saturation of the hyperpolarising response.

The activation of synapses by hyperpolarising receptor potentials has been the subject of some speculation (Grundfest, 1961 ; Fuortes, 1971). That the retinal photoreceptor cells hyperpolarise upon illumination suggest that excitatory transmission to second order retinal cells may occur upon hyperpolarisation. The supposition, in the case of the hair cells, that no signal is produced when the cell is at rest and two different signals, ie. two different transmitters, are produced by the two directions of bending has been proposed, (Fuortes, 1971). It is now widely accepted however, as has been already described for the retina, that the stimulus (light, movement of stereocilia), must cause a reduction in the rate of tonic transmitter release by the pre-synaptic cells. Accordingly, impulse activity in the afferent fibres which innervate the hair cells is directly modulated by the hair cell receptor potential, activity being increased when the hair cells are depolarised and decreased when they are hyperpolarised (Sand et.al. 1975).

In the cells so far discussed exhibiting non-impulsive activity, regenerative action potentials are not a necessary step in the transmission of the receptor response because the distances are very short. It must not be assumed however that the close proximity of synaptic region and transducer site is a requirement for the lack of impulsive activity in a particular neurone. Electrotonic

transmission of receptor potentials may also take place over greater distances, at which point the distinction between confinement of graded activity to the cell body and dendrites of a cell and the allocation of spiking activity to its axon becomes somewhat obscured.

2.3 Crustacean Non-spiking Thoracic-coxal Muscle Receptor Organ.

Spanning the thoracic-coxopodite joint in each leg of a number of species of decapod crustacea is a single muscle receptor complex. It is innervated at its proximal end by two large and either one or two small diameter afferent fibres. These thoracic-coxal muscle receptor organs (TCMRO's) were again first described by Alexandrovicz (Alexandrovicz and Whitear, 1957 Alexandrovicz, 1958.) and their remarkable response characteristics elucidated by Bush and his co-workers (Ripley, Bush and Roberts, 1968 ; Bush and Roberts, 1968, 1971 ; Roberts and Bush, 1971.)

The muscle receptor consists of a long, thin bundle of muscle fibres enclosed within an elastic connective tissue sheath. The attatchment of the TCMRO is so arranged across the thoracic-coxal joint that it is stretched by backward movement of the leg ("remotion") and due to its elasticity, shortens with forward movement ("promotion").

In contrast to the crustacean chordotonal organs and most other arthropod mechanoreceptor neurones, the cell bodies of the TCMRO afferents lie within the central nervous system. From the cell bodies of the two largest afferents extends a thin process which gradually enlarges until 1-3mm outside the thoracic ganglion they reach in Carcinus maenas, a maximum diameter of 40-70µm. Both fibres then run as uniform cylinders for several millimeters, often over a centimeter in the posterior legs of large crabs, before branching peripherally to innervate the short proximal tendon of the receptor muscle (the T fibre), or two flanking connective tissue strands (the S fibre). Fine structural analysis revealed that the T fibre terminations are in series with the receptor muscle while those of the S fibre are in parallel with it.

Similar to the sensory neurones of the crustacean abdominal muscle receptor organ but unlike the vertebrate muscle spindle afferents, these two afferent fibres of the TCMRO are large enough to impale with microelecrodes. They have a resting membrane potential of -40 to -80mV which decreases upon increasing the length or tension of the muscle receptor organ. Simultaneous intracellular recording of the membrane potential at different points along the nerve show that the responses of all the TCMRO neurones to receptor muscle stretch are decrementally conducted down the fibre without the intervention of all-or-nothing spikes. These responses have the characteristics of receptor potentials, i.e. they are graded with rate and amplitude of stretch.

The response of both the S and T fibres to a constant-velocity ramp function stretch show an initial dynamic component, dependant upon the rate and extent of the stretch followed by a decline to a maintained level of depolarisation dependant upon the final amplitude of the stretch. The T fibre response shows a much greater velocity sensitivity than the S fibre, On the other hand, the steady state membrane potential of the S fibre varies almost linearly with receptor muscle length. Being in series with the receptor muscle , the T fibre response is also strongly depedent on the tension within the receptor muscle strand. Under conditions of "tension clamp" (Bush, Godden and Macdonald, 1975 ; Bush and Godden, 1974), the S fibre response followed the slow length changes required to produce a ramp-shaped tension change in the receptor muscle, while the T fibre followed exactly the ramp tension change. Isometric contraction of the receptor muscle, resulting from efferent activation, will also give graded depolarisation in the T fibre with little or no response in the S fibre.

Like other muscle receptor organs previously discussed, the TCMRO is under efferent control. Stimulation of the motor nerve to the TCMRO under isometric conditions results in slow tension development within the muscle. The importance of this efferent supply to the receptor muscle, particularly at short initial lengths, may again be appreciated in maintaining the sensitivity of the T fibres response over a wide range of receptor muscle lengths.

The functional similarity with other muscle receptors is apparent. The frequency/time response of the caterpillar MRO to a slow constant-velocity ramp stretch shows great similarity to the S fibre response to a similar slow velocity ramp stretch (see Weevers, 1966 ; Bush and Roberts, 1971). As the velocity of the ramp stretch increases, the response of the caterpillar MRO changes in form to resemble the dynamic response of the T fibre. As has been previously mentioned, this composite response of the caterpillar MRO to a ramp stretch is perhaps not surprising since there is only one bilateral MRO per segment. The greater dynamic sensitivity of the crustacean TCMRO T fibre of course suggests a comparison with the fast crustacean abdominal muscle receptor organs and the vertebrate primary spindle afferents, all of which show rapid adaption characteristics. The static sensitivity of the S fibres may be compared with the slowly adapting slow crustacean abdominal receptors and the secondary spindle afferents.

The striking feature of the response of the S and T fibres is of course their lack of all-or-nothing impulses. Instead, graded, depolarising receptor potentials spread elecrotonically along the two large fibres to the CNS where they modulate motoneurone discharge. What then are the properties of these large receptor cells which allow the transmission of an analogue receptor potential over a distance of several millimeters without apparently any loss of information or reduced efficacy of response ?

As might be expected from the unresponsive nature of the membrane of such afferent fibres to an imposed potential change, the current/voltage relationships are almost linear over a wide range of current values (Roberts and Bush, 1971). The value of specific membrane resistance, R_m is high, in Scylla for S fibres of radius 45 μm the value is approximately 900,000 ohm cm^2. Assuming the S and T fibres to have a specific axoplasmic resistance, R_i, similar to that found in other crustacean nerve fibres of around 60 ohm cm, it is not surprising given the high membrane resistance that the lengh constant, λ, of Scylla S fibres in large individuals to be approximately 5.8cm. In Carcinus the length constant is in the order of 1-2 cm (Cannone and Bush, 1980). These values for space constant are large when compared to standard values for frog muscle fibre and a 1μm diameter mammalian nerve at 1.5 and 0.2 mm respectively, and even more remarkable when compared with large diameter (75μm) lobster axons with length constants of 1.6 mm. Even squid giant axons with diameters of around 200μm (Sepia) and 500μm (Loligo) have length constants only up to 1.0 and 2.0 cm respectively (Weidman, 1951 ; Gorman and Mirolli, 1972).

In Carcinus given such a space constant and assuming infinite cable properties, the amplitude of response recorded from an afferent S or T fibre as it enters the thoracic ganglion would be about 80%-90% of that recorded at the receptor muscle some 5mm distally. Hence minimal loss of the receptor signal is seen, even over distances up to 1.0cm and more.

The efficiency of this analogue signal is obvious. That these non-impulsive responses are the normal mode of signalling for this sense organ is demonstrated by their participation in a "resistance reflex" through their ability to evoke impulses in motoneurones to the coxal promotor muscles of the leg (Bush and Roberts, 1968).

Synaptic transmission in this stretch reflex pathway shows similar characteristics to that seen in other non-impulsive neurone systems (cf. vertebrate hair cells), in that it is continuously graded or tonic in nature. The transfer characteristics of this synapse, plotted as the amplitude of the postsynaptic response against the imposed presynaptic potential (Blight and Llinas, 1980), gives a sigmoidal curve, This is very similar in form to that demonstrated for the input-output characteristics of the vertebrate hair cell (Hudspeth and Corey, 1977) and also for the pre-post synaptic transfer characteristics of the squid giant synapse injected with TEA and bathed in TTX (Katz and Miledi, 1967). The slopes of the logrithmic relations between pre and post synaptic responses obtained from the T fibre/motoneurone preparation and the squid giant synapse are almost identical. The value of 12.5mV of presynaptic depolarisation for a tenfold increase in postsynaptic response in the crab compares with the value of 11.5mV obtained from the squid synapse. Such "high gain" synaptic transmission is capable, through small changes in presynaptic membrane potentials, to strongly modulate the firing of tonically active motor units and hence evoke powerful reflex effects.

CHAPTER 3.0 STRETCH RECEPTORS IN SOFT-BODIED INVERTEBRATES.

In stark contrast to the wealth of information upon stretch receptors among the vertebrate and articulated invertebrate phyla, there have been few previous reports of stretch receptors in soft-bodied invertebrates. As many members of these phyla are highly mobile and possess a thick sheet of longitudinal muscle facilitating rapid shortening, it is of obvious adaptive value that the length of the animal and/or the tension within the longitudinal muscle be closely monitored.

3.1 Body wall and locomotion.

In considering the role of stretch receptors in soft-bodied invertebrates, the structural basis of the body plan in these phyla must be borne in mind. It is quite different from that of vertebrates or articulated invertebrates in which numerous fixed points of reference are provided by an internal or external skeleton for a receptor monitoring position, tension or movement. In soft-bodied invertebrates the musculature is arranged around either a thick jelly-like, mucoid material, the mesoglea as in the case of coelenterates ;a solid mass of mesenchyme as in platyhelminthes and rhynchocoels ; or as in the case of oligochaetes and polychaetes, a hydrostatic skeleton formed by the internal pressure of the coelomic

fluid. In the phylum annelidia a characteristic metameric division of the body into repeating segments is apparent accompanied by a more complex body wall musculature and a consequent wider repertoire of locomotory habits and behaviours than that shown by the Coelenterates the Turbellaria or the Nemertea. In the classes polychaeta and oligochaeta the body wall is essentially composed of two sheaths of muscle fibres, a thin outer circular layer and a thicker inner longitudinal layer, a layer of oblique muscle may or may not be present. Polychaete locomotion is achieved by the combined action of the parapodia, the lateral fleshy projections from each segment, and the body wall musculature. Slow crawling movement results entirely from the action of the parapodia, whilst undulatory movements produced as a result of alternate waves of contraction of the longitudinal muscles give the worm the ability to crawl and swim rapidly. Movement in the oligochaetes, most extensively studied in the earthworm, is not undulatory but involves alternate cycles of extension, anchorage and contraction of the body, resulting from antagonistic contractions of circular and longitudinal muscles. The third class in the phyla, the hirudinae, are behaviourally far more advanced than their oligochaete cousins and show a much wider repertoire of locomotory behaviour. The leech body wall consists of three distict muscle layers, from the outside inwards, a thin circular layer, a thinner oblique layer and finaly a powerfully developed, thick layer of longitudinal fibres. Dorso-ventral muscle bundles also cross the body cavity in Beach segment.

The terrestial locomotory behaviour of the leeches involves essentially the same type of contraction pattern as that shown by the earthworm, exept that the points of attatchment are provided by the posterior and anterior suckers. Refinements of this basic pattern of terrestial locomotion produces such varied behaviours as inchworm and veniform crawling and various exploratory and "alert" behaviours (see Sawyer, R.T. in Muller, Nicholls and Stent, 1981). In swimming the dorso-ventral muscles are maintained in a tonic state of contraction, flattening the body, whilst waves of contraction pass alternatively down the ventral and dorsal longitudinal muscle bands.

In all three groups, muscles act against an internal hydrostatic pressure,and, except in the case of the polychaete parapodia, the lever is not exploited as a method of locomotion. Hence the abscence of any fixed points of reference in such mobile and plastic bodies presents unique problems for a receptor monitoring length or tension. Bearing in mind such limitations we may now review the evidence for the existence of such organs.

3.2 The leech nervous system

Most early work upon the analysis of the annelid nervous system was concerned with the giant, fast, through-conducting axons of the earthworm which project along the ventral nerve cord. Such neurones are involved in rapid withdrawal responses and hence provide for the

simultaneous excitation of the longitudinal body wall muscles in each segment.

Over the past fifteen years a single annelid, the medicinal leech, Hirudo medicinalis has been used in the detailed cellular analysis of simple reflexes and of more complex behaviours (see Muller, Nicholls and Stent,1981 for review). The annelid nervous system was introduced to the age of modern neurophysiological techniques by Stephen Kuffler and David Potter working at Harvard Medical School. Their work on the leech neuroglial cells, as well as providing the basis for investigation of glial cells in the vertebrate CNS, also expounded the excellent advantages of the leech CNS as an experimental preparation (Kuffler and Potter, 1964).

Being a segmental animal, the leech has a segmented nervous system. The ventral nerve cord consists of a segmentally repeating chain of ganglia each joined by two lateral and one thin medial bundle of nerve fibres which form the connectives. Apart from some specialization at the head and tail ends, where fused ganglia form head and tail "brains", each of the 21 segmental ganglia are virtually identical to each other. Each of these segmental ganglia contain approximately 400 nerve cell bodies (Macagno, 1980) segregated by septa into 6 descrete groups or packets, each is enveloped by a single packet glial cell. These stereotyped ganglia, unlike the opaque nervous systems of their oligochaete relatives, are quite transparent and the nerve cell bodies are all accessible to microelectrodes. The accessibilty of the leech nervous system

made it a focus of interest for neuroanatomical and embryological research in the late 19th century. Such noteworthy workers as Retzius, etc. all made valuable contributions to the early neurobiology of the leech. From such early work, together with the important work of Baylor, Kuffler, Nicholls and Stuart in the second half of this century, it was apparent that it is possible to unambiguously identify individual neurone cell bodies on the basis of their sizes, shapes and positions they occupy within each ganglionμ and that each type of identified cell corresponded to a specific modality of sensory receptor or motoneurone. Thus within each ganglion there are 14 cells which are solely responsible for conveying touch, pressure and noxious mechanosensory information to the CNS (Nicholls and Baylor, 1968). Each cell occupies a constant position within the ganglion, shows characteristic electrical properties and may be identified from ganglion to ganglion and from one experimental preparation to the next. The body wall muscles are similarly specifically innervated by a limited number of excitatory or inhibitory motoneurones (Stuart, 1970).

It is this simplicity and economy which allows the same cell to be located again and again in different preparations and makes it possible to describe in detail for each sensory modality or motoneurone type, its response and electrical characteristics, their central and peripheral branching patterns and consequent receptive fields, the morphology of their central and peripheral endings and their response to a variety of denervation/ regeneration proceedures.

3.3 Locomotory rhythm and the role of afferent feedback.

Such extensive definition of the properties of individual sensory and motoneurones has made the leech preparation very favourable for the analysis of simple reflex synaptic interactions within the CNS, and also for analysing more complex behavioural acts such as swimming and the constriction-dilation rhythm of the leech heartbeat. It has been possible for example to identify and characterise a roster of neurones involved in the leech swimming behaviour. For this complex act, sensory neurones, swimm initiating neurones, pattern generating neurones, motoneurones and modulatory neurones have been identified, their response and electrical properties characterised, their synaptic interactions described and their arbours traced (Kristan, Stent and Ort, 1974a,b ; Ort, Kristan and Stent, 1974.)

The swimming rhythm of the leech was an early subject for the investigation of locomotion in animals. The studies of Sir James Gray and his colleagues in the late 1930's were concentrated upon the mechanism which maintained the normal locomotory rhythm of different animals. Two theories had been put forward, firstly, that the locomotory rhythm was determined by "a closed chain of peripheral reflexes, each phase of a complete locomotory cycle setting up automatically the particular pattern of peripheral stimulation requisite for the elicitation of the succeeding phase in the cycle" and secondlly, that the rhythm is "fundamentally, a property of the CNS which is, however, susceptible to modification

or extinction by peripheral stimulation", (Gray, Lissmann and Pumphrey, 1938).

The range of movements shown by the leech during terrestial locomotion (alternating changes in length) or during swimming (dorso-ventral undulations) are such that proprioceptors could provide information from the completion of one movement for the initiation of the next. Hence locomotion could be due to a closed chain of peripheral reflexes. This was illustrated by Gray et.al. (1938) in the terrestial ambulatory rhythm of the leech. Here, tactile information arising from fixation of the posterior sucker initiated a wave of contraction of the circular muscles and a simultaneous inhibition of the longitudinal muscles. Fixation of the anterior sucker resulted in a wave of excitation over the longitudinal muscles . In the absence of tactile stimulation from either the ventral surface of the body wall or the suckers, the leech swam. In the same paper however, it was shown that the waves of longitudinal muscular contraction in the swimming leech could pass over a completely de-afferented section of the ventral nerve cord whilst complete co-ordination of the rhythm was maintained. Similar experiments with the earthworm showed that the locomotory wave would cross a section of nerve cord with the body wall completely removed, (Gray and Lissmann, 1938). Such observations suggested that the propagation of the swimming wave in the leech and the locomotory rhythm in the earthworm was not due to the transmission of peripheral reflexes but by the nerve cord itself. No evidence was obtained to show the existence of an electrical impulse rhythm inherent in the isolated nerve cord of either leech or

earthworm which resembled the typical swimming and ambulatory rhythms. It was concluded, somewhat fortuitously perhaps, that a swimming rhythm in the leech emerges only if the general excitability of the nerve cord is maintained at an adequate level thus allowing its intrinsic rhythm to prevail. Similarly, some level of clearly defined tactile or proprioceptive stimulation from the suckers and ventral surface of the body was importand in co-ordinating the sequential activity of the longitudinal and circular muscles during the leech ambulatory rhythm (Gray et al, 1938).

During a normal ambulatory or swimming cycle drastic changes occur in the length and overall shape of the leech. It seems reasonable to suspect therefore that proprioceptive reflexes may be involved in, and play a significant role in the co-ordination of the whole cycle. The responses recorded by Gray et al. (1938) from an anterior segmental nerve in response to passive stretch of the respective body wall segment must be regarded circumspectly. The stretched piece of body wall included both muscle and overlying skin and therefore the recorded response must undoubtedly contain the activity of touch and pressure mechanosensory neurones (Nicholls and Baylor, 1968). Similarly, the responses recorded from the segmental nerves of the leech by Laverack (1969) to ramp and sinusoidal indentations of the body wall are likely to be mechanosensory in nature. Indeed two units of differing thresholds were distinguished which most likely correspond to the low-threshold touch sensitive mechanosensory neurones and the higher threshold pressure sensitive mechaonsensory neurones. On the other hand it seems likely that the

effect demonstrated by Gray et.al. on increasing the load to the longitudinal muscles upon the frequency of the ambulatory rhythm of the leech is mediated by stretch or tension receptors within the body wall. Hence Gray et al (1938) showed that the duration of contraction of the longitudinal muscles during a normal locomotory cycle is in the order of 2.0 sec. When the load on the longitudinal muscles is increased to 7.0 g the contraction time is increased to 5.0 sec. If this isotonic contraction is prevented, an isometric force of up to 80 g may be developed with a contraction lasting up to 60 sec. The leech thus appears to possess a resistance reflex essentially similar to that found in vertebrates and higher invertebrates.

The importance of tension within the longitudinal musculature for the maintenance of the peristaltic locomotory rhythm in the earthworm was also demonstrated by Gray and Lissmann (1938). The rhythmical activity of a preparation suspended in air was lost when the body weight was supported in water. If tension was applied during the ambulatory rhythm when the longitudinal muscles were relaxing, then the rate of relaxation was increased. When it was applied during longitudinal muscle contraction, then the contraction was vigorously re-inforced. It is difficult to assess from such experiments the importance of the contribution of stretch receptors over that of the pattern of afferent input from other cuticular receptors in the regulation of the normal locomotory rhythm. This problem was partially overcome however by Gray et.al. (1938) who showed that the frequency of the undulatory body wave which travels down the body of the leech during swimming (the swim cycle) was

reduced by an increase in the viscosity of the media.

This effect of proprioceptive feedback upon the locomotory rhythm of the leech has been further investigated by Kristan and colleagues in their detailed analysis of the swimming activity, (Kristan et.al., 1974a,b ; Ort et.al., 1974 ;Kristan and Stent, 1976). A number of facts had been established by previous work regarding the role of the leech nervous system in the generation of the swimming movement. Namely, i). The presence of the head or tail ganglia was not required for the generation of the swimming rhythm since after decapitation or disconnection from the ventral nerve cord, leeches swim, often more readily and for longer periods. Hence the rhythm is produced within the abdominal segments. ii). The passage of the rhythm is mediated by the ventral nerve cord, as co-ordination of front and rear body portions is lost after cutting of the connective. iii). Moreover, the neuronal activity responsible for the co-ordination of the swimming behaviour is intrinsic to the CNS, as the locomotory wave is capable of crossing a section of the ventral nerve cord with the connective intact but with the entire body wall of several midbody segments removed. iv). That this intrinsic rhythm was susceptible to peripheral feedback however was shown by the decrease in the frquency of the swim cycle in a media of high viscosity.

Direct evidence that the rhythmic locomotory pattern of the leech was produced by a central pattern generator (i.e. neuronal elements within the CNS not requiring sensory input for their rhythm) was

soon forthcoming, . Despite earlier unsuccessful attempts (Gray et.al., 1938) it was shown that rhythmical electrical activity reflecting the antiphasic contractions of dorsal and ventral longitudinal muscles exhibited by the leech during swimming, could be recorded from an isolated leech nerve cord deprived of all peripheral sensory input (Kristan and Calabrese, 1976). The relationship between the rhythmically active segmental motoneurones, interneurones and the actual swimming movements can be studied by means of a semi-intact leech preparation in which front and rear body segments (in which the head and tail ganglia have been surgically disconnected from the nerve cord) are connected by an exposed but immobilised section of the ventral nerve cord from which the body wall has been removed (Kristan et.al. 1974). The intact parts of this preparation swim normally whilst from the exposed peripheral and central nervous system may be recorded bursts of motoneurone activity which would have commanded appropriate movements had the body wall muscles been intact.

Extracellular recordings from the segmental nerves of such a preparation revealed a motoneurone pattern of activity clearly related to that of the swim cycle. This swim cycle consisted of a four phase pattern of motoneurone impulse bursts. In each segment an excitatory motoneurone impulse burst to the dorsal longitudinal muscle is followed by a burst of impulse activity from the inhibitory motoneurones to the dorsal longitudinal muscles. This is followed by activity in the excitatory motoneurones to the ventral longitudinal muscles followed by activity from the ventral inhibitory motoneurones. Hence the troughs and crests of an

undulatory body wave are produced by a rhythmic contraction-relaxation cycle of the segmental dorsal and ventral longitudinal muscle commanded by such excitatory and inhibitory motoneurone activity. The period of such a segmental contractile rhythm ranges from about 400-2000 msec. The rearward progression of the body wave is the result of co-ordination of the contractile cycles of neighbouring segments such that the contractile cycle of each segment leads that of the next posterior segment. This intersegmental phase lag, coupled with the fact that the time taken for the rearward progression of the body wave over the length of the animal is equal to the period of the segmental contractile cycle, results in the leech maintaining one full wavelength over the length of its body at all swimming speeds (Kristan et.al. 1974a).

What then is the influence of sensory feedback upon the activity of the central pattern generator ? If a leech, surgically decapitated, is suspended in a water filled dish by pinning the denervated 6th and 7th segments to a hard rubber pillar and the denervated 15th and 16th segments to a second rubber pillar, the sequence of swimming movements may be recorded photographically from the body silhouette (Kristan and Stent, 1976). With such an arrangement a suitable distance may be found between the two pillars at which a single complete wavelength is maitained over the length of the animals body and the swimming is regular. If the distance between the supporting pillars is reduced so that the mid-body segments encounter mechanical resistance preventing the transition from the crest to the trough of the swimming wave, normally achieved between these two points, then the swimming undulations are no

longer regular. In such a case, because transition from the crest to the trough was not achieved by the mid-body segments, immediate re-initiation of crest formation was produced. This re-initiation of the body wave was transmitted to the head portion upon which no mechanical restrictions were imposed, so that its downward movement, which normally signifies the end of the swimming wave, was delayed and crest formation was re-initiated by moving upwards. Hence a proprioceptive feedback system exists which monitors the realisation of the swimming wave as it proceeds down the body. If this movement is not achieved, the fact is communicated forwards and a new body wave is initiated to reset the disrupted rhythm. The effect of afferent activity in such a case would be to stabilise the centrally generated swimming rhythm by synchronising the rhythmical afferent input with the output of the central pattern generator neurones. Hence the proprioceptive reflexes would only effect the rhythm if the actual body movements did not match those commanded by the central pattern generator.

It seems likely that the proprioceptive feedback reported from such experiments would be based upon detection of longitudinal body wall stretch. The effect of such body wall stretch upon the swimming rhythm was investigated in the semi-intact preparation and the isolated leech nerve cord preparations by recording the activity of the four motoneurone classes which produce the rhythmical longitudinal muscle activity (Kristan and Stent, 1976; Kristan and Calabrese, 1976). Stretching the dorsal edge of a body wall flap during swimming in a semi-intact preparation caused an increase in the duration of the rhythmic bursts of the dorsal excitatory

motoneurone and also of the ventral inhibitory motoneurone. The effect upon the ventral longitudinal excitors and the dorsal longitudinal inhibitors was opposite in effect in that dorsal body wall stretch decreases activity in these cells. Stretching the ventral body wall has the obvious opposite effect upon such motoneurone activity from that of dorsal body wall stretch. It was also apparent that body wall stretch of a posterior segment has the opposite effect upon motoneurone bursts from an anterior segmental ganglion than upon the motoneurone bursts in the stretched segment. A finding which supports the earlier, behavioural, observations upon the forwards conduction of the stretch receptor input.

A further observation may be made concerning the afferent feedback to the swimming rhythm. In the intact animal, as the period of the swim cycle increases (i.e. the frequency of the swimming wave decreases), the intersegmental delay of the longitudinal muscle contractions producing the swimming wave also increases along the body of an intact leech (Kristan et.al., 1974a). If, however, the body wall is opened and recordings are taken either from a semi-intact or isolated ventral cord preparation then the intersegmental delays of the motoneurone bursts which would have produced the longitudinal muscle contractions no longer show any dependance upon the swimming wave frequency (Kristan and Calabrese 1976). It seems reasonable to conclude that the co-ordination of the motoneurone swim cycle period with the intersegmental delay between two adjacent segments is due to sensory input which is absent from the denervated ganglia of the semi-intact or isolated preparations. As may be recalled, correlation of the front-to-rear intersegmental

delay with the swim cycle period results in the leech maintaining a single wavelength along its body at all swimming speeds. Thus while afferent stretch information is not necessary to maintain the contractile rhythm, it is necessary to maintain the correct phase relationship between the intersegmental motoneurone burst delays and the swimming wave frequency.

Although there is physiological and behavioural evidence for the existence of stretch receptors in soft bodied invertebrates (Gray and Lissman, 1938; Kristan and Stent, 1976) the elements responsible had not previously been anatomically identified. The small population of peripheral neurones recently described, with dendrites closely associated with the longitudinal muscle of the body wall of the leech are strong candidates as stretch receptor neurones (Blackshaw, 1981; Blackshaw, Nicholls and Parnas, 1982). This investigation deals with the unusual morphological and physiological properties of these neurones. The response of the cells to changes in body wall length is described as well as aspects of their efferent innervation and post-synaptic projections.

METHODS

i) Dissection and preparation

Experiments were performed on adult specimens of the medicinal leech Hirudo medicinalis , obtained either from a commercial supplier (Biopharm, Swansea) or reared from laboratory matings and kept in copper free, dechlorinated water maintained at 22.5 degrees C in an incubator prior to use. All experiments were carried out at room temperature (20 -25 degrees centigrade).

The medicinal leech is a segmented animal. Each of its 21 midbody segments are innervated via paired segmental nerves by one of a chain of identical segmental ganglia which are joined by paired connectives to form a ventral nerve cord (see Muller, Nichols and Stent, 1981). A considerable literature exists upon the proceedure for the removal of, and the recording from the segmental ganglia of the leech (See Nicholls[1] and Baylor, 1968; Stuart, 1970.). Suffice to say that leech ganglia and portions of body wall with attatched nervous structures, dissected from the animal and maintained in artificial leech C.S.F, remain viable and give good electrophysiological recordings for many hours.

The preparation used throughout the present study consisted of 3 -5 body wall segments dissected out of the animal with a single segmental ganglion attached to the central most segment by the paired nerve roots on one side, (Nichols and Baylor, 1968 ; Stuart, 1970 ; Blackshaw, 1981b), see also figure 1. An intact animal was pinned out, dorsum uppermost and a longitudinal incision made along

the dorsal midline of the animal. Left and right body wall flaps were then deflected and pinned appart and the viscera and gut contents removed to reveal the underlying ventral nerve cord and longitudinal muscle. At this stage a segmental ganglion was cleared of its enclosing ventral blood sinus (the "stocking") and the paired nerve roots on either side freed towards the body wall. A piece of body wall, several segments long and extending from the dorsal midline to the ventral midline was then removed together with a segmental ganglion attatched to the central-most segment and pinned, skin side uppermost, to the Sylgarded floor of a 5ml transparent culture dish.

All experiments were performed upon midbody segments 14 - 17 of the animal. A particular advantage of the leech preparation is the ability to identify an individual neurone from segment to segment and from preparation to preparation. In the present study all experiments were performed upon a particular stretch receptor neurone which was constantly located in the ventral body wall adjacent to the excretory duct, the nephridiopore. A preparation was developed which enabled routine intracellular recordings to be made from either the peripheral cell body or centrally projecting axon of the neurone and a detailed description is given in Results section 1.0.

The 5ml culture dish containing the body wall section was placed on a glass slide supported between the two arms of a U - shaped metal stirrup. The preparation was viewed with dark - field optics.

To this end, a fibre optic cold light source (Fort, Lux 150), projected light onto a 45 degree angled mirror under the metal supporting stirrup. Light was then reflected vertically upwards through a "Leitz" dark field condenser which could be focused in order to provide the required dark field illumination of the prepartion. All apparatus was supported on a vibration free table and with the exception of the cold light source was enclosed within an earthed Faraday cage.

ii) Solutions

The body wall and ganglia were bathed in standard leech Ringer solution of the following composition; (mM) NaCl, 115.0; KCl, 4.0; $CaCl_2$, 1.8; tris maleate neutralised with 3N NaOH to pH 7.4, 10.0. Most experiments were performed in Ringer fluid containing 1.8 mM Ca^{2+}. In some experiments however, synaptic activity was enhanced by raising the Ringer Ca^{2+} concentration to 8.0 mM. A high Ca^{2+} concentration raises the firing threshold of leech neurones and therefore reduces spontaneous synaptic activity and acts antagonistically at the nerve ending in increasing transmitter release, (Nicholls and Purves, 1979). High Mg^{2+} solutions (15mM) were used to block chemical synaptic transmission. In such solutions, NaCl was replaced with an equivalent concentration of $MgCl_2.6H_2O$. This blocking effect of magnesium at the chemical synapse was antagonised by the use of a 15.0mM $CaCl_2$ Ringer solution.

During experiments the composition of the fluid in the recording bath was changed by turning a tap controlling the flow of solutions under gravity from either of two reservoirs whilst an intracellular recording was maintained from the preparation.

Tetrodotoxin was dissolved in normal leech Ringer and used at a concentration of 300 nM.

iii) Electrical recordings

Intracellular recordings were made using conventional glass microelectrodes which were pulled from thin walled filament glass (Clarke Electromedical instruments, GC150TF, outside diameter 1.2mm) on a Camden Instruments horizontal micropipette puller. They were filled with 4.0 M potassium acetate and had final resistances of between 20 - 50 Meg Ohm. The internal filament enabled the tips to be filled by capillary action upon standing the end of the electrode in electrolyte. The shanks were then backfilled from a syringe and fine needle.

The electrode was inserted in a holder made from a short piece of bent glass tubing which was in turn securely supported upon a small platform attached to a universal movement ball and socket joint. The ball and socket joint was mounted on a "Huxley" micromanipulator which provided the facility for course and fine adjustement in three planes of movement.

Intracellular recordings were made either from a single neurone, or paired intracellular recordings were obtained from synaptically related cells. Hence two recording channels were available with corresponding paired microelectrodes and micromanipulators. A silver/silver chloride wire connected the electrodes to negative capacitance high-input impedance amplifiers. An Ag/AgCl electrode was placed in the recording bath and acted as a reference electrode against which potentials were measured. One recording channel (unity gain WPI model M701) allowed current to be injected into the cell via the microelectrode, whilst voltage was simultaneously monitored via a modified bridge circuit. The second recording channel (10 times gain, Getting model 5 microelectrode amplifier) also utilised a bridge balance as well as incorporating a direct current passage mode, which enabled direct current to be injected via the microelectrode. Included in the bridge circuit of both amplifiers was the ability to measure electrode resistance with reference to the bath indifferent electrode. Both signals were further amplified on D.C. coupled Tectronix 5A20N and 5A22N differential D.C. amplifiers and displayed on a dual trace Tectronix storage oscilloscope. Two voltage calibrators allowed 1.0 mV or 10.0 mV calibration steps to be applied to the negative side of either of the two differential amplifiers.

Two Devices isolated stimulators (MK. III) were used for intracellular current injection. With the WPI microelectrode amplifier in the low current injection range the maximum output voltage at the probe was +/- 2.5 V. The maximum current which could therefore be injected via the microelectrode in this mode was 2.5 V

divided by the electrode resistance. The maximum current available therefore with electrodes of 50 Megohm and above was $2.5V/50\times10^{6}$ A = 5.0 nAmps. For iontophoresis of dye from the electrode tip, higher current values were often required, characteristically 10–20nA for injection of Lucifer yellow. This was achieved using a breakaway box (WPI, model BB-1). A maximum current of 200 V could be applied to the breakaway box. In this case the internal current injection facility of the WPI amplifier was bipased allowing a larger current to be applied across the electrode. The voltage applied to the breakaway box to pass 10nA of current across an electrode of 50 Megohm resistance is given by V = Electrode resistance + 2 Megohm times current ($V = 50+2\times10^{6}$ times 10×10^{-9})

A current monitor output was also available from the electrometers and displayed on the oscilloscope as a voltage signal directly proportional to the current injected via the microelectrode. For the WPI amplifier, used in the low current injection range the current to voltage calibration was 1 V = 5×10^{-8} amperes. The isolated stimulators were in turn under the command of a Devices digitimer, (model 3290). The digitimer provided 5 decade counters and 3 logic circuits. The first decade counter controlled the cycle/recycle period whilst the other counters controlled the switching of the isolated stimulators which determined the duration and amplitude of the individual current pulses. Pulses from the cycle/recycle circuit of the digitimer to the oscilloscope external trigger input also synchronised current injection pulses with the oscilloscope sweeps.

Extracellular recordings were obtained by means of glass tipped suction electrodes placed on the dissected segmental nerves. The electrodes consisted of a 1.0 ml disposable syringe with an attatched 40 x 0.8 mm needle. Glass tips, pulled by hand from thin-walled electrode glass and fire polished to the correct diameter were connected to the needle by a short piece of flexible plastic tubing. With the inner plunger of the syringe removed, suction could be applied to the back of the electrode, drawing Ringer up into the plastic tubing and into contact with the needle. With this arrangement, the metal needle acted as the central recording eletrode.

Voltage changes were measured across the electrode mouth with respect to a ground wire wound around the plastic tube and glass tip. This reference wire was insulated along its length appart from a bared end which was positioned as close as possible to the mouth of the glass tip of the electrode. The output of the suction electrode was amplified 1000 times on an Isleworth A101 preamplifier and led to an A.C. coupled Tectronix 5A20N differential amplifier before final display on the storage oscilloscope. A box diagram of the electrical apparatus is shown in figure (i).

iv) Muscle tension and length recording.

Stretch was applied to the muscle associated with the ventral stretch receptor neurone. A thin strip of ventral longitudinal muscle containing the associated peripheral SRN cell body and

dendrites was dissected out with its innervation via the anterior segmental nerve root intact. This preparation hence contained the entire stretch receptor neurone with projecting axon and central arbour. The longitudinal muscle layer of the leech forms a continuous sheet along the length of the animal. The muscle strip used in the present experiments was approximately 2.0 mm wide and consisted of muscle fibres from the segment under study together with fibres from one or two denervated segments either side, a length of approximately 2.0 cm in all. One end of this muscle strip was securely clamped to the arm of an "Advance" type VI muscle puller. The muscle puller was controlled by a ramp function generator, which provided for the independent setting of both amplitude and velocity of displacement. Ramp function stretches (stretch, hold and release) or ramp releases (release, followed by hold and subsequent re-stretch) of the muscle puller arm of final displacement of between 0.5 and 5.0 mm were available at velocities of displacement ranging between 6.0 and 28.0 mm per second. A second output from the ramp function generator was available for display of the ramp stretch stimulus on the oscilloscope screen. Measurements of the ramp stretch displacement were made directly from a scale alongside the moving puller arm. Synchronisation of the muscle ramp stretch and oscilloscope beam sweep were again controlled from the digitimer.

The other end of the longitudinal muscle strip was either pinned to the floor of the recording chamber or securely attached to the beam of an isometric tension transducer. This allowed tension to be monitored from the muscle strip either when at a constant length or

throughout the period of the muscle stretch. Output from the tension transducer was amplified 1000 times and monitored, along with ramp muscle stretch SRN response.

All final signals were led to a Racal Store 4DS instrumentation recorder and recorded on 6.25 mm magnetic tape. Four recording channels were available. Three FM recording channels and a single DR channel, bandwidth between 100HZ and 300 KHZ which was used for recording extracellular signals. Tape speeds between 15/16ths and 60 inches per second were available which gave FM recording bandwidths which ranged between 625 Hz at 15/16th inches per second and 40 KHz at 60 inches per second. All data could be subsequently analysed "off line" on a Nicolet (model 201) digital oscilloscope. In the relevant experiments signal averaging was performed off line on Neurolog NL 200 spike trigger and NL 750 averager units.

v) Coherence analysis

In order to investigate the effect of stretch receptor neurone input upon motoneurone activity, intracellular recordings were made from the SRN soma whilst recording extracellular motoneurone activity from the segmental nerve roots. The coherence of activity between several motoneurone spike trains was then assessed according to methods of Brillinger, Bryant and Segundo, (1976), see also Appendix.

A weak gamma particle source was used to trigger a randomly occuring sequence of hyperpolarising or depolarising square wave current pulses which were injected into the peripheral SRN soma. Subsequent random hyperpolarising or depolarising voltage deflections were recorded from the SRN cell body and stored on magnetic tape along with the on-going extracellular spiking activity of between 2 and 4 motoneurone units which were recorded from the medial anterior segmental nerve root, (see also Results section 5.4). A random afferent input and multi-unit spiking output was therefore available for off-line analysis using methods of Brillinger et al. (1976).

(vi) Intracellular dye injection and histological processing

In morphological studies, the peripheral cell body of the SRN was injected with either Horseradish peroxidase (HRP), or Lucifer yellow (LY).

Horseradishperoxidase

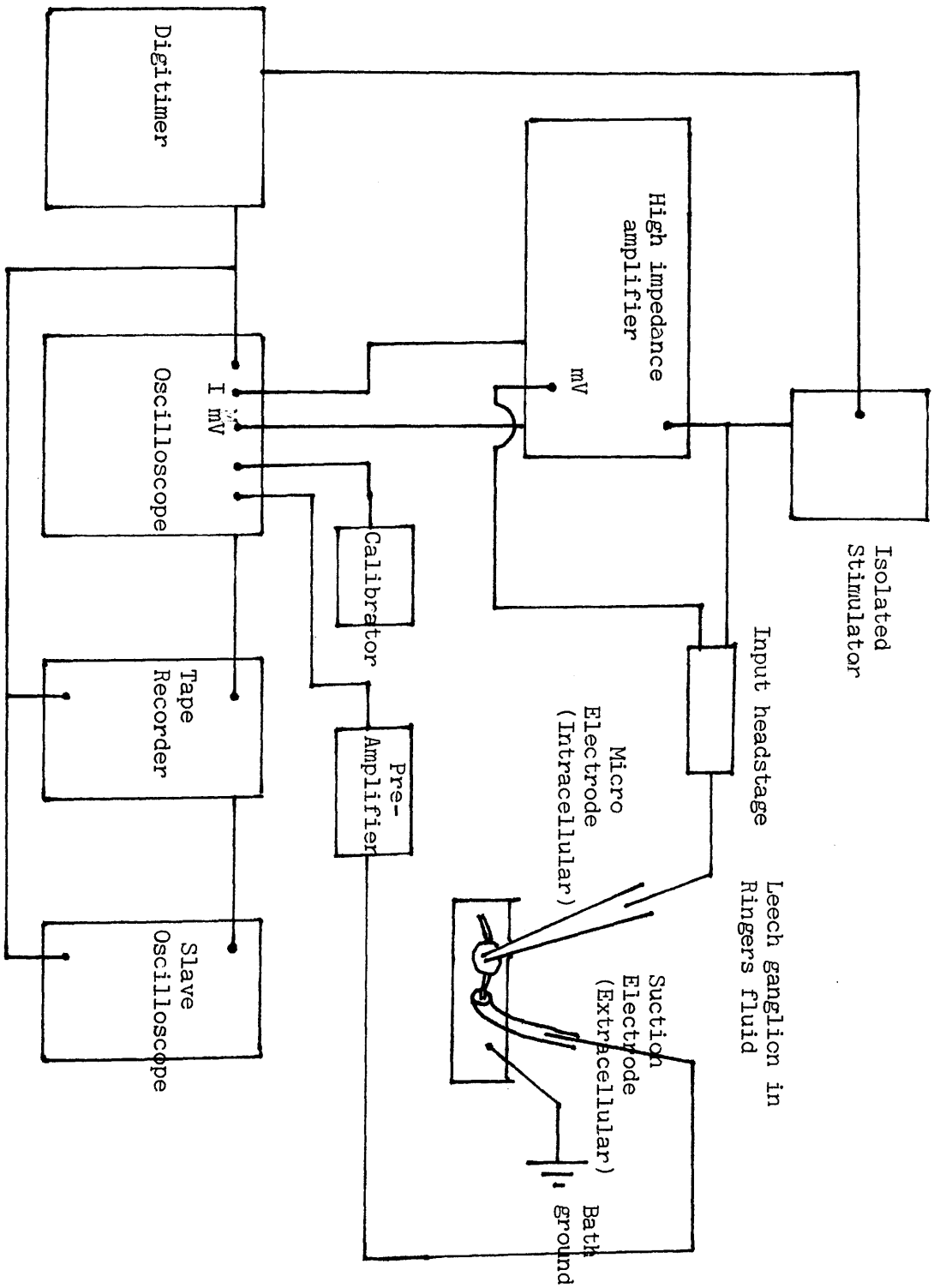
Electrodes were pulled from thick walled fillament glass (Clarke Electromedical, GC120F) and filled with 2.0% HRP (Sigma type VI) in 0.2 M KCl solution containing 0.2% fast green dye. They were bevelled on a WPI rotary beveller to a final resistance of between 30 and 100 Megohm. HRP was injected either using pressure from a compressed air source or iontophoreised, typically with 5 nA, 350 ms

depolarising current pulses at 1.0 Hz for 15 mins. Preparations were left overnight in glucose supplemented Ringer solution or culture medium at 4.0 degrees C. In a few instances the neurone was re-injected with HRP the following day and left for a further 4 - 6 hours. At the end of the incubation period, preparatioms were fixed in a 1.0% glutaraldehyde solution in tris maleate buffer for 1.0 Hr. After this period preparations were washed in buffer to stop the fixing reaction and left for a further 15.0 mins. in fresh buffer. Preparations were then incubated for 30.0 mins. in diaminobenzidine on an agitating plate shaker. The HRP was reacted by adding three drops of 1.0% hydrogen peroxide to the diaminobenzidine solution whilst constantly mixing the reaction medium. After a few minutes a brown precipitate formed within the injected neurone and when of sufficient intensity in relation to any background reactivity the reaction was halted by repeatedly flushing the preparation with buffer. Any further dissection of overlying muscle or fascia was done at this stage. The preparation was then dehydrated by bathing in 100% methanol, with a sequence of at least 4 - 5 changes to fresh alchohol allowing approximately 5-10 minutes between changes. Then cleared in methyl salicylate and mounted between coverslips in Picolite mounting medium. The preparation was viewed as a hole mount with either Nomarski or bright field optics. Colour or black-and-white photographs were taken with either an Olympus O-M2 single lens reflex camera on a Microinstruments DIC microscope or a Leitz photomicroscope. Camera lucida reconstructions were made using a Wild compound microscope with drawing tube attachment.

Lucifer Yellow

Electrodes were again pulled from thick walled glass and filled with 10.0% Lucifer yellow (Stewart, 1978) in either 0.1 M lithium chloride or distilled water. They were bevelled to give final electrode resistances of between 20 and 75 Meg. ohm. Most successful LY injections were performed using pressure, although in some experiments iontophoreisis was also used, typically 50 - 100 nA, 400 ms hyperpolarising current pulses at 1.0 Hz for 15 - 20 mins. Iontophorisis of dye into the peripheral SRN soma was less successful because of the large size of the cell and hence the amount of dye needed to fill it and the consequent technical difficulty of holding an intracellular penetration of the peripheral neurone for a sufficient length of time.

Injected neurones were left for between 1 and 3 hours before they were fixed overnight in 4.0% formaldehyde in 0.1M phosphate buffer. Preparations were then washed in phosphate buffer, dehydrated in 100% methanol, cleared in methyl salicylate and mounted between coverslips in Fluormount, a non-fluorescent mounting medium. Preparations were then viewed on a Leitz fluorescent: microscope, using ultra-violet epi-illumination with a BG 12 blue excitation filter and 530 or 510 nM cut-off filter. The morphology of injected neurones was reconstructed from photographic slides taken at a number of focal levels through the preparation. The bleaching time of the fluorescent tracer (5 -15 mins) did not allow a direct camera lucida drawing of the preparation to be made.



Figre (i). Block diagram of arrangement of apparatus for recording from single cells in a leech ganglion. Recordings could be made either intracellularly via micro electrodes or extracellularly from suction electrodes placed on the nerve roots.

RESULTS

SECTION 1.0 MORPHOLOGY

The leech is a segmented animal. Each of its 21 midbody segments, 5 annuli wide, is innervated by one of a chain of segmental ganglia which are joined by paired connectives to form the ventral nerve cord.

The body wall is made up mainly from three layers of muscle. An outer circular layer overlies a thin oblique muscle layer, and they both overlie an inner longitudinal muscle layer. This longitudinal muscle layer is the thickest and has been classified as "helical smooth" muscle, (Hansom and Lowy, 1960).

The tendancy within the invertebrates for nerve cell bodies to be grouped together in ganglia, which on the evolutionary scale first manifests itself among the primitive flatworms, continues throughout the phylum annelidia and many would argue reaches its organisational climax in the Hirudinae. For example, the organisation of nerve cell bodies within the segmental ganglion of Hirudo is typical for most of the higher invertebrates : the nerve cells are arranged in an outer cortical layer surrounding a region of nerve cell branches and synapses which constitutes the central synaptic neuropile. In the segmental ganglion of Hirudo the modalities, receptive fields and response characteristics of a number of sensory neurones and the identification of a number of motoneurones has been well documented (Nicholls and Baylor, 1968 ; Stuart, 1970).

Not without a shout however are the more limited populations of peripherally located neurones. In Hirudo for example, three types of ciliated sensory cells occur at the sensillae spots around the middle annulus of each body segment (Phillips and Friesen, 1982). A number of nerve cell bodies of unknown function are also known to occur at the branch points of the segmental nerves (Gaskell, 1914. in Kristan and Stent, 1976). Among these peripherally located neurones are the small population of cells described by Blackshaw et. al. (1982). Approximately a dozen of these neurones are found within each segment and each cell occupies a specific site within the body wall, (see later). Their morphology is unique and their close association with the segmental longitudinal muscle has raised the possibility of their function as stretch receptors.

Experiments were performed on one of these neurones that innervates ventral longitudinal muscle. The position of the cell body of this stretch receptor neurone (SRN) in relation to the segmental nerve branches and segmental boundaries is shown in figure 1. This shows the ventral portion of a piece of body wall which includes a typical midbody segment, the central annulus of which is indicated by the two sensillae. It is innervated by its segmental ganglion via a pair of nerve roots. The cell body of the SRN was visualised for intracellular recording and dye injection by removal of the skin and circular and oblique muscle overlying the anterior nerve root in the area indicated by the rectangular box. The SRN lies consistently along the first major branch of the anterior nerve root, designated branch AA (Ort et. al. 1974). The cell body lies within the sheath of the nerve and is conspicuous as an oval

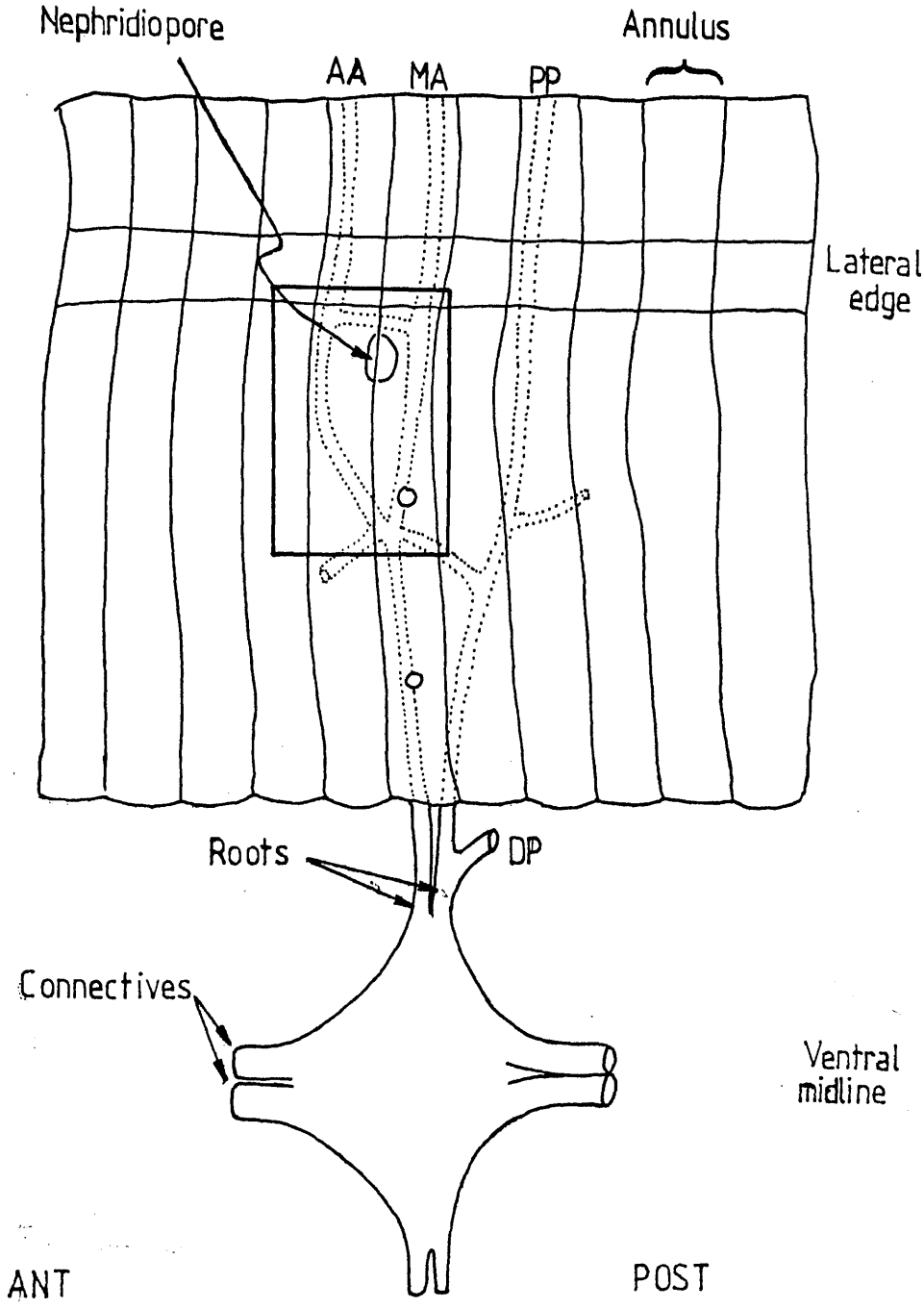


Figure 1. Schematic diagram of a ventral portion of the body wall of the leech containing a typical midbody segment and attached segmental ganglion to show the location of the ventral SRN cell body and dendrites in relation to the perpheral nerve roots (dotted) and segmental boudaries (not to scale). The neurone is consistently located in each middbody segment in the area indicated by the rectangular box. Its cell body lies within the sheath of the first major branch of the anterior nerve root (AA) at a level adjacent to the nephridiopore (the excretory duct opening) which lies on the anterior edge of the central annulus of the segment. Nomenclature of nerve roots as in Ort et al. (1974), AA, anterior anterior; MA, medial anterior; PP, posterior posterior; DP, dorsal posterior.

swelling of the AA root directly adjacent to the nephridiopore (the opening of the excretory duct) situated in the groove on the anterior edge of the central annulus. The consistent siting of this particular SRN next to the nephridiopore made it very convenient for locating the cell and all experiments were performed on this particular neurone.

Intracellular injection of the enzme Horseradish peroxidase (HRP) or of the fluorescent dye Lucifer Yellow (LY) was used to study the morphology of the SRN.

1.1 Peripheral morphology

A total of 40 cells were injected intracellularly with HRP and a further 9 cells filled with LY.

Figure 2 is a camera lucida drawing of the entire outline of the SRN after injection of HRP into the cell body. The cell body lies between a distal fan shaped dendrite, which is associated with a lateral band of longitudinal muscle fibres and a proximal fan shaped dendrite which is associated with a more medial band of longitudinal muscle fibres. A 10µm diameter axon extends centrally from the proximal dendrite and enters the segmental ganglion 3-4 mm distant in the anterior nerve root. Detail of the peripheral area of the SRN is shown in figure 3 and in the camera lucida drawing in figure 4.

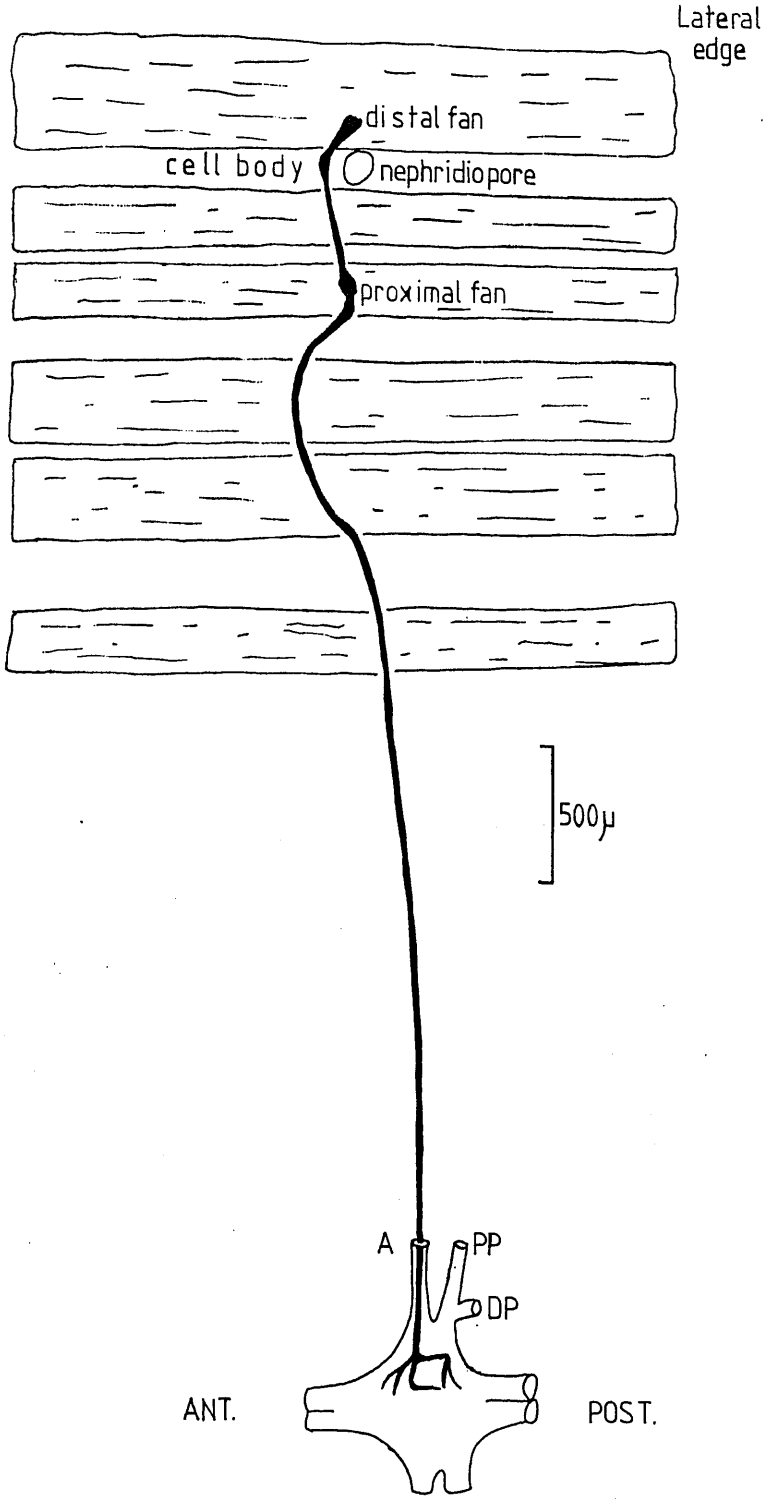
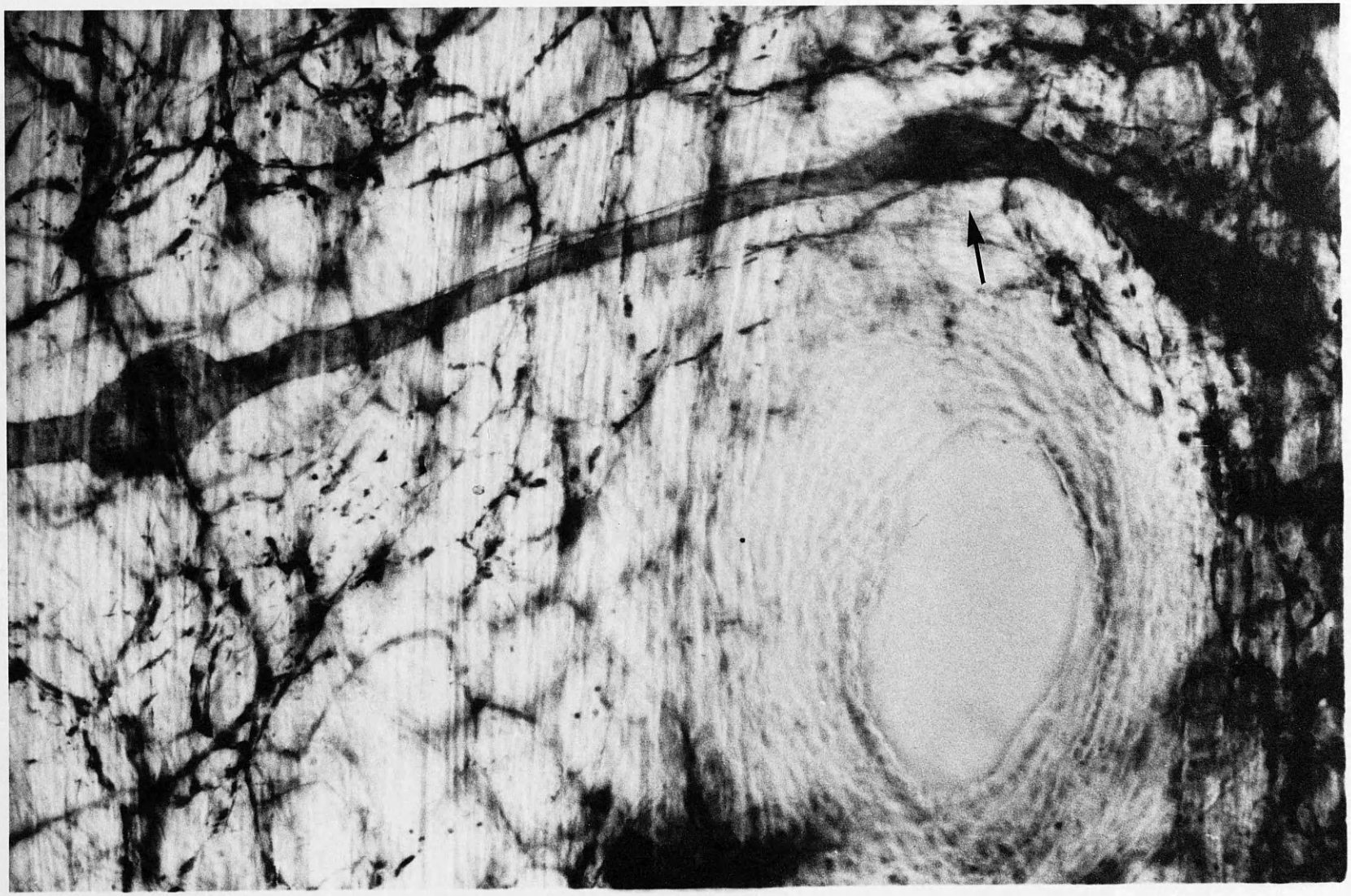


Figure 2. Camera lucida drawing of the entire ventral SRN reconstructed from the whole mount preparation shown in figs. 3 and 5. Horseradish peroxidase was injected into the peripheral cell body and allowed to travel centrally. The relationship of the distal and proximal fans of the SRN to the separate bands of longitudinal muscle is shown. The distal fan is associated with a lateral bundle of longitudinal muscle fibres. The proximal fan lies in association with a more medial and separate band of longitudinal muscle fibres. A, anterior nerve root. PP, posterior posterior nerve root (Ort et al. 1974).

Figure 3. Whole mount preparation of a piece of body wall viewed with Nomarski optics which contains the peripheral cell body and dendrites of the SRN. The cell body (arrow, 30μm by 60μm) was injected with Horseradish peroxidase and lies directly adjacent to the nephridiopore which appears as the prominent oval pore on the right of the plate. The peripheral dendrites appear as flattened fan-shaped swellings of the axon and are associated with different bands of longitudinal muscle which may be seen running horizontally across the section.



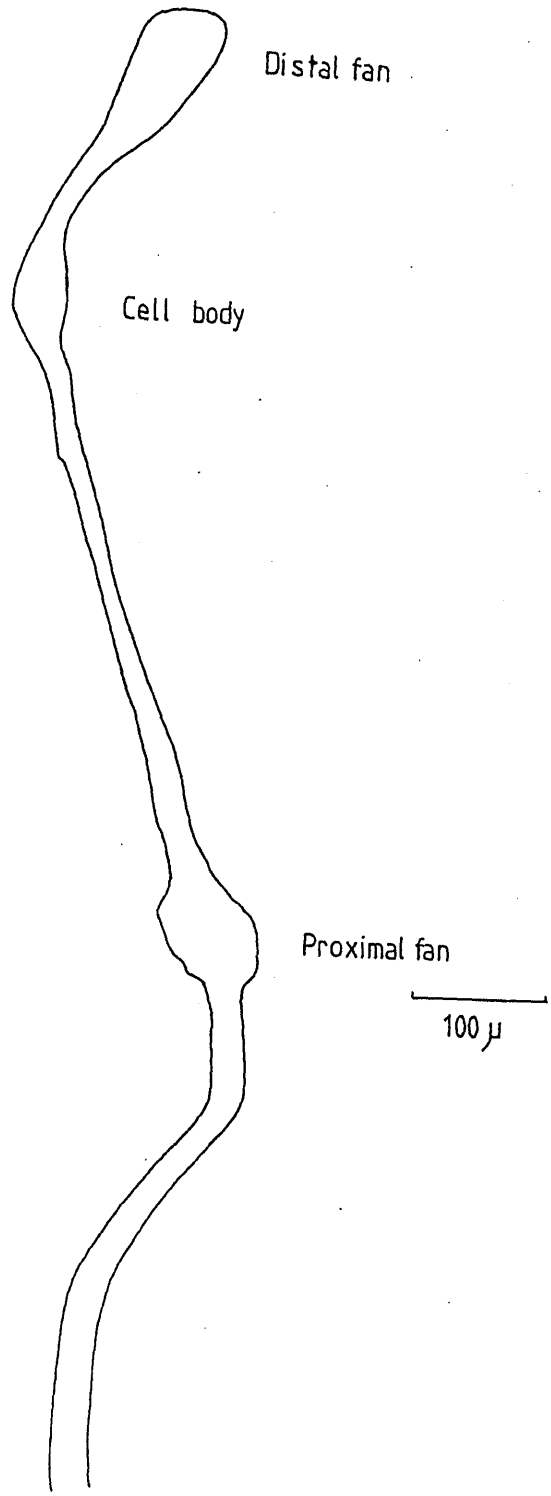


Figure 4. Camera lucida drawing of the peripheral SRN cell body and dendrites shown in figure 3. The distal fan-shaped dendrite (distal fan) extends 200µm from the cell body and is approximately 70µm across at its widest point. The cell body and a 15µm process separate the distal fan from a second fan-like dendrite (proximal fan). A 10µm diameter axon extends centrally towards the segmental ganglion from the proximal fan.

Figure 3 is a whole mount preparation of a piece of body wall containing the SRN cell body and dendrites in which the cell body was injected intracellularly with HRP. The cell body (30μm x 60μm) lies adjacent and directly anterior to the nephridiopore, which appears as the prominent oval pore. The distal fan-shaped dendrite (distal fan) extended some 200μm from the soma and is approximately 70μm across at its widest point. From the cell body a 15μm diameter process extended centrally within the sheath of the peripheral nerve and at a distance of approximately 400μm formed a second fan-like dendrite (proximal fan). In longitudinal section both fans appeaed as thin, flattened profiles, 2-3μm deep. Electron micrographs show the two opposing surfaces of the fan to be quite different (Blackshaw, McKay and Thompson, 1984). In unstained preparations one side appeared deeply invaginated with glial cell processess while the opposite surface of the terminal appeared flat. In HRP stained preparations the flat surface appeared to be characterised by rows of small mushroom-like outgrowths less than 1μm in diameter.

Figure 5 shows at higher magnification a whole mount preparation of the body wall containing the proximal fan of the SRN stained intracellulaly with HRP and viewed with Nomarski optics. When the fan is viewed and focused at the level of the flat surface, the mushroom-like outgrowths confer a distinctly knobbly appearance to the dendrite. These knobbles are arranged in rows across the central region of the fan in a band approximately 25μm wide (Blackshaw and Mackay, in preparation).

Figure 5. A whole mount preparation of a piece of body wall containing the proximal SRN dendrite in which the cell body was injected with Horseradish peroxidase and the preparation viewed with Nomarski optics. Small mushroom-like outgrowths project from the surface of the fan and confer a distinctive knobbly appearance. The outgrowths are arranged in rows across the central region of the fan in a band approximately 25μm wide. Bands of longitudinal muscle may be seen in close association with the right hand edge of the dendrite. Scale bar 15μm.

Both fans are in close association at their edges with specific bands of longitudinal muscle fibres. In figures 3 and 5 bands of longitudinal muscle may be seen running horizontally across the section. In light micrographs, bands of longitudinal fibres are seen to penetrate the nerve sheath at the level of the fans and become closely associated at the edges of the dendrite.

1.2 Central morphology.

Figure 6 shows a whole mount preparation of a midbody ganglion after injection of LY into the peripheral cell body of the SRN. As arborisation of the SRN does not occur at any one depth within the ganglion, only certain portions of its arbour are in focus at any particular level. Figure 7 is a camera lucida reconstruction of the central arborisation of the SRN from sections taken at approximately 8 different levels through the ganglion. The large axon divides within the ganglion into three main branches which distribute, with abrupt change in diameter, finer, club-like secondary branches to areas of the ipsilateral neuropile. None of the neurones examined showed branches extending across the midline of the ganglion or into the neighbouring connectives.

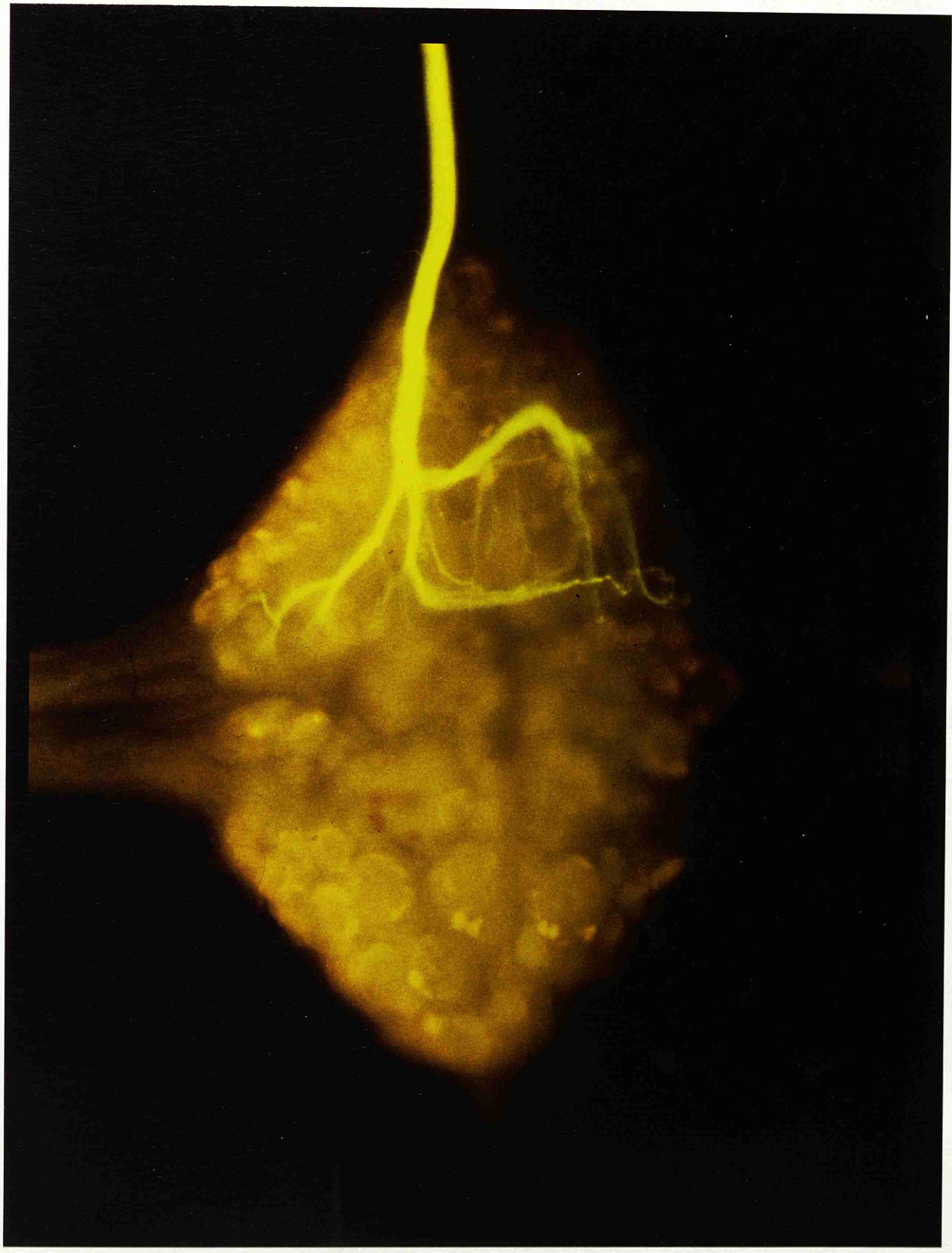


Figure 6. Whole mount preparation of a midbody ganglion after injection of Lucifer Yellow into the peripheral cell body of the SRN and photographed under U.V. illumination. The central arbourisation of the SRN is clearly visible. Since branching of the SRN within the ganglion does not occur at any one depth, only certain portions of the arbour are in focus at any particular photographic level. Scale bar 250μm.

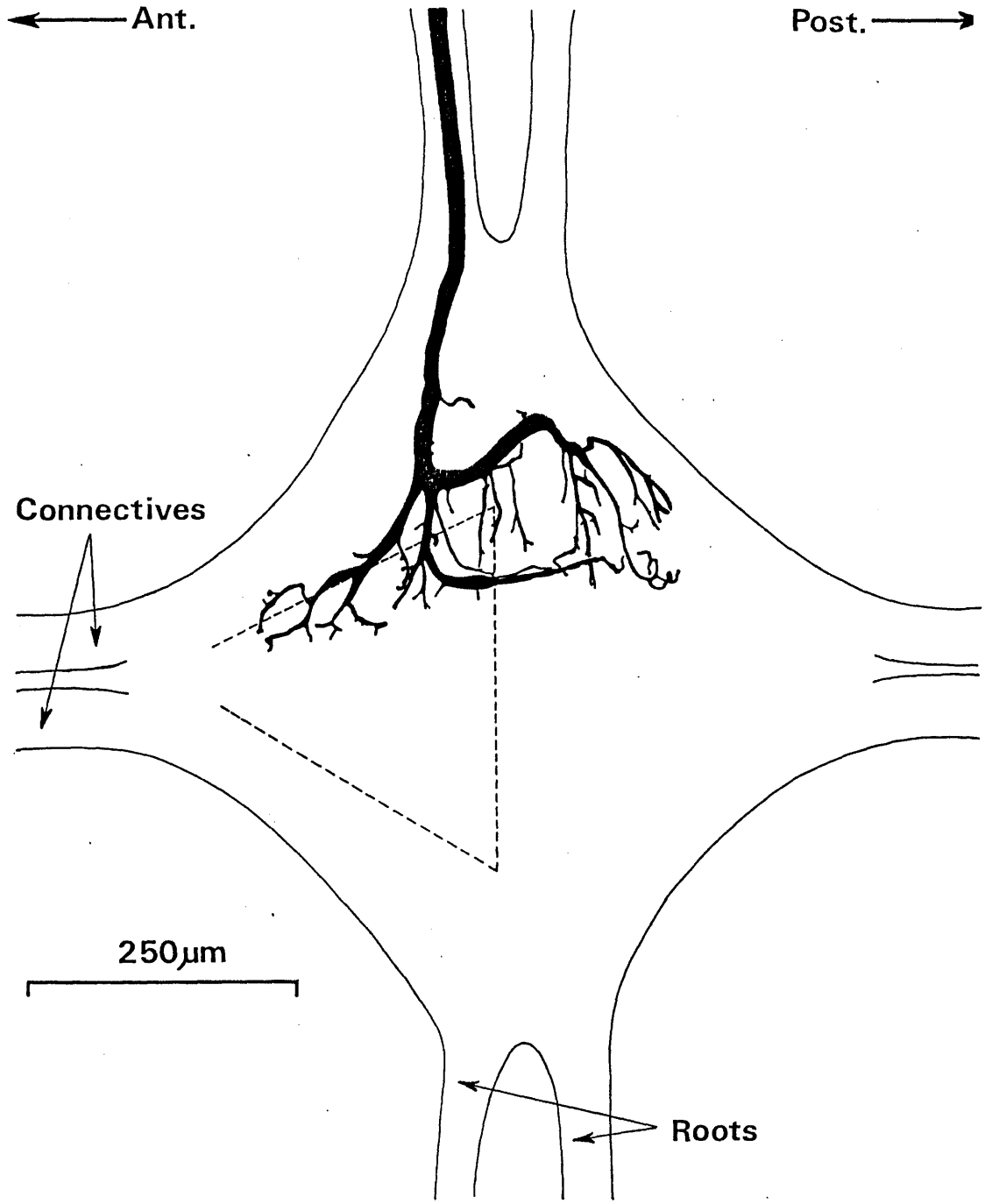


Figure 7. Cameral lucida drawing of the termial arbourisation of the SRN within the segmental ganglion reconstructed from the whole mount preparation in fig. 6. The SRN axon enters the segmental ganglion in the anterior nerve root and characteristically divides into three major branches which distribute finer, club-like secondary branches to areas of the upsilateral neuropile. None of the 49 neurones examined distributed branches across the midline of the ganglion or into neigbouring connectives.

SECTION 2.0 Electrical properties

Intracellular recordings were made from the SRN cell body and its axon using conventional K-acetate filled glass microelectrodes, (see Methods).

2.1 "Resting" Membrane Potential

The membrane potential recorded from the SRN cell body in 67 preparations ranged between -30 mV and -60 mV with a mean value of -47.5 mV. Measurments were taken either upon penetration of the cell when a steady state level was reached, the E_m often increasing for several seconds after penetration as the electrode "sealed" into the cell body, or prior to withdrawal of the electrode as a "withdrawal potential".

2.2 Action potential

Figure 8 shows the response of the neurone to depolarising and hyperpolarising square wave current pulses injected into the cell body. Depolarisation of the cell body by the passage of 3-4 nA of

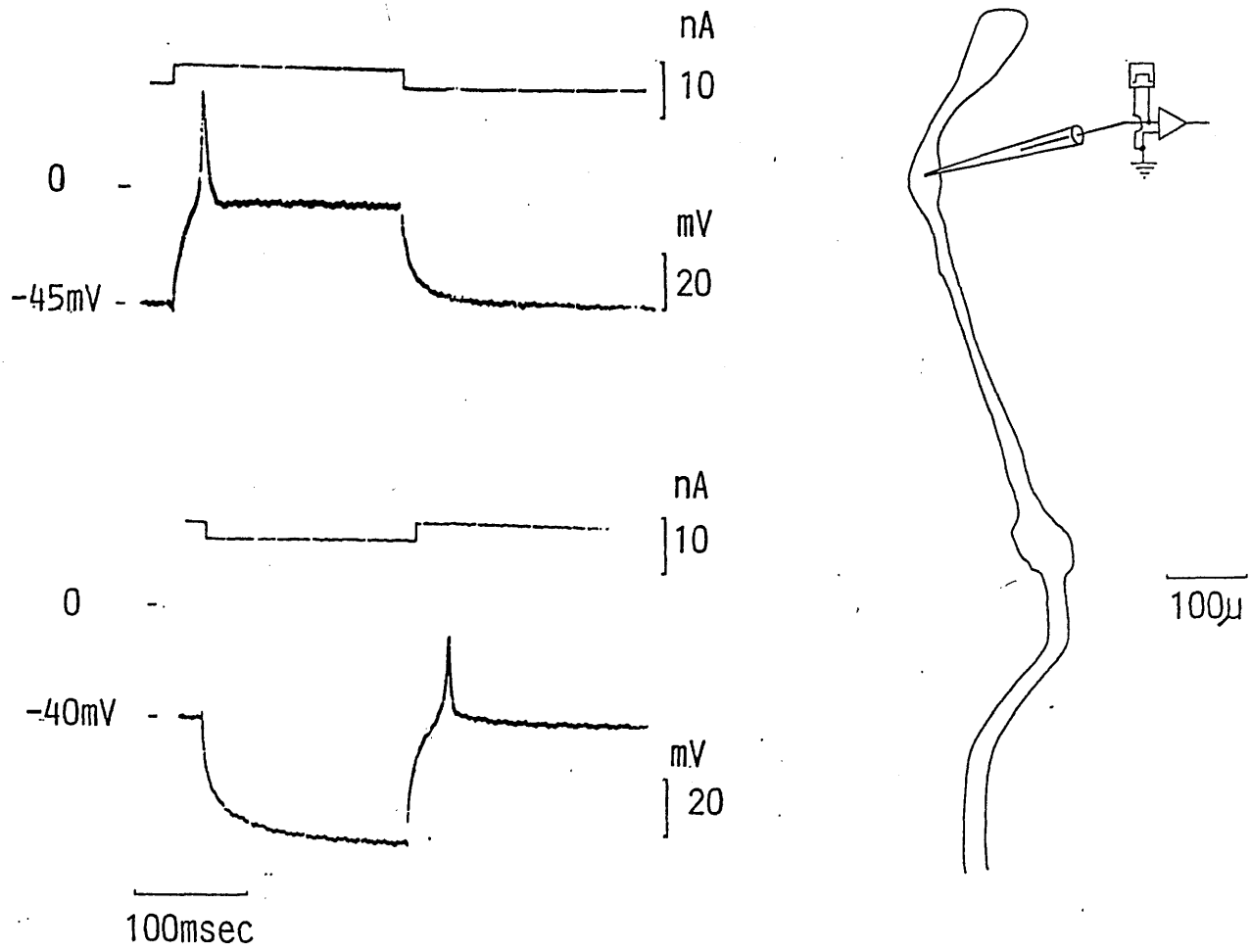


Figure 8. The response of the neurone to injection of depolarising or hyperpolarising current. Current was injected into the peripheral cell body of the SRN (right hand diagram). a, Response of the cell body (bottom trace) to a 3-4 nA square wave depolarising current pulse (top trace). A single action potential arose from the cell body which frequently overshot zero by about 30 mV. b, Response of the SRN cell body (bottom trace) to a 3-4 nA square wave hyperpoilarising current pulse (top trace). The cell responded with an all-or-nothing action potential upon termination of the current pulse. Neither the anode-break nor the depolarisation-evoked SRN action potentials were followed by an after-hyperpolarisation.

current elicited an overshooting action potential. Not all action potentials elicited however from the cell body upon injection of depolarising current showed this transient reversal of membrane potential. Figure 12 shows that an all-or-nothing action potential may be elicited upon depolarisation of the cell body by approximately 1 nA. This action potential did not overshoot zero. A further example is shown in figure 13.

The response of the neurone to a hyperpolarising square wave current pulse is also shown in figure 8. The cell responded with an all-or-nothing action potential upon termination of the current pulse. This anode break excitation of the cell was quite characteristic and provided a usefull additional means of identification. It was often easier to elicit an action potential to anode break than to depolarisation of the cell body. Action potentials recorded from the SRN cell body following a hyperpolarising pre-pulse were rarely overshooting. The absolute level of depolarisation to which the action potential extended generally was far lower for an anode break action potential than for a depolarisation- evoked action potential.

Repetetive activity recorded from the cell body was rare. Figure 9 shows the result of increasing the amount of depolarising current injected into the cell body. In this preparation injection of > 10 nA D.C. current resulted in the cell body giving repetetive overshooting action potentials. This was never seen to occur after a hyperpolarising current pulse of the same amplitude.

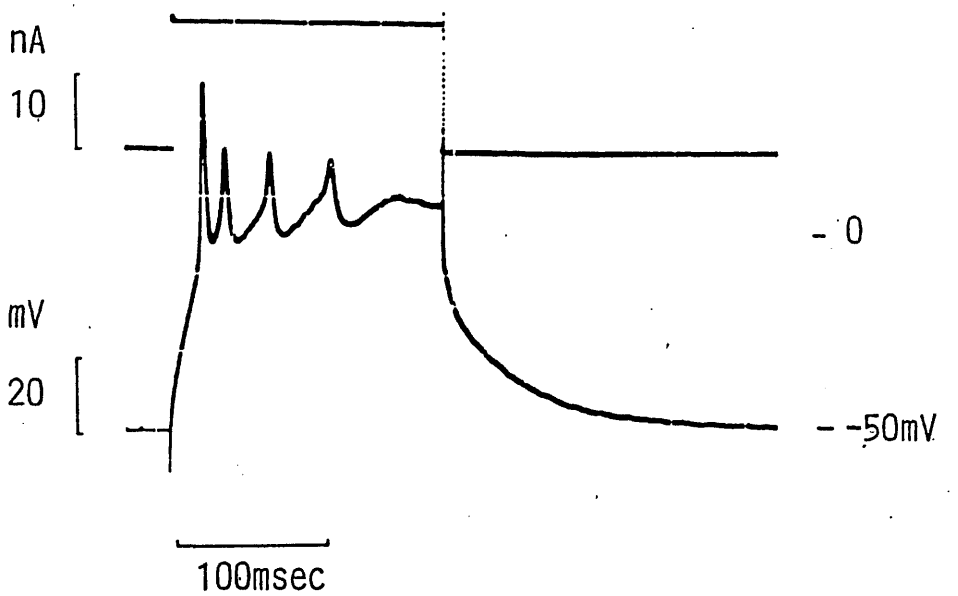


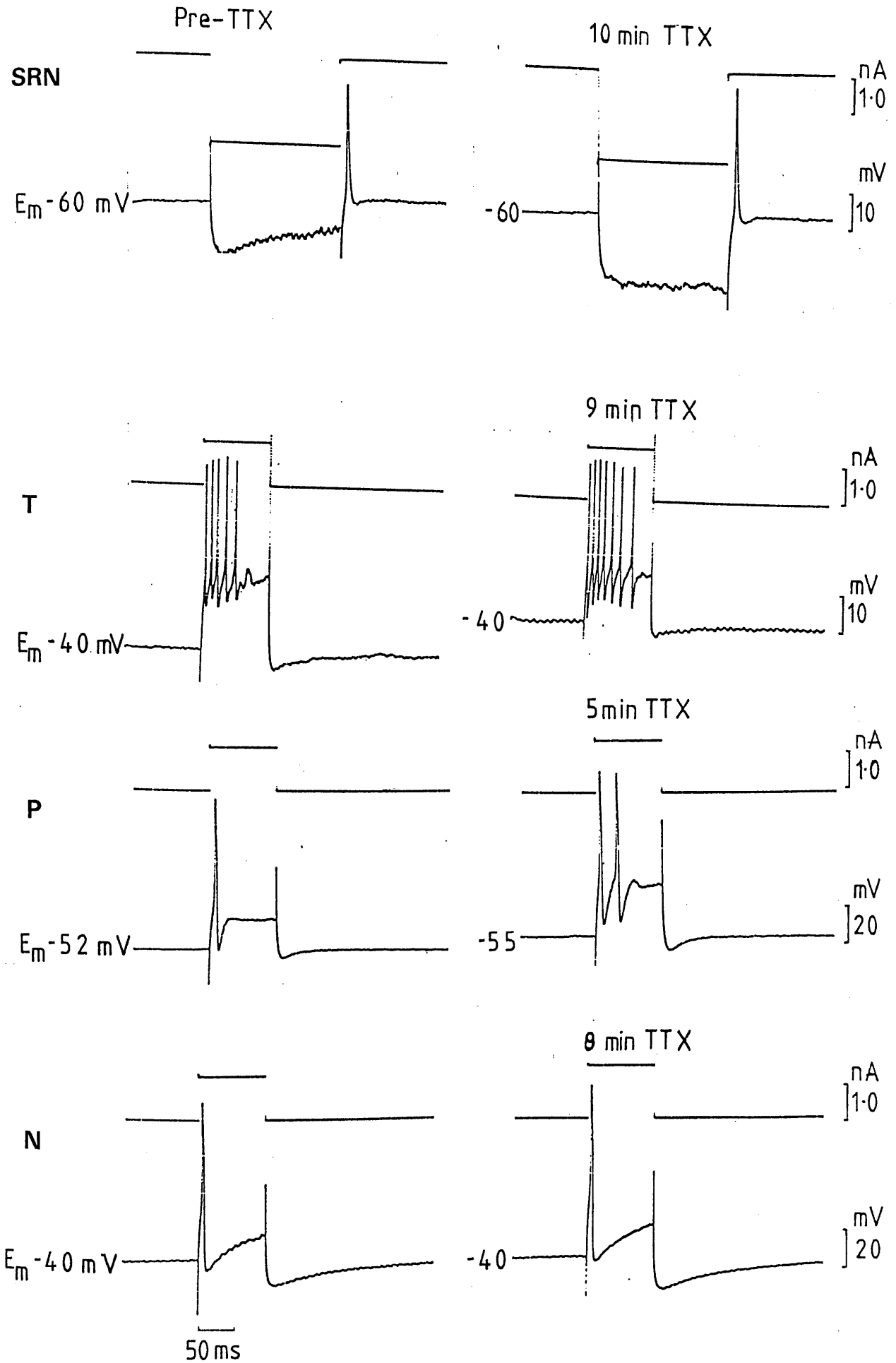
Figure 9. Repetative firing recorded from the SRN soma. Repetative activity recorded from the SRN cell body was rare. In this preparation injection of >10 nA of depolarising current into the cell body (top trace) elicited several action potentials (bottom trace). Repetative firing of the SRN upon anode break excitation was never observed after a hyperpolarising current pulse of similar amplitude.

2.3 Tetrodotoxin sensitivity of the action potential

It is well established that the neurotoxin extracted from the ovaries of the puffer fish, tetrodotoxin (TTX), will prevent impulse transmission by selectively blocking the early transient voltage-sensitive conductance increase for sodium (Narahashi, Moore and Scott, 1964). In concentrations ranging between 800 nM (Pasztor and Bush, 1983 ; crustacean oval organ afferent fibres) and 0.1 mM (Narahashi et al, 1964 ; lobster giant axons) TTX acts to reduce the early inward sodium current and hence reduce the intracellular action potential amplitude over the time course of a few minutes until spiking activity is completely abolished.

Figure 10 illustrates the effect upon action potential amplitude of adding 300 nM TTX to the bathing fluid. Recordings were made from the SRN cell body and from the cell bodies of Touch, Pressure and Noxious mechanosensory neurones located in the segmental ganglion. Action potentials were elicited by current injection into the cell bodies before and during subsequent exposure to TTX over a period of 10-20 minutes. No effect of TTX upon the amplitude of action potentials from the SRN, T, P, or N cells was seen. No apparent change in the action potential was noticed even in cells left for up to 20 min. in TTX. In all experiments the capsule surrounding the segmental ganglion was removed, hence in the case of the T, P, and N cells no physical barrier existed in the access of TTX to the cell bodies and it was assumed that the nerve sheath offered little or no resistance to the diffusion of substances around the cell body of

Figure 10. Tetrodotoxin (TTX) insensitivity of the SRN, T, P, and N cell action potentials. Action potentials were elicited from the peripheral SRN cell body and the central cell bodies of the touch (T), pressure (P), and nociceptive (N) mechanosensory neurones both in the absence (left column) and presence (right column) of 300 nM TTX. The SRN somatic action potential (bottom trace), elicited by a hyperpolarising current pulse (top trace) was unaffected after 10 minutes exposure to TTX. Similarly no effect was seen upon the amplitude of the action potentials recorded from the T, P, or N cells after exposure to 300 nM TTX for 9, 5, and 8 minutes respectively.


Pre-TTX
10 min TTX
SRN
nA
1·0
mV
10
E_m - 60 mV
-60
9 min TTX
T
nA
1·0
mV
10
E_m - 40 mV
-40
5min TTX
P
nA
1·0
mV
20
E_m - 52 mV
-55
8 min TTX
N
nA
1·0
mV
20
E_m - 40 mV
-40
50 ms

the peripheral SRN.

2.4 Non propagation of the action potential

Using dark field illumination it was possible to visualise the large diameter axon of the SRN as it entered the segmental ganglion in the anterior nerve root. It generally occupied a position on the anterior border of the root and with care stable intracellular recordings could be obtained. Confirmation of its identity was made by passing current into the SRN cell body through a second microelectrode whilst recording the subsequent potential changes within the axon with the first electrode.

Figure 11 illustrates the result of passing depolarising or hyperpolarising current into the cell body whilst recording simultaneously from the SRN axon and vice versa. In this preparation, paired intracellular recordings were made from the cell body and from the axon 3 mm away in the anterior nerve root. In panel a, depolarisation of cell body elicited an all-or-nothing action potential, (25 mV amplitude) which was transmitted passively along the axon, its amplitude and width being distorted in proportion to the space and time constants of the membrane (see later). The sub-threshold responses of the cell body are similarly transmitted along the axon in relation to the membrane electrical constants. In the present case the amplitude of the all-or-nothing signal recorded from the axon had fallen to 25% that recorded in the cell body. At the same distance along the axon however the sub-threshold potential had fallen to only 36% of that recorded in the cell body.

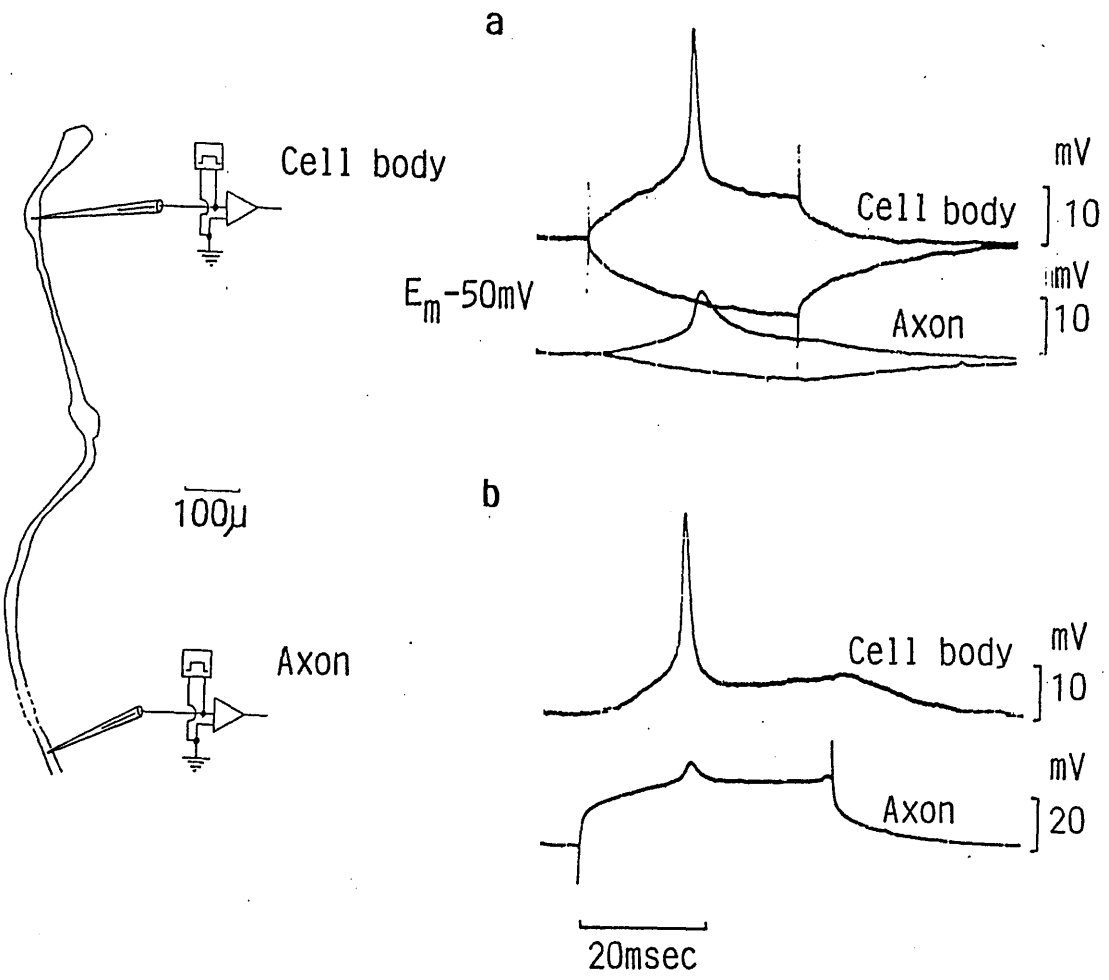
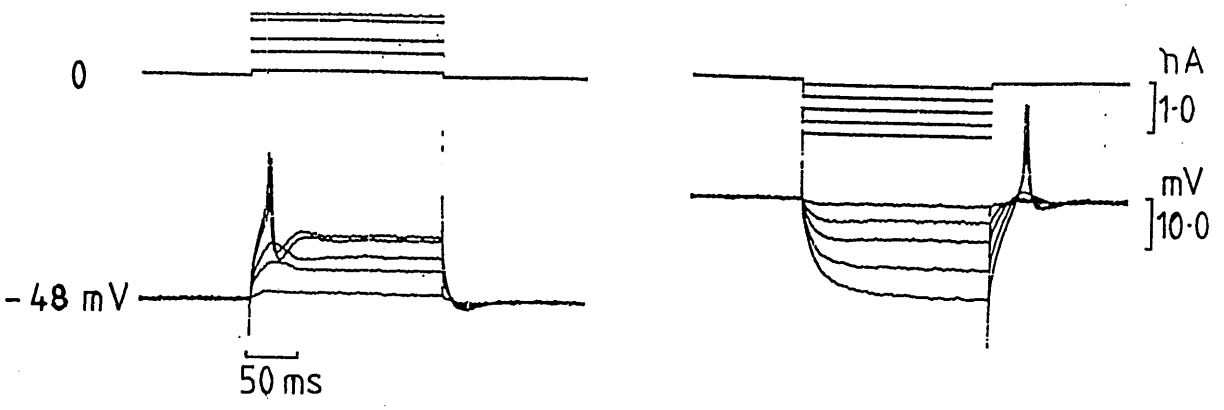
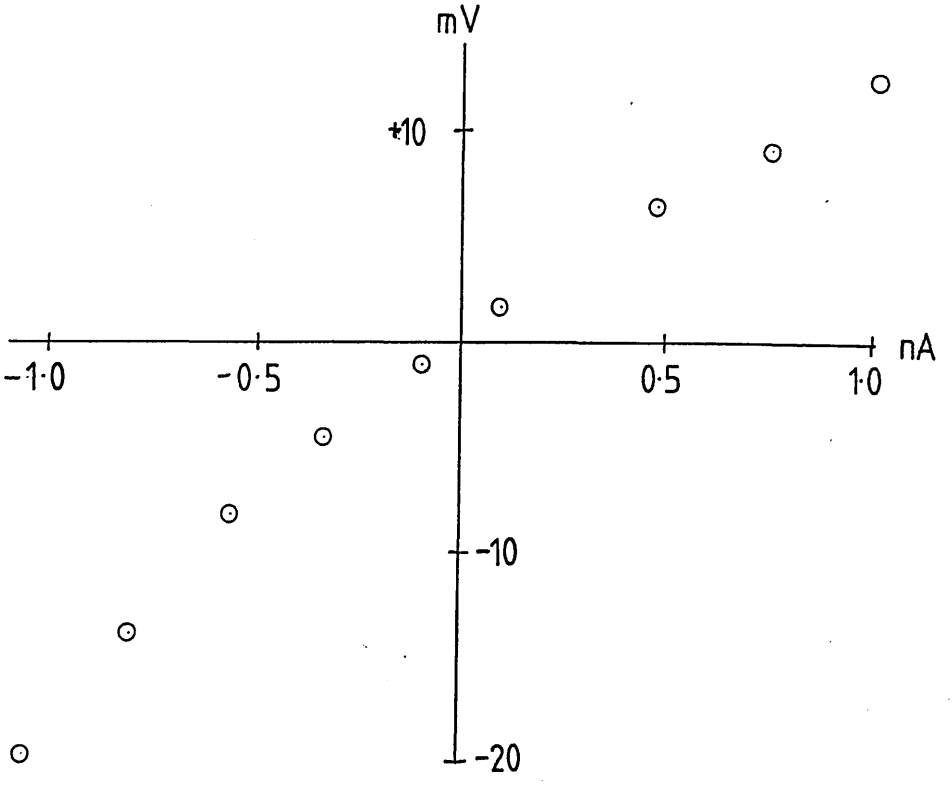


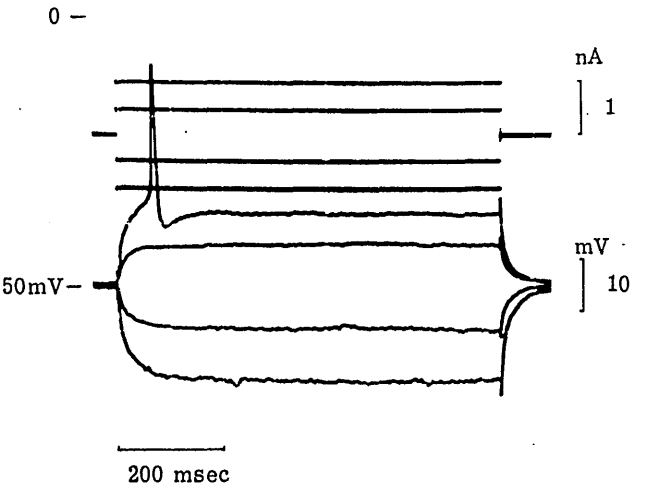
Figure 11. Non-propagation of the action potential. Paired intracellular recordings were made from the SRN cell body and from the axon 3mm away in the anterior nerve root (left hand diagram). a, Response of the cell body (upper trace) and the axon (lower trace) to square wave depolarising and hyperpolarising current pulses (not shown) injected into the peripheral SRN cell body. Depolarisation of the cell body elicited an all-or-nothing action potential which was transmitted passively along the axon. b, Injection of depolarising current (not shown) into the axon (lower trace) initiated an all-or-nothing action potential from the cell body (upper trace) which was reflected in the axon recording. It was not possible to elicit an all-or-nothing action potential from the axon by increasing the amplitude of injected current into either the axon or the cell body.

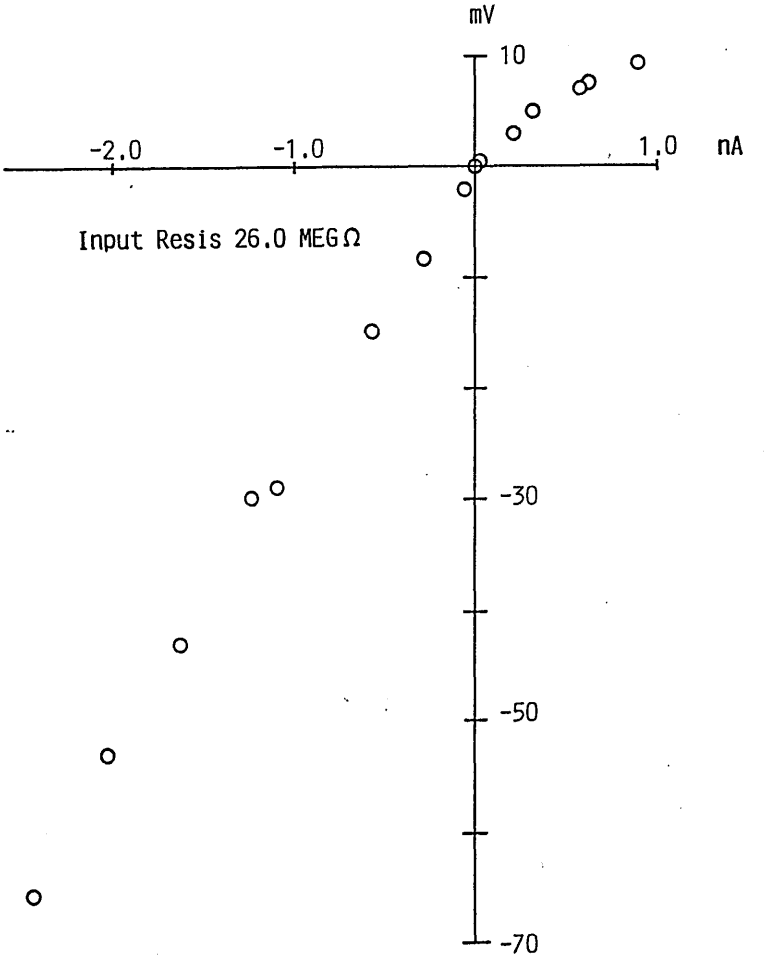
Figures 12 and 13. Input resistance (R_{in}) of the SRN. R_{in} was measured from the SRN soma using a single microelectrode to inject current pulses and record, via a bridge circuit, subsequent potential changes. The steady state current-voltage relationships for two different cells are shown. Both show an almost ohmic relationship over the greater part of the voltage range. Figure 12, traces above show example responses of the SRN cell body to increasing depolarising square wave current pulses (left hand figure) and increasing hyperpolarising current pulses (right hand figure) used to construct the lower graph. Figure 13, Top figure shows examples of the superimposed responses (bottom traces) to four depolarising and hyperpolarising square wave current pulses (upper traces) used to construct the graph in the lower figure.



Input Resis 14·5 MEGΩ




0 –
nA
1
mV
10
50mV–
200 msec


mV
10
-2.0
-1.0
1.0
nA
Input Resis 26.0 MEGΩ
-30
-50
-70

In figure 11b depolarising current was injected into the axon whilst recording simultaneously from the cell body. Passive spread of current from the axon into the cell body initiated an all-or-nothing action potential in the cell body which was reflected in the axon recording.

It was not possible in any preparation to elicit an all-or-nothing action potential from the axon by increasing the amplitude of injected depolarising or hyperpolarising current.

2.5 Membrane characteristics.

The input resistance of the neurone, R_{in}, was measured from the cell body using a single microelectrode to inject small current pulses and record, via a bridge circuit, the subsequent potential changes. The steady-state current-voltage relationships for two cells are shown in figures 12 and 3. Both curves show an almost ohmic relationship over much of the voltage range, one of the cells however (fig. 13) shows some rectification within the depolarising current range. The input resistances for these two cells, calculated from the slope of the linear portion of the curves were 14.5 Meg ohm and 26.0 Meg ohm respectively. R_{in}, determined for a total of 7 cells, ranged between 14.2 Meg ohm and 26.0 Meg ohm giving a mean value of 18.2 Meg ohm (+/- 4.7 S.D. see also table 1 for other values of R_{in}). These results compare with values for the input resistance of Retzius cells within the ganglion ranging between 18.2

Meg ohm (1 cell, this study) and a mean of 20.7 Meg ohm for four cells in the ganglion (Fuchs, Nicholls and Ready, 1981. Values of input resistance for isolated cells in this study was much higher, 129 +/- 36 Meg ohm for Retzius cells isolated in culture for 3 days).

From evidence presented in figure 11 that action potentials, generated in the cell body region of the neurone, are not actively propagated down the axon, it appeared that transmission of information from the peripheral sensory dendrites to the CNS might depend upon the passive properties of the axon as in the case of the crustacean non-spiking TCMRO (Roberts and Bush, 1971) and hence may require rather specialised cable properties.

Figure 14 shows the experimental arrangement used to determine the electrical length constant (λ) of the cell and sample records obtained. Current was injected into the neurone from an electrode placed in the cell body at point "a". The current and potential change monitored at this point are shown in the upper two traces of panels A and B. The voltage changes in response to current pulses injected at "a" were recorded by inserting two further electrodes at position "b", 240µm from the point of current injection and at position "c" 4300µm from the cell body. The potential changes observed at positions "b" and "c" are shown as the bottom traces in panels A and B respectively.

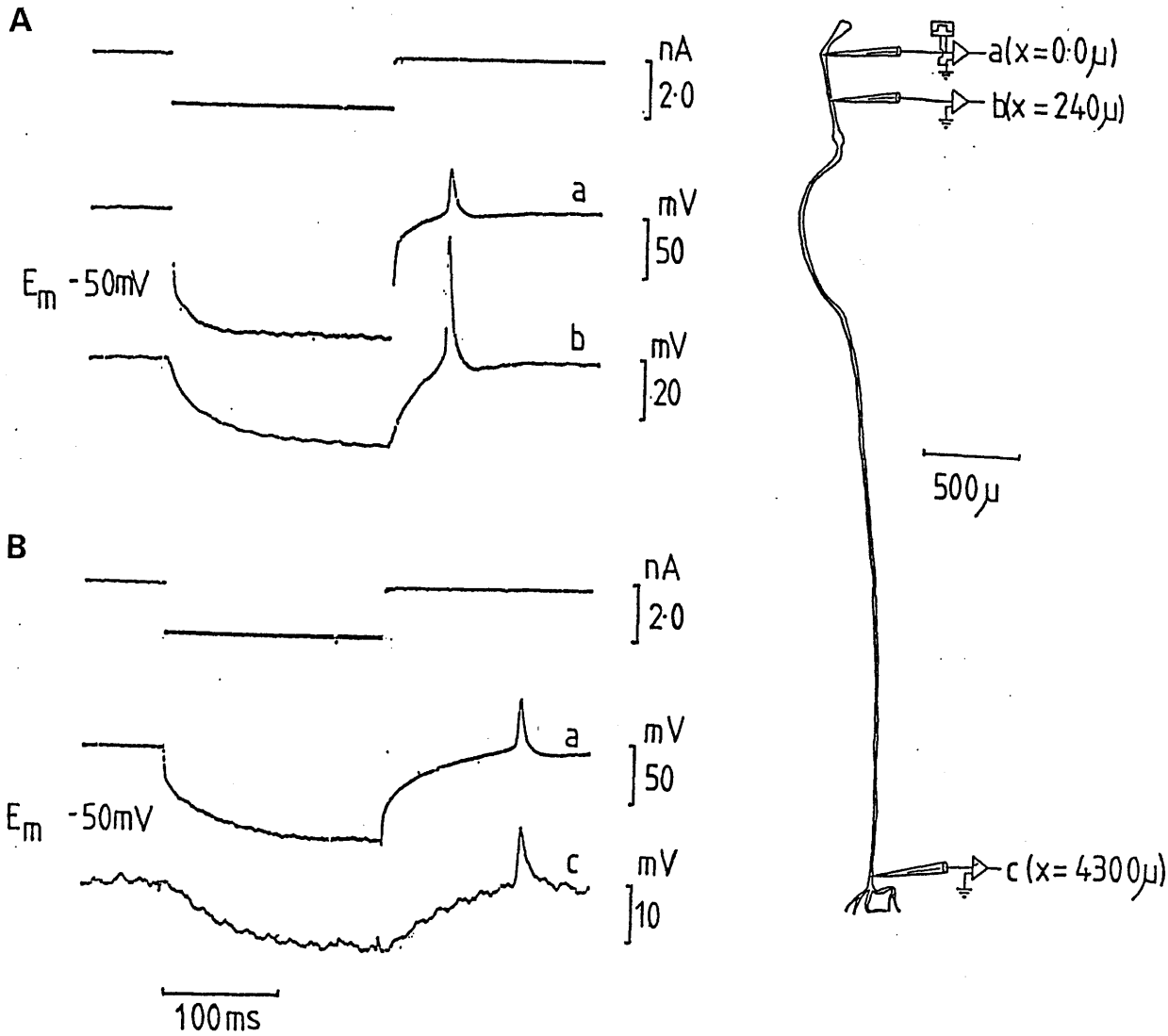


Figure 14. Space constant of the SRN. The space constant of the SRN was calculated from the decay of hyperpolarising current pulses injected into the SRN soma at position "a" (right hand diagram). The injected current and potential change monitored at this point are shown in the upper two traces of the left hand panels A and B. The potential changes were monitored at two further positions within the neurone, at position "b", 240μm from the point of current injection and at position "c", 4300μm from the cell body (right hand diagram). The potential changes recorded at these two positions in response to current injection at "a" are shown in the bottom traces in the left hand panels A and B respectively. The space constant calculated for this cell using the simple infinite cable model was 4.1mm.

The value of the length constant was calculated from the simple infinite cable model. This assumes that the potential recorded will fall exponentially with distance from the site of current injection. The relation is:-

$$V_x = V_o \exp^{(-x/\lambda)}$$

where V_x and V_o are the distal "c" and proximal "b" potential changes recorded at a separation x. From the above equation:-

$$\text{length constant} = x \,/\, \ln V_o/V_x$$

Given values from figure 12 (x = 4.06mm, V_o = 28.1mV, V_x = 10.4mV) the length constant was calculated as 4.1 mm. (see also table 1 for other values of λ)

Specific membrane resistance (R_m) for this neurone was calculated using the formula:-

$$R_m = 2\pi a r_m$$

which assumes that the afferent fibre may be represented as a cylindrical cable of infinite length and that $r_m = R_{in}\lambda$, where r_m is the transverse membrane resistance and a is the fibre radius, measured experimentally from HRP-filled whole mount preparations. The value of R_m for the fibre using this equation was 18.3 K Ohm cm^2

Specific longitudinal resistance (R_i) was calculated from the relation:-

$$R_i = \pi r_i a$$

again assuming a cable of infinite length and that $r_i = R_{in}/\lambda$, where r_i is the longitudinal axonal resistance and a again is the fibre radius. The value calculated was 27.2 Ohmcm.

The behaviour of this afferent fibre as an infinite cable will also be reflected in the time course of the potential charging curves recorded in response to an injected current pulse. In a spherical cell, without dendrites or an axon, the rise and fall of potential in response to an injected current pulse may be described by the exponential function:-

$$V = iR(1-e^{-t/\tau})$$

where time, t=0, represents the beginiing or end of the injected current pulse. The time constant (τ) is the product of the membrane resistance and capacitance and is the time for the potential to rise or decay to (1 - 1/e) or 63% of its final value. For current flow in a cable however, the situation is more complicated (Hodgkin and Rushton, 1946). In this situation the capacitative and resistive elements of each segment of the cable interfere with each other so that the potentials recorded at various distances along the cable do not rise and fall exponentially. In the present experiments, the electrotonic potential was recorded at the point of current injection at which point the time constant is given

Table 1 Passive membrane properties

Cell	Em(mV)	Rin(Mohm)	λ(mm)	τ(ms)	Rm(Kohm cm)	Ri(Ohm cm)
1	-45	14.2	2.45	-	8.7	29.1
2	-55	14.5	-	-	-	-
3	-42	16.7	4.4	-	23.5	22.7
4	-50	26.0	-	-	-	-
5	-50	24.0	-	-	-	-
6	-50	17.3	4.1	25.2	22.0	33.1
7	-53	15.0	-	-	-	-
mean	-49	18.2				

by the time for the potential to rise to 84% of its maximum amplitude. The time (τ) for a hyperpolarising potential change, in response to a hyperpolarising current pulse injected at the same site in the axon, to fall to 84% of its final amplitude was measured as 25.2 msec.

2.6 Distal axon properties

Intracellular recordings made from the SRN axon at a distance of some 4 mm from the cell body as it enters the segmental ganglion invariably showed an E_m identical to that recorded from the cell body, (c.f. figs. 11 and 14). Inspection of the intracellular record from the axon however reveals small, transient events superimposed upon the membrane potential. Figure 15a shows 4 consecutive sweeps of an intracellular record taken from the SRN axon in the anterior nerve root immediately adjacent to the ganglion. The small transient events, approximately 2mV in amplitude were superimposed upon the membrane potential of -50 mV. These events were not normally seen in an intracellular recording made from the cell body of the same neurone. Such an intracellular recording generally showed no on-going activity whilst activity was often present in a simultaneous intracellular recording from the axon some 4 mm away.

Figure 15b shows these transient events, recorded from the axon in a different preparation on a faster time base. 5 consecutive sweeps show three events <5mV in amplitude. They have characteristic

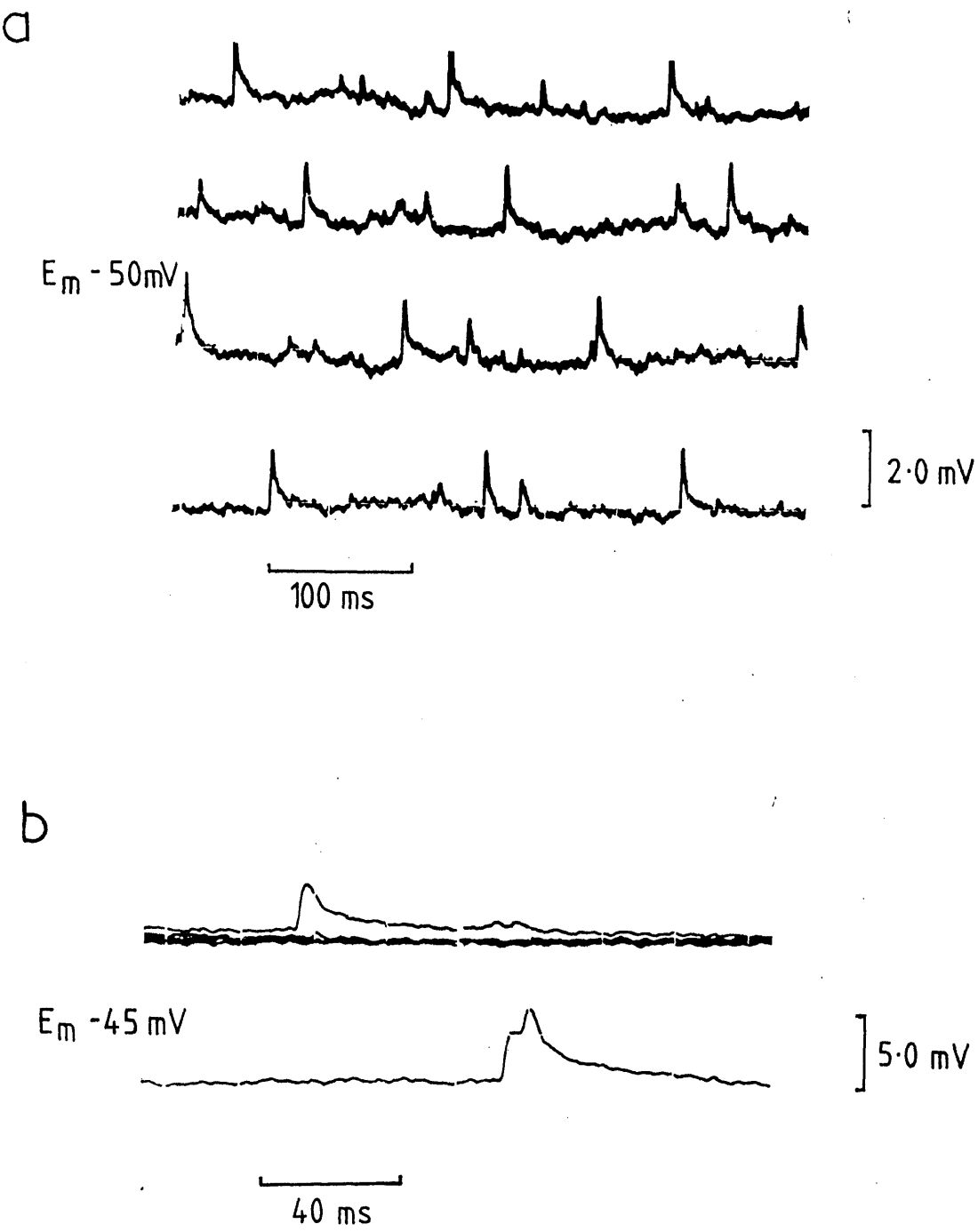


Figure 15. Distal axon properties. a, Four consecutive sweeps are shown of an intracellular record made from the SRN axon in the anterior nerve root adjacent to the segmental ganglion. Small (approximately 2.0 mV) transient events show characteristic post-synaptic potential (psp) activity (fast rise time, slow exponential decay and summation). b, Similar transient events, recorded from a different preparation on a faster time base. Five consecutive sweeps show three events approximately 5.0 mV in amplitude. Summation of two potentials is clearly seen in the lower trace.

features of post-synaptic potentials (psp's), i.e. fast rise time and slow exponential decay, the rapid rise time being an indication of the proximity of the synaptic site. Summation of two potentials seen in the lower trace is indicative of subthreshold post-synaptic activity. Frequently, psp's of different amplitude were seen, indicating a number of presynaptic inputs of varying efficacy in close proximity, eg panel a.

At the post-synaptic membrane of a conventional fast excitatory synapse (eg. the neuro-muscular junction) the excitatory post-synaptic potential (epsp) mediated by the release of a pre-synaptic transmitter substance (eg. Ach), results from a non-specific increase in permeability of the post-synaptic membrane to sodium, potassium and to a negligible extent to calcium. A feature common to both the epsp and the inhibitory posy-synaptic potential (ipsp) is the ability to reverse the electrical gradient responsible for the peak Na and K currents which give rise to the post synaptic event. It is possible therefore at a suitable potential level to reduce or reverse the direction of the current flow and hence the polarity of the observed psp.

In figure 16 the electrotonic potentials produced as a result of the injection of square wave depolarising currents into the axon are shown for two different cells at E_m. Superimposed upon the electrotonic potential curves are a number of epsp's which reduce in size as each successive charging curve reaches a higher level of depolarisation. This is also shown in figure 17a. In this cell, from

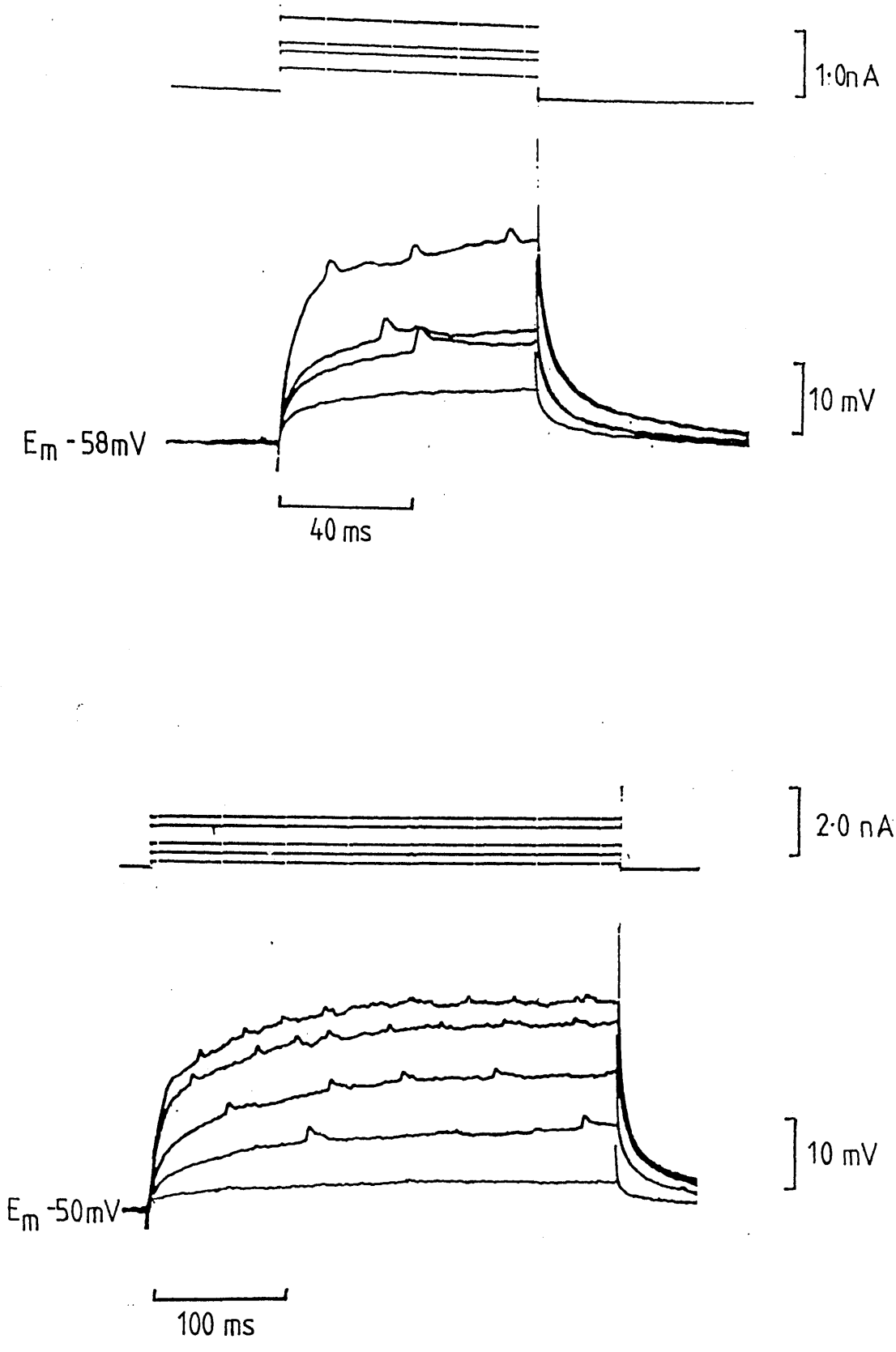


Figure 16. Change in psp amplitude upon alteration in SRN E_m. Square wave current pulses were injected into the SRN axon (top traces, both panels) and the susquent poential changes recorded via the same microelectrode (lower traces) for two different SRN's at E_m. The amplitude of the psp's superimposed upon the electrotonic potential curves decreases as the injected current depolarises the axon by degrees in a manner characteristic of chemical synapses.

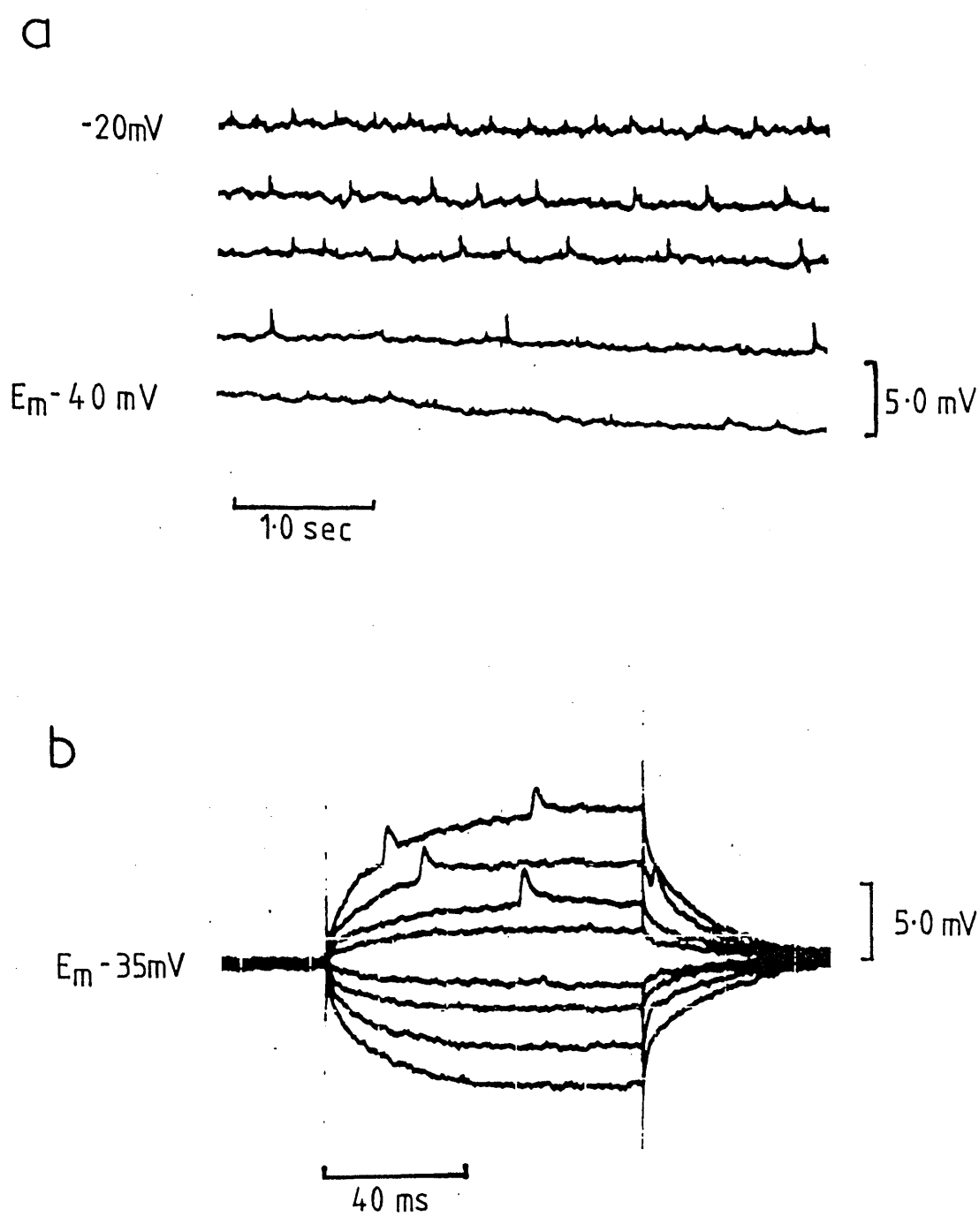


Figure 17. Changes in psp amplitude with alterations in SRM E_m. a, Five consecutive intraxonal recordings are shown in which depolarising current was injected into the SRN axon (not shown) and the E_m held at five successive levels of depolarisation between πrestingπ E_m (−40 mV) and =20 mV. The psp amplitude decreases by degrees as the SRN E_m is depolarised. A further effect of altering the SRN E_m is that the frequency of the synaptic events was dramatically altered (compare top trace with second bottom trace). b, Several superimposed traces are shown of the intraxonaly recorded SRN potentials in response to square wave depolarising and hyperpolarising current pulses (not shown). Hyperpolarisation of the SRN by a few mV completely abolished the synaptic activity.

another preparation, direct current was injected through the recording electrode, via a bridge circuit, into the axon. In this way the membrane potential of the axon was held at successive levels of depolarisation and the effect on the epsp amplitude observed. Again the epsp amplitude decreases by degrees as the cell depolarises. It was not possible in these experiments to move the membrane potential above -20 mV, possibly due to the rectifying properties of the electrodes used, and hence observe reversal of the epsp's.

Figure 17b shows the result of moving the membrane potential to more hyperpolarised levels. If, according to the idea that the peak current underlying the epsp is proportional to the difference between the Em and the reversal potentials for the ionic species envolved, then one should expect a larger amplitude epsp at more hyperpolarised potentials. This was not possible to demonstrate since hyperpolarising the cell below approximately -33 mV completely abolished the synaptic activity. Figure 16a, 16b and 17a also show that epsp activity was infrequent at E_m and increased in frequency as the E_m reached more depolarised levels.

Further evidence for the synaptic nature of the transient events is given in figure 18. The trace in panel a, and the bottom two traces in panel b are are intracellular recordings of activity from the SRN axon, taken near to the point of origin of the anterior nerve root. In panel a the preparation was bathed in normal leech Ringer solution (see methods). Panel b shows activity in the axon 5

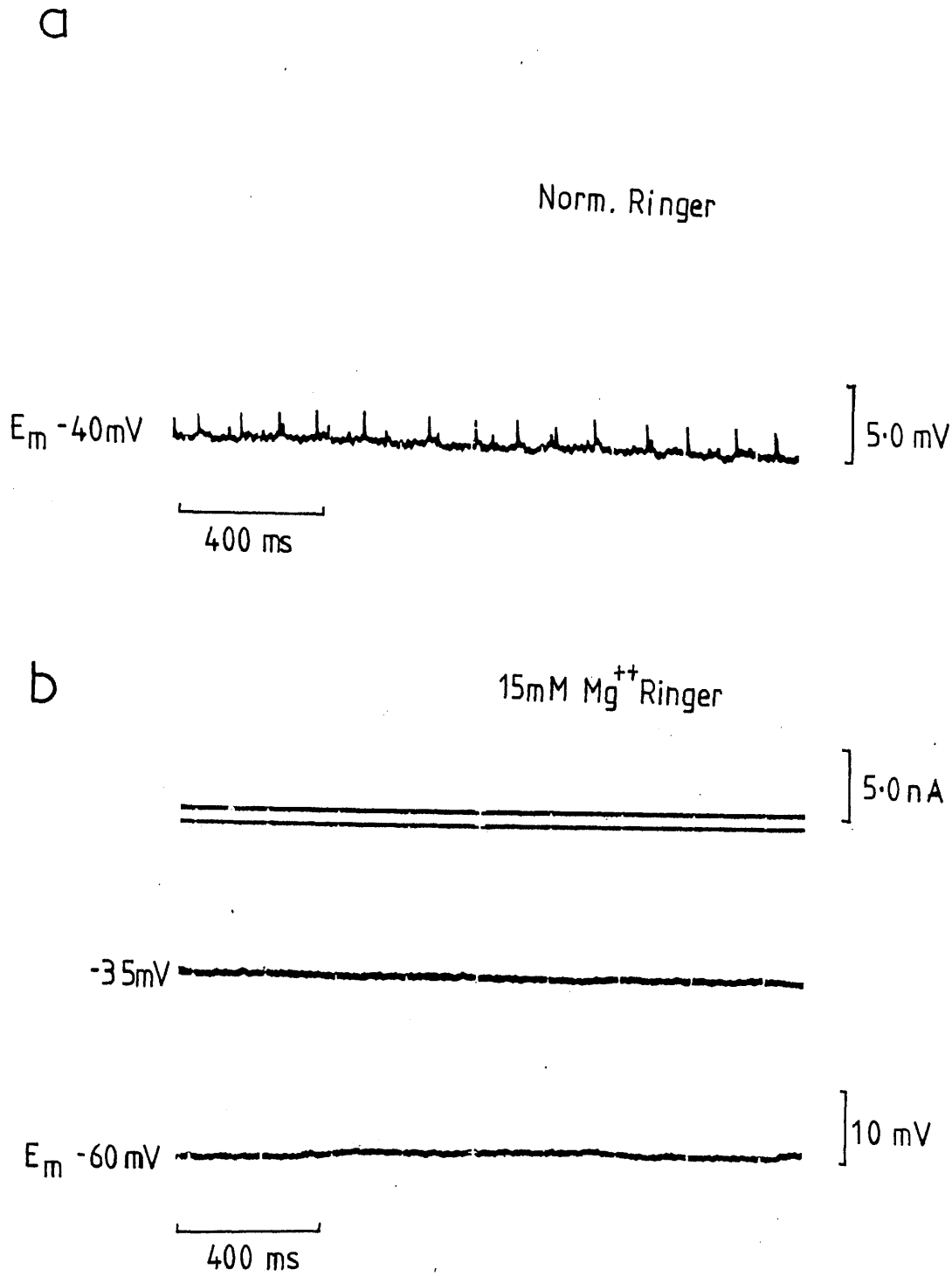


Figure 18. Abolition of psp's in high Mg^{++} solution. Intraxonal recordings were made from the SRN axon adjacent to the segmental ganglion. a, Synaptic activity recorded from the SRN axon in normal leech Ringer solution. b, Activity in the SRN axon from different preparation 5 mins. after substitution to a high (15mM) Mg^{++} Ringer solution (lower trace). All transient activity is abolished at E_m (-60mV). Direct depolarising current (top trace) was injected into the SRN axon and the SRN E_m lowered to -35 mV (middle trace). Synaptic activity remained absent at the new E_m in the presence of 15mM Mg^{++}.

mins after substitution to a high Mg Ringer solution, (15 mM Mg^{++}), a concentration known to block chemical synapses in the leech (Baylor and Nicholls, 1969). All transient activity is inhibited in the 15mM Mg^{++} Ringer solution, both at E_m and upon depolarising the SRN axon membrane potential to -35 mV.

SECTION 3.0 RESPONSE CHARACTERISTICS

3.1 Segmental nerve suction electrode recordings

Previous studies on stretch-sensitive units in the segmental nerves of annelids have generally employed the conventional technique of extracellular recording using platinum hook electrodes, with the consequent disagreeable proceedures associated with mineral oil pools etc. (Gray et al. 1938.; Laverack, 1969.) In such experiments, spiking activity in the segmental nerves may be recorded in response to passive stretch or deformation of the body wall. In the preparations used, it was not possible to determine either the identity of the sensory elements or their location, either within the segmental muscles or the overlying cutaneous tissue. It is such responses that have often been interpreted as proprioceptive in nature (see Dorset D.A., in Mill, P.J., 1976).

In the present study, extracellular activity was recorded from the anterior segmental nerve in response to a ramp function stretch of the ventral body wall. Figure 19a shows a suction electrode recording of activity from the distal end of the cut anterior segmental nerve in response to a single ramp function stretch of the body wall of final amplitude 1.8mm. In this and the subsequent

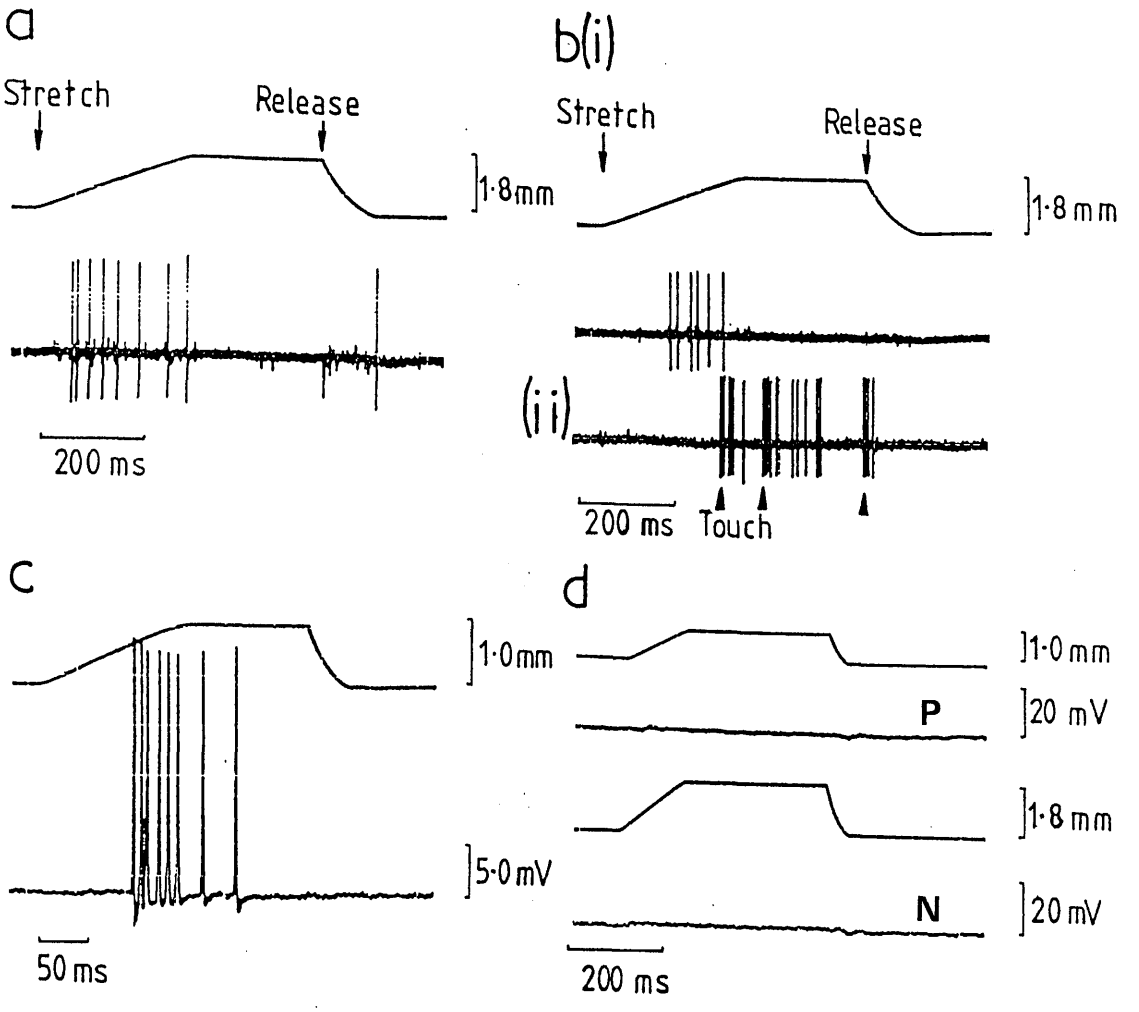


Figure 19. Extracelluar recordings from the anterior segmental nerve root. Extracelluar activity was recorded with suction electrodes from the anterior segmental nerve root in response to ramp function stretch of the ventral body wall. a, Extracellular activity (lower trace) in response to a ramp function stretch of the body wall (upper trace) of final amplitude 1.8mm. bi, Extracelluar activity recorded from the anterior nerve root in a different preparation in response to a ramp stretch of the ventral body wall. bii, A continuation of the extracellular record shown in bi, but in which the ventral body wall was touched with a glass probe at a stimulus strength known solely to excite touch mechanosensory endings (Nicholls and Baylor, 1968). The extracellular spike amplitude recorded in response to stretch of the body wall matched exactly the amplitude of spikes recorded in response to touch of the body wall. c, Intracelluar recording (lower trace) from the central soma of the touch cell which innervates the ventral portion of the body wall showing its response to a 1.8mm amplitude ramp function stretch of the ventral body wall (upper trace). The intracellularly recorded response profile of the T cell closely resembles that recorded extracellularly from the anterior nerve root. d, Intracellular recordings from the ventral P mechanosensory neurone (upper pair of traces) and N mechanosensory neurone (lower pair) in response to stretch of the ventral body wall of final amplitudes of 1.0 and 1.8 mm respectively. The body wall distortion produced by stretch of the longitudinal muscle was insufficient to activate the higher threshold mechanoreceptors.

experiments shown in figure 19, the body wall preparation remained essentially intact with the skin still covering the three underlying muscle layers.

The response shown in figure 19a is very similar to that reported by Laverack (1969, fig. 1b.) to a constant velocity triangular indentation of the body wall.

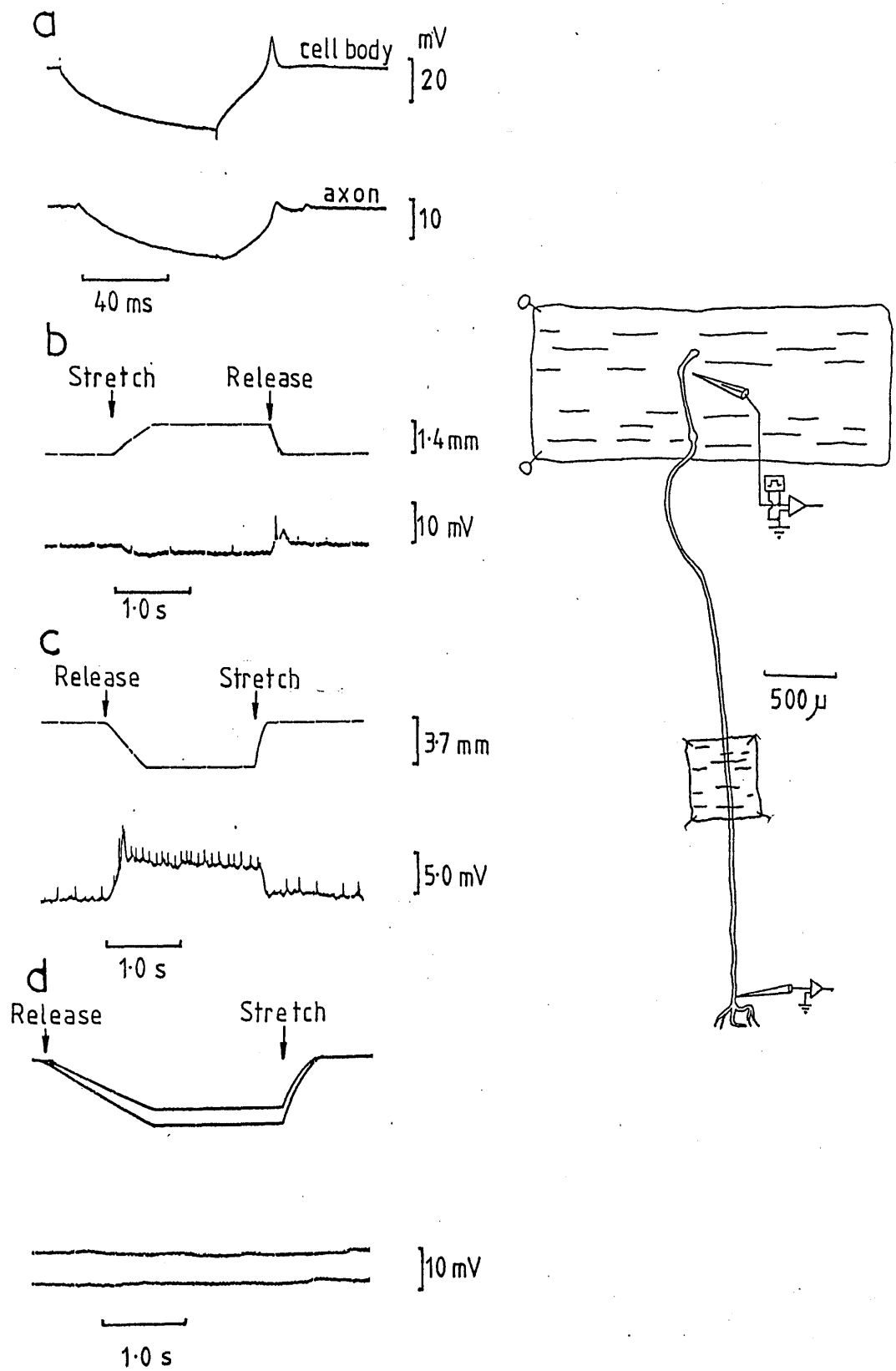
In figure 19b(i) extracellular activity was recorded with a suction electrode from the anterior nerve root in response to a ramp stretch of the body wall. Figure 19b(ii) is a continuation of the extracellular record in figure 19b(i) but in which the body wall was gently touched with a glass probe at a stimulus strength known solely to excite touch mechanosensory endings (Nicholls and Baylor, 1968). A high frequency discharge was seen in response to touch of the body wall which exactly matched the amplitude of the response previously recorded to stretch of the body wall. Figure 19c shows an intracellular recording of the response of the touch mechanosensory neurone which innervates ventral skin, to a ramp function stretch of the ventral body wall. The response profile of the T cell closely matches that recorded extracellularly from the anterior root in response to a ramp stretch. It therefore seems likely that spiking activity recorded in the anterior nerve root in response to stretch of the body wall is from T cell mechanosensory units innervating the skin. It is known that distortion of the skin by 30μm or less, or eddies in the bathing medium (which may result from stretch of the body wall), is sufficient to elicit a response from the touch

mechanosensory neurones, (Nicholls and Baylor, 1968). The degree of body wall distortion produced by stretch was insufficient to activate higher threshold pressure (P) and noxious (N) mechanoreceptors present in the skin as shown in figure 19d.

From the size of the SRN axon it might be expected that its action potential, if conducted to the CNS and recorded extracellularly, would be larger than those of the T cells'. However, extracellular spikes larger than those of the T cell were not seen. From the evidence presented in section 2.4 and the lack of any spikes larger than those of the T cell it seemed likely that the cell may respond with a decrementally conducted signal. The response of the neurone to stretch of the ventral body wall was therefore recorded intracellularly.

3.2 Intracellular axonal recordings.

The response of SRN to body wall stretch was studied by applying ramp function stretches to the ventral longitudinal muscle whilst recording intracellularly from the SRN axon 3-4mm distant as it enters the segmental ganglion in the anterior nerve root. The preparation consisted of the segmental ganglion and anterior nerve root containing the SRN and a thin strip of ventral longitudinal muscle at the level of the cell body and dendrites of the neurone (figure 20).


a
cell body
mV
20
axon
10
40 ms
b
Stretch
Release
1·4 mm
10 mV
1·0 s
c
Release
Stretch
3·7 mm
5·0 mV
1·0 s
d
Release
Stretch
10 mV
1·0 s
500 µ

All overlying oblique, and circular muscle and skin had been dissected away and the majority of the longitudinal muscle, leaving only the longitudinal muscle bands associated with the SRN. One end of this muscle was pinned to the Sylgarded floor of the recording chamber whilst the other was securely attached to the arm of the puller device. The movement transmitted to the cell body and dendrite region of the SRN by such an arrangement of the puller made it very difficult to maintain an electrode impalement and thereby record its response from the cell body. It was therefore necessary to record the response of the neurone intracellularly from its axon in the anterior nerve root. The axon recording site was carefully isolated from all movement of the longitudinal muscle by securely pinning, half way along the anterior nerve root, a separate region of the longitudinal muscle, through which the anterior root ran. This second strip of longitudinal muscle, pinned with cactus spines, was separate from the more lateral piece of longitudinal muscle to be stretched associated with the SRN dendrites, hence no movement was transmitted either to it or the anterior nerve root which it suppported.

3.3 SRN response to changes in body wall length.

Initial confirmation of the identity of the SRN axon in the anterior root was made by passing current into the cell body whilst recording the consequent potential change from the SRN axon with a second electrode, (figure 20a). In subsequent experiments it became

possible to unambiguously identify the SRN axon without the need to impale the cell body, both from its regular position on the anterior margin of the nerve root and from the characteristic high frequency epsp activity often present (see section 2.6). It could be further identified by the single, decrementally transmitted action potential evoked upon anode break excitation of the axon, (see also section 2.4)

The length of the strip of longitudinal muscle to be stretched was adjusted by means of a rack and pinion device on the puller so that it matched the "resting" length of a segment in an intact anaesthetised animal, (approximately 5mm).

The response of the neurone, either to stretch of the ventral longitudinal muscle (upward deflected ramp), or to release of the previously stretched ventral longitudinal muscle (downward deflected ramp), is shown in figures 20b and 20c. The response of the SRN to a ramp stretch of the longitudinal muscle consisted of a hyperpolarising potential, maintained for the duration of the stimulus with a marked excitatory response apparent upon release of stretch. Similarly the neurone responded to a ramp release of the longitudinal muscle with a depolarising receptor potential again maintained for the duration of the ramp stimulus. In control experiments intracellular recordings were made from other unidentified axons in the anterior root (figure 20d). No axon apart from the SRN was seen to respond to a ramp release or stretch of the body wall. Niether were any movement artifacts seen when the

recording electrode was randomly placed randomly into the nerve sheath and vigourous movements applied to the longitudinal muscle. I was confident therefore that the responses observed were from the SRN axon and free of contamination from movement artifact. The response of the SRN to stretch of the longitudinal muscle is shown in greater detail in figure 21. Single ramp function stretches were applied to the longitudinal muscle whilst recording intracellularly from the SRN axom 3-4 mm away in the anterior nerve root. Three ramp stretches of final amplitude 1.0, 1.4 and 1.8 mm were applied to the strip of muscle, allowing a time interval of approximately 20 sec. between consecutive pulls. The neurone responded with hyperpolarising potentials which were maintained for the duration of the stretch stimulus, with final amplitudes of response of 1.2, 1.7 and 2.0 mV respectively from the initial E_m of -40 mV. Upon release of stretch there was a marked excitatory response. This was characterised by small spike-like events. They did not overshoot zero and were thougth to be spikes initiated in the cell body and transmitted decrementally along the (SRN) axon.. They were distinct from the epsp events seen in the same fibre (see section 2.6).

The response of the SRN to ramp releases of the ventral longitudinal muscle in a different preparation is shown in figure 22. In this preparation high frequency psp activity was present superimposed upon a rather low initial membrane potential (-30 mV). The response of the neurone was studied by applying a single ramp release to a previously stretched segment of longitudinal muscle. The response showed two distinct phases, an initial dynamic component, followed by a lower amplitude static response maintained

Figure 21. SRN response to imposed stretch of the ventral longitudinal muscle. a, Ramp function stretches were applied to the longitudinal muscle whilst reording intracellularly from the SRN axon 3-4mm away in the anterior nerve root. b, Three imposed ramp stretches of final amplitude 1.0, 1.4 and 1.8 mm were applied one afer the other to the strip of longitudinal muscle allowing a time interval of 20 seconds between stretches (upper trace, three stretches superimposed, top stretch final amplitude 1.8mm) The SRN responded with hyperpolarising potentials, maintained for the duration of the stretch stimulus with final amplitudes of response of 1.2, 1.7 and 2.0 mV respectively (lower three traces, top intracellular response record corresponds to the top stretch trace).

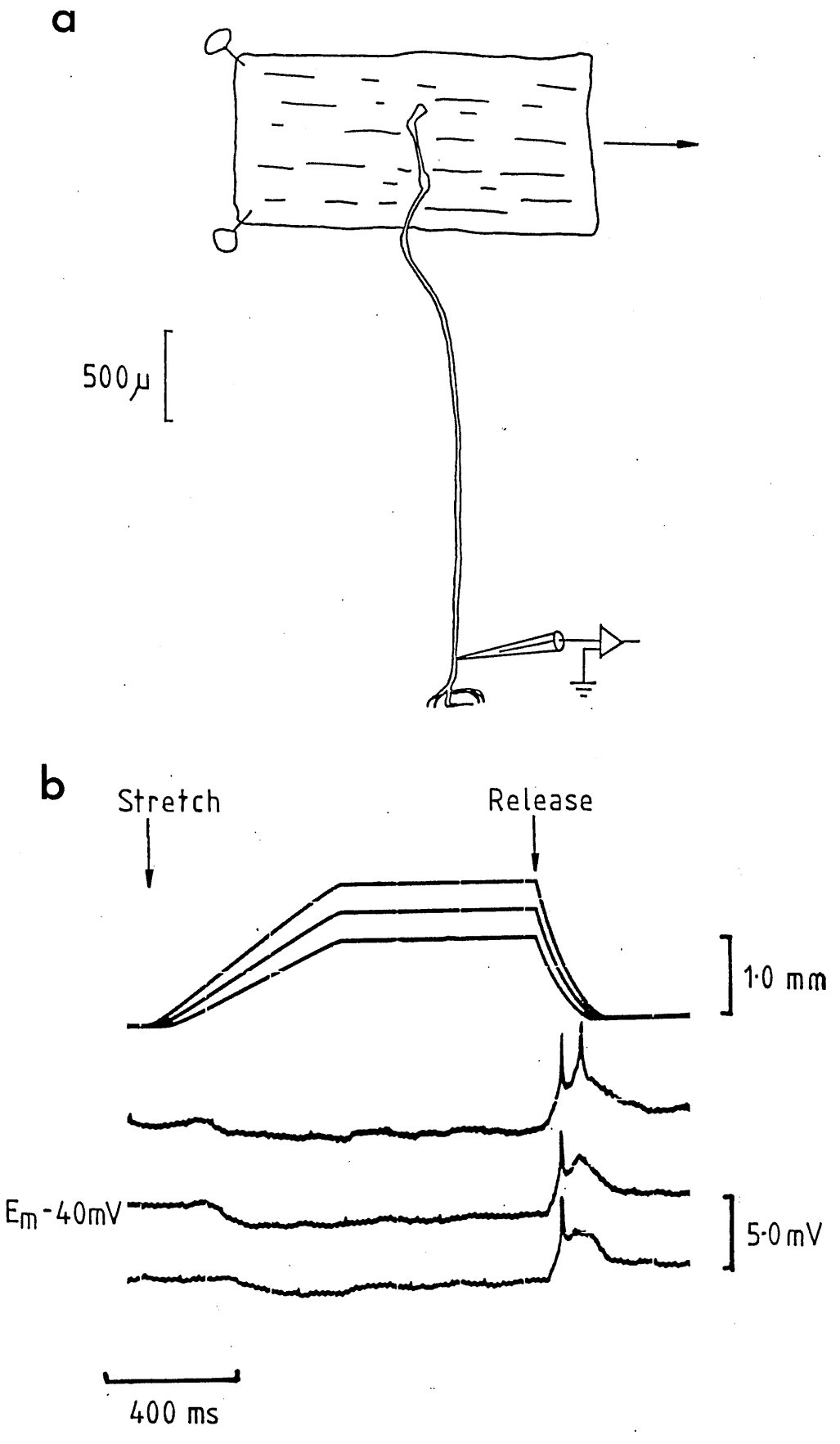
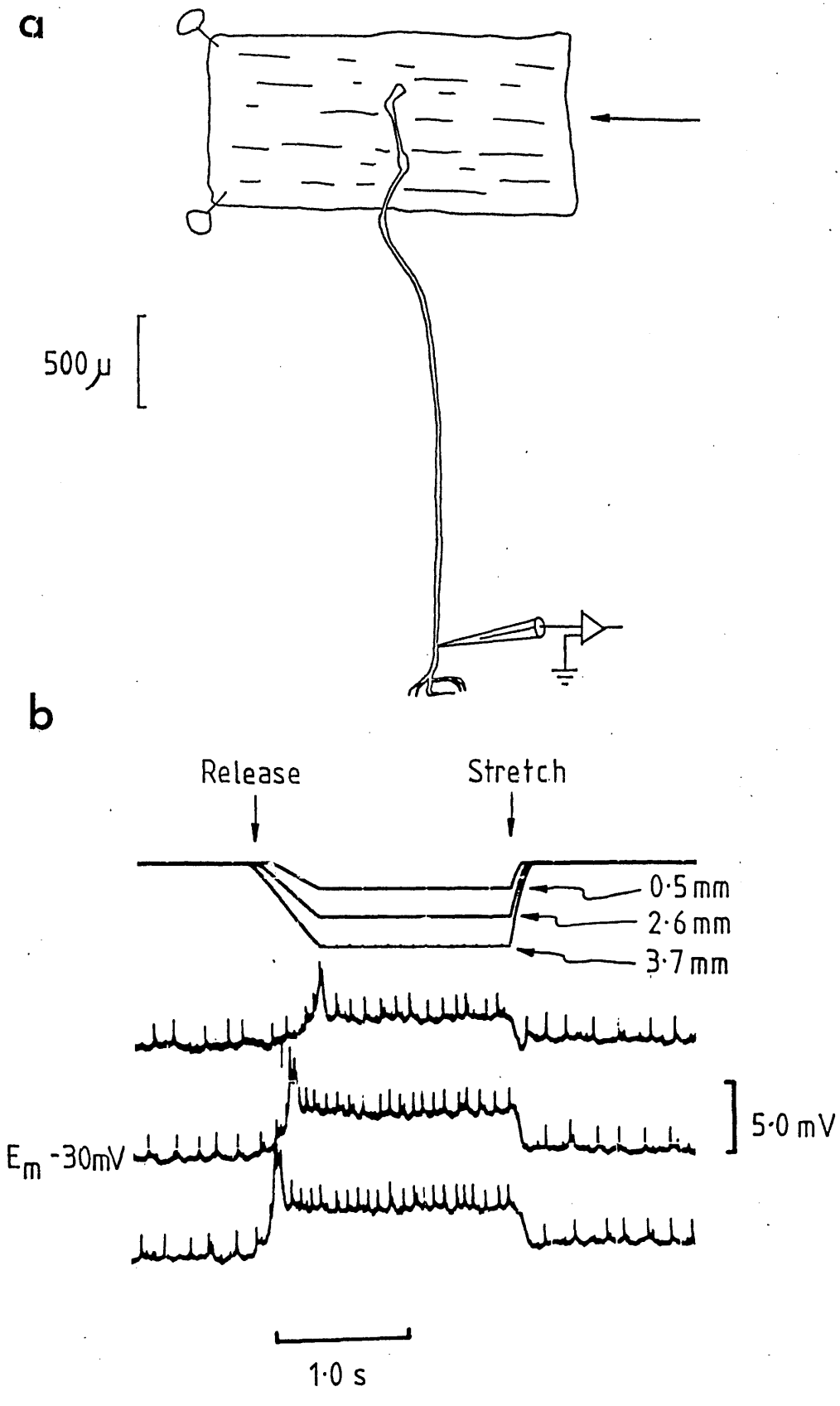

a
500μ
b
Stretch
Release
1·0 mm
Em -40mV
5·0 mV
400 ms

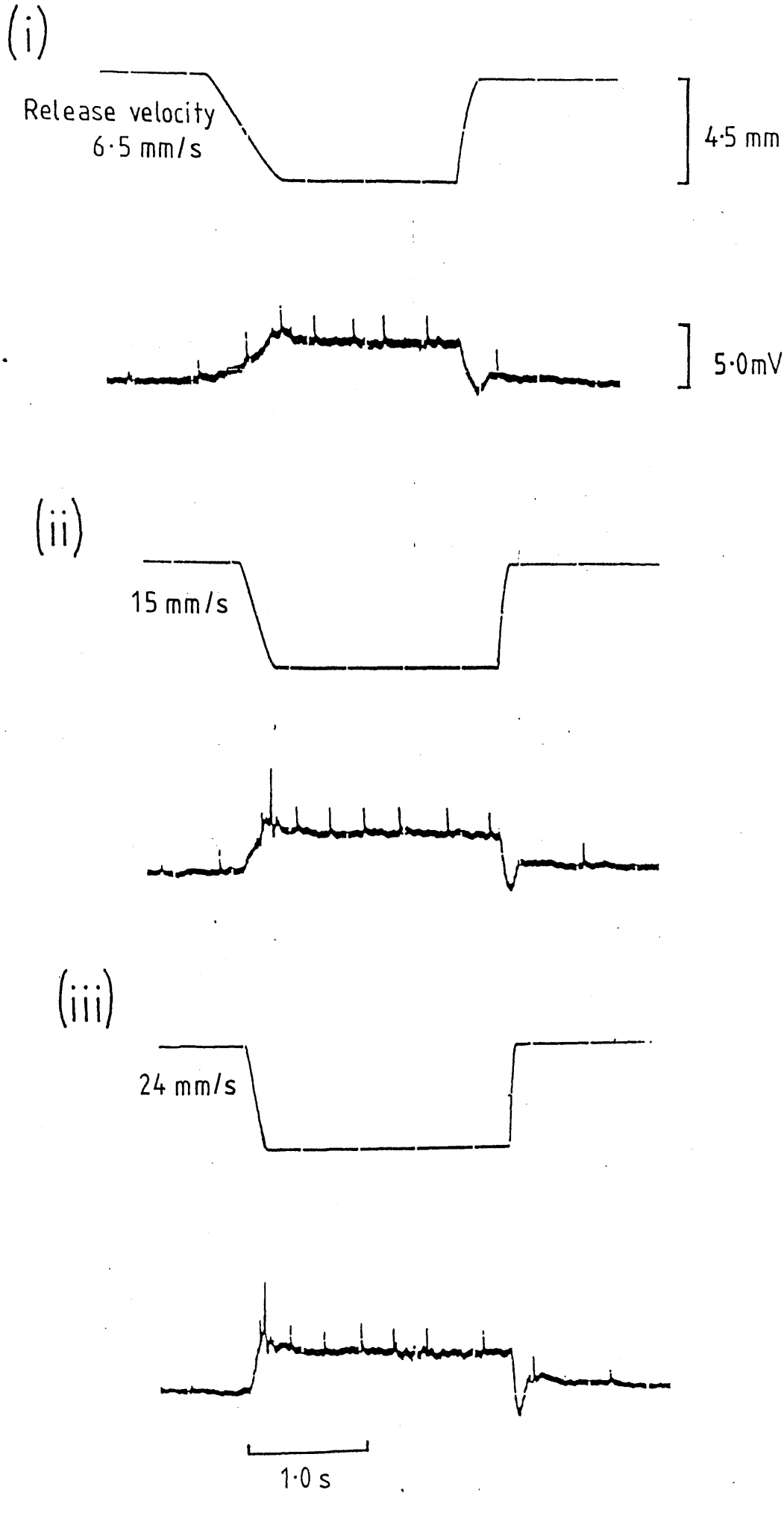
Figure22. SRN response to imposed release of stretch of the ventral longitudinal muscle. a, The response of the neurone to release of the longitudinal muscle was studied by applying single ramp releases to a previously extended piece of longitudinal muscle whilst recording intracellularly from the SRN axon 3-4mm distant in the anterior nerve root. b, Three, separate ramp releases of the longitudinal muscle of final amplitude of displacement of 0.5, 2.6 and 3.7mm were applied (upper trace three records superimposed). The neurone responded with a depolarising potential maintained for the duration of the ramp stretch stimulus. The final amplitude of the responses were 1.8, 3.0 and 3.1 mV respectively. Two distinct phases were present in the SRN response, i) an initial dynamic component followed by ii) a lower amplitude static response whose amplitude was proportional to the amplitude of the ramp release (lower three traces, The bottom intracellular record corresponds to the lower of the three ramp releases).


a
500 μ
b
Release
Stretch
0·5 mm
2·6 mm
3·7 mm
5·0 mV
E_m -30mV
1·0 s

for the duration of the ramp stimulus. The final amplitudes of the static responses to the ramp length changes of 0.5, 2.6 and 3.7 mm were 1.8 3.0 and 3.1 mV respectively.

Study of the response of the SRN to release of stretch entailed, prior to the ramp release, stretching the longitudinal muscle strip 1-2 mm beyond what was considered its natural resting length. Therefore the initial dynamic component of the SRN to a ramp release may be considered to be the same as that seen to the re-shortening phase of ramp stretch. This dynamic component of the SRN response is striking and only occurs in response to the release phase of either the ramp stretch, or to the ramp release. Figure 23 shows a sequence of three ramp releases of identical final amplitude (4.5 mm) but with three different initial velocities of displacement of 6.5, 15 and 24 mm/second. The response of the SRN recorded from the axon is shown in the bottom trace of each panel. As expected, the final amplitude of the static response of the cell was the same in each trial, (+3.0 mV). The initial dynamic response of the SRN however displayed a marked velocity sensitivity. At the two highest velocities of longitudinal muscle release a single SRN cell body spike was recruited. The appearance of this cell body spike and its contribution to the dynamic response is shown in greater detail in figure 24. In this figure three intracellular recordings (bottom) are shown in response to three consecutive ramp stretches of the longitudinal muscle, with different final amplitudes and different rates of stretch. The lower intracellular record corresponds to the smaller amplitude ramp stretch. A marked dynamic response was seen at the end of the stretch in response to release of the extended

Figure 23. The dynamic component of the SRN response. The intraxonal response of the SRN (bottom traces each panel) are shown to three ramp releases (top traces in each panel) of identical final amplitude of displacement (4.5mm) but with three different initial velocities of displacement. The initial dynamic component of the SRN displayed a marked velocity sensitivity. As the rate of release of the longitudinal muscle was increased the rate of depolarisation of the initial response component also increased. Single spikes were superimposed upon the peak of the dynamic components of the responses in panels (ii) and (iii). These were distinct from the lower amplitude synaptic activity superimposed upon the receptor and membrane potentials and were considered to be cell body spikes, initiated in the periphery in response to rapid change of longitudinal muscle length and transmitted decrementally to the axon recording site.


(i)
Release velocity
6·5 mm/s
4·5 mm
5·0mV
(ii)
15 mm/s
(iii)
24 mm/s
1·0 s

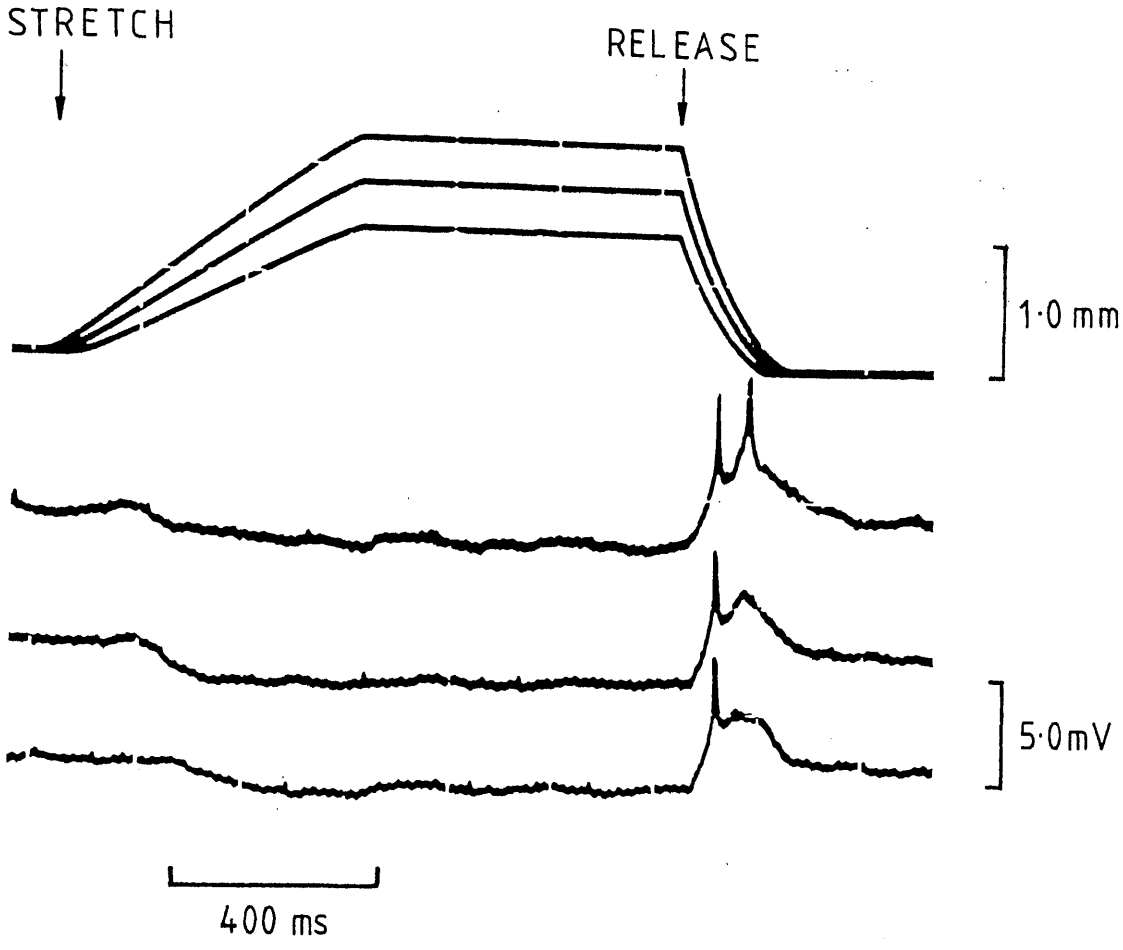


Figure 24. The contribution of the cell body spike to the dynamic component of the SRN response. Three consecutive sweeps are shown of an SRN intraxonal recording (bottom three traces) in response to three consecutive ramp stretches of the longitudinal muscle of different amplitudes and velocities of stretch and release. The lower intracellular record corresponds to the smallest amplitude ramp stretch. A marked dynamic component was seen in response to release of the extended longitudinal muscle. As the velocity of the release increased (uppermost trace shows the highest velocity if release) the number of cell body spikes superimposed upon the dynamic component gradually increases.

ventral muscle. As the velocity of the release increased, (uppermost ramp trace shows the highest release rate velocity) the number of cell body spikes superimposed upon the prominent dynamic component gradually increased.

At least two components in the response of the SRN to ramp length changes are therefore apparent. i) A static "amplitude modulated" response, dependent upon the magnitude of the ramp length change, and ii) A dynamic response in which the rate of depolarisation of the SRN E_m is dependent upon the release rate of the longitudinal muscle. Superimposed upon this dynamic component may be one or more cell body spikes. The contribution of these various components of the SRN response is shown in figure 25. Here "typical" responses to a ramp release of the longitudinal muscle of 3.7 mm and a ramp stretch of 1.8 mm are shown.

From the maintained hyperpolarising receptor potential of the SRN in response to a longitudinal muscle stretch and a similarly maintained depolarising receptor potential in response to a ramp shortening of the body wall, it appeared that the membrane potential of the SRN at any particular momemt reflected the length of the longitudinal muscle with which its dendrites are in close association. Figure 26 shows the response of the SRN E_m to stepwise lengthenings and shortenings of the longitudinal muscle strip, produced by hand manipulations of the puller arm via a rack and pinion device. In figure 26a(i) five consecutive stretching movements (not shown), approximately 1mm in amplitude, evoked five

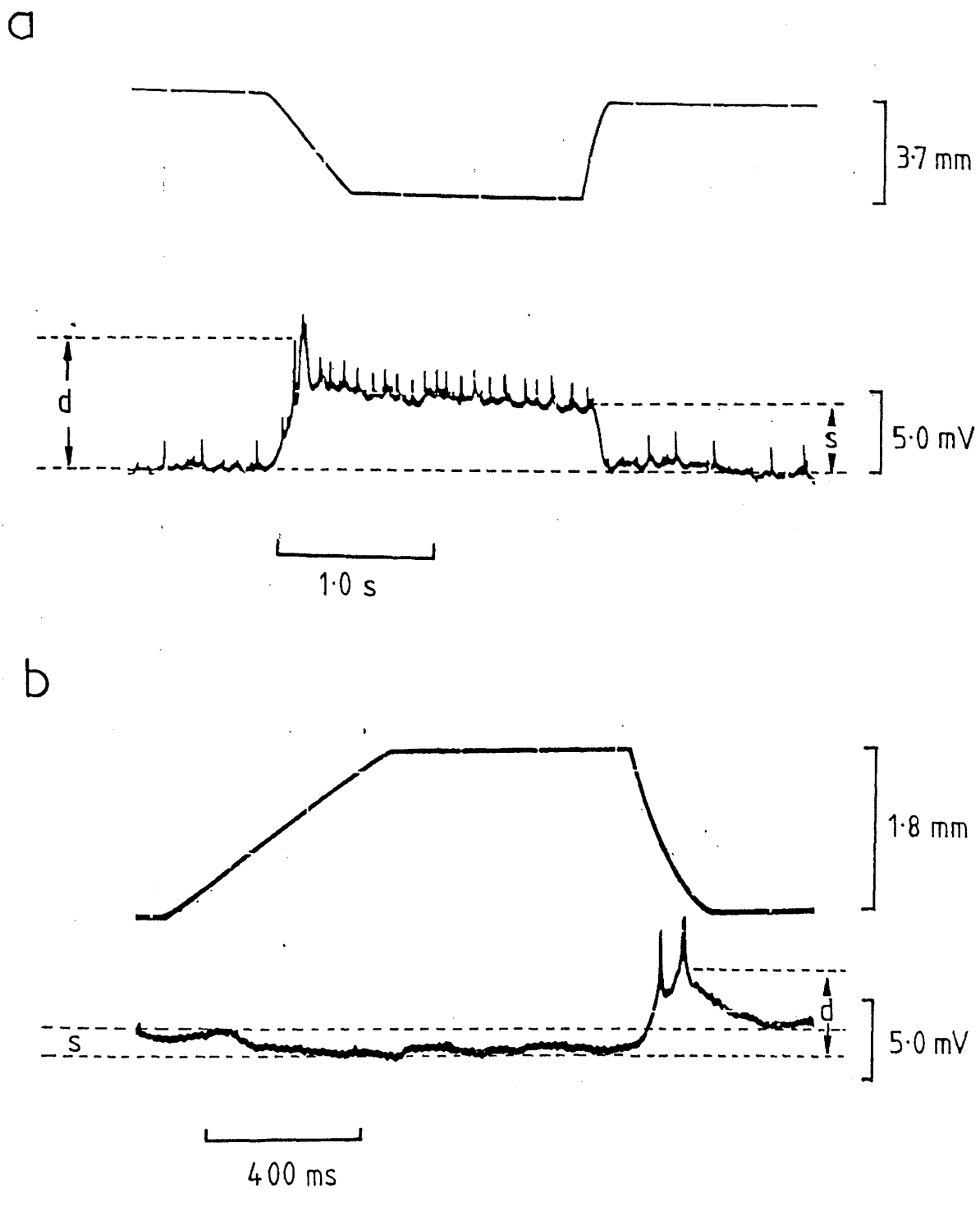
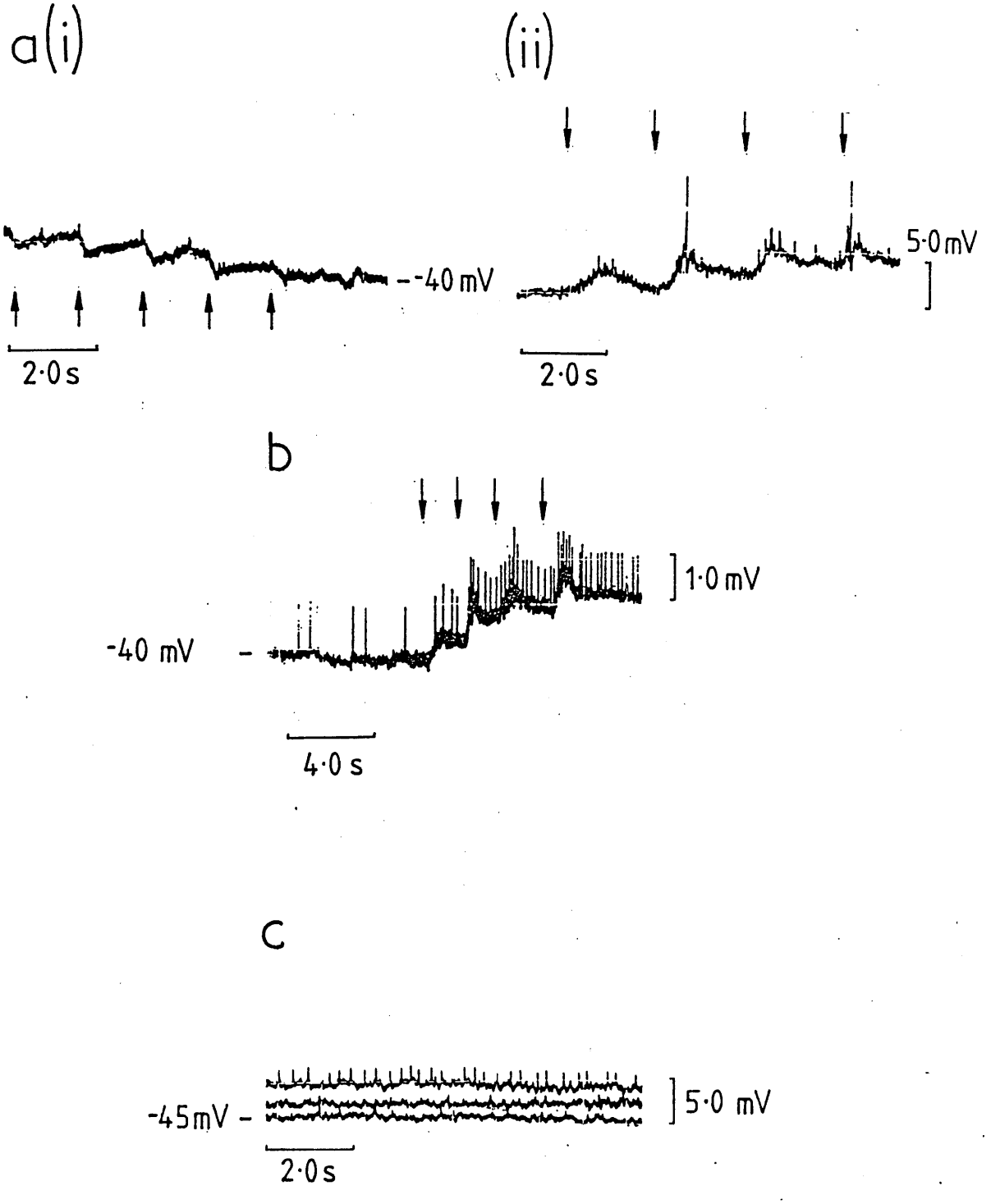


Figure 25. "Typical" responses of the SRN to an imposed ramp stretch and an imposed ramp release. a, Intracellularly recorded response of the SRN (lower trace) to a single ramp release of the longitudinal muscle of final displacement 3.7mm (upper trace). The amplitude of the initial dynamic component (d) is given as the peak of the unadapted static response plus the dynamic overshoot. The static response (s) shows the amplitude of the adapted static component of the response prior to re-lengthening of the longitudinal muscle. b, The response of the SRN (lower trace) recorded intraxonally to a single imposed ramp stretch of the longitudinal muscle of final displacement 1.8mm (upper trace). s, d, static and dynamic components of response. The amplitude of the dynamic component is given minus the contribution from the prominent cell body spikes.

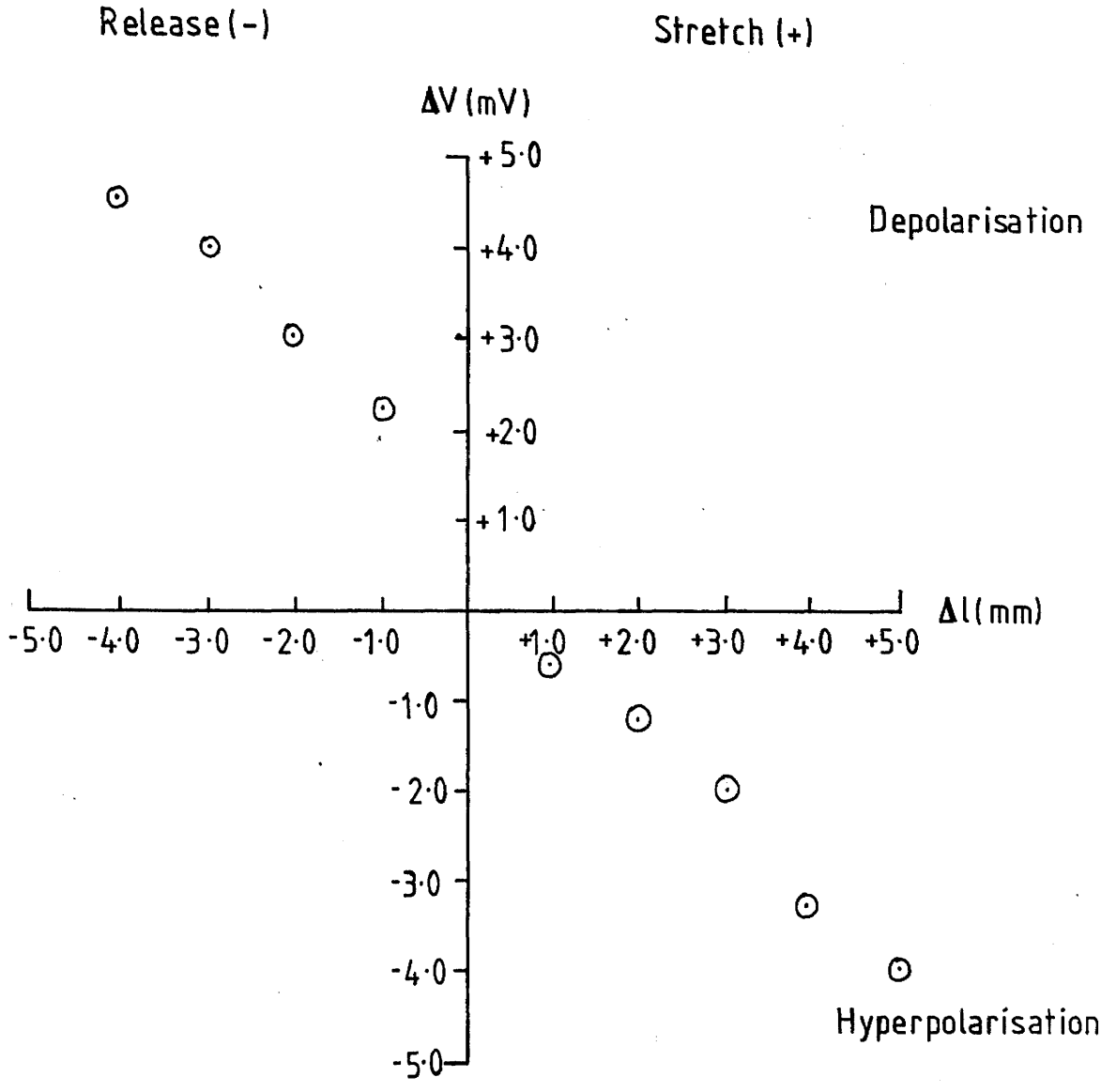
successive hyperpolarisations of the SRN membrane potential. A few minutes later, four consecutive stepwise shortenings of the longitudinal muscle (fig 26aii) produced stepwise depolarisations of the SRN. The static response of the SRN, measured 1.0 second after the length change, is plotted against the change in longitudinal muscle length in figure 26d. The response varried in an approximately linear fashion with the longitudinal muscle length. Figure 26b shows a different preparation in which four successive shortenings were again applied to the longitudinal muscle whilst the E_m was monitored intracellularly from the axon. Again the four successive longitudinal muscle shortenings evoked stepwise depolarisations of the SRN E_m. In this preparation low frequency epsp activity was also apparent at the initial E_m which increased in frequency by degrees as the longitudinal muscle was progressively shortened. Figure 26c is a third preparation in which the static response of the SRN to three different static lengths of the longitudinal muscle is shown. From its initial arbitary resting length and corresponding SRN axon membrane potential of -45 mV the longitudinal muscle was shortened in two, 1mm steps and the resultant depolarisation of the E_m recorded. In this preparation epsp activity was initiated upon depolarisation of the SRN axon to approximately -40 mV.

By inhibiting the synaptic activity, observed in many preparations superimposed upon the SRN membrane potential, by bathing the preparation in a high Mg^{++} Ringer solution, it was possible to record the underlying changes produced in response to changes in body wall length uncontaminated by the synaptic

Figure26. The static response of the SRN. a(i), Intraxonally recorded response of the SRN to five consecutive lengthenings (not shown) of the ventral longitudinal muscle. The muscle strip was lengthened by approximately 5.0mm in five manually applied steps of 1.0mm each (arrows). Five consecutive step hyperpolarisations of the SRN E_m were evoked in response to the imposed length changes. a(ii), A few minutes later the muscle strip was re-shortened by approximately 4.0mm in four manually applied steps of 1.0mm each (arrows). The cell responded with four step depolarisations of the membrane potential. b, Response of the SRN from a different preparation to four consecutive manually applied shortenings of the longitudinal muscle (arrows). c, Intraxonally recorded static response of the SRN from a different preparation to three different longitudunal muscle lengths (not shown). From an initial arbitary resting muscle length and corresponding E_m of -45 mV the longitudinal muscle was shortened in two 1.0mm steps and the resultant depolarisations in membrane potential are shown (three traces superimposed, upper trace corresponds to shortest longitudinal muscle length). d, The static response of the SRN measured 1.0 second after commencement of the imposed length change. Voltage and length changes are plotted as displacements from the initial E_m of the neurone (-45 mV). Data from traces in figures 26a (i), (ii). The static response of the cell varied in an approximately linear fashion with the longitudinal muscle length.


a(i)
(ii)
-40 mV
5·0 mV
2·0 s
2·0 s
b
1·0 mV
-40 mV
4·0 s
c
-45 mV
5·0 mV
2·0 s

d

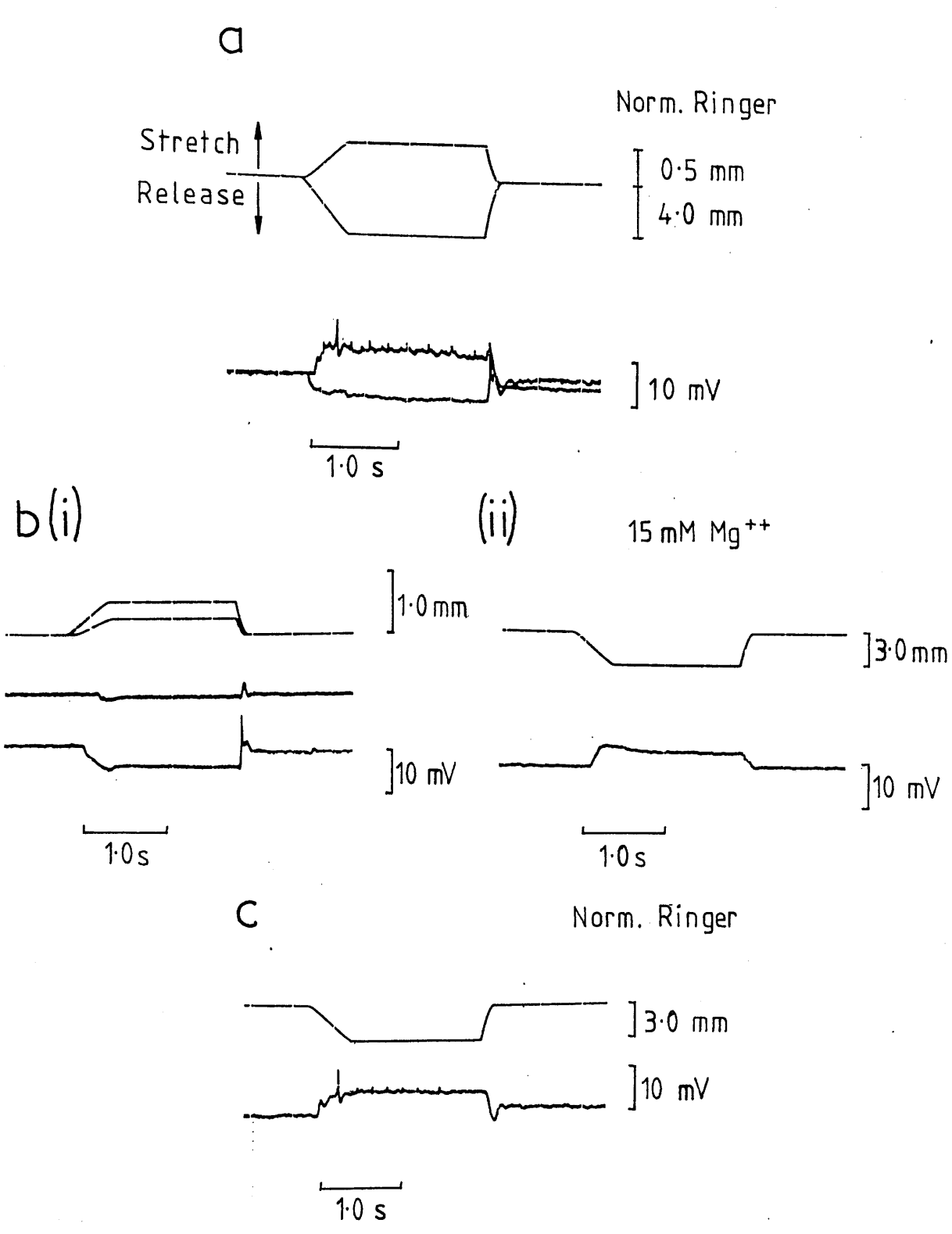

Release (-)
Stretch (+)
ΔV (mV)
+5·0
+4·0
+3·0
+2·0
+1·0
-1·0
-2·0
-3·0
-4·0
-5·0
-5·0 -4·0 -3·0 -2·0 -1·0
+1·0 +2·0 +3·0 +4·0 +5·0
Δl (mm)
Depolarisation
Hyperpolarisation

transients.

Figure 27 illustrates a preparation in which the response of the SRN was recorded intracellularly from its axon to a sequence of ramp stretches and releases in both the presence and absence of 15 mM magnesium. The SRN responses observed in fig 27a are typical of those recorded in normal Ringer solution. Superimposed upon the depolarising receptor potential evoked in response to a ramp release are a number of small epsp's. An SRN cell body spike was also initiated upon the shortening phases of the ramps. In fig 27b, high (15mM Mg^{++}) Ringer solution was substituted for the normal bathing medium and after 10 minutes the responses of the same cell again recorded to ramp length changes of the ventral longitudinal muscle. Under these conditions receptor potentials were observed in isolation. Upon returning the preparation to normal, Mg^{++} free, Ringer solution, small excitatory synaptic events were again observed superimposed upon the depolarising receptor potential (Fig. 27c).

The response of the SRN to ramp stretches and releases in the presence of 15mM Mg^{++} is shown in greater detail in figure 28. In these experiments 15 mM Mg^{++} Ringer was used throughout and the response of the SRN to changes in body wall length was again monitored intracellularly from its axon in the anterior root. Figure 28a shows the hyperpolarising response of the SRN to a single ramp stretch of final displacment 0.4 mm. In figure 28b the depolarising receptor potentials produced in response to ramp releases of 2.6 and

Figure 27. Response of the SRN in the presence and absence of Mg^{++}. Intracellular recordings (lower traces in each panel) were made from the SRN axon in response to a series of imposed ramp stretches and releases (upper traces in each panel) in the presence and absence of 15mM Mg^{++}. a, Typical depolarising and hyperpolarising responses (lower traces superimposed) of the SRN E_m to imposed ramp release and stretch stimuli in normal (zero Mg^{++}) Ringer solution. Low frequency psp activity was superimposed upon the depolarising receptor potential evoked in response to imposed release of the longitudinal muscle. b, 15 mM Mg^{++} Ringers solution was substituted for the normal bathing medium and after a 10 min. soaking period the responses of the same neurone were recorded to imposed ramp muscle length changes. Under these conditions the distortion induced receptor potentials could be recorded in isolation. b(i), Two hyperpolarising receptor potentials (lower traces) in response to two separate imposed stretches of the longitudinal muscle (upper trace, stretches superimposed, bottom intracellular record corresponds to uppermost ramp stretch). Note the remaining cell body spike on the dymnamic portion of the release phase of the stretch. b(ii), Depolarising receptor potential (lower trace) in response to a single ramp release of the longitudinal muscle (upper trace). Synaptic activity is abolished in the high Mg^{++} Ringers solution. c, Preparation returned to normal Ringers solution. Depolarising response of the SRN (lower trace) to a single imposed ramp release of the muscle (upper trace). Synaptic activity is re-established upon the depolarising response of the SRN.


a
Norm. Ringer
Stretch
Release
0·5 mm
4·0 mm
10 mV
1·0 s
b(i)
(ii)
15 mM Mg++
1·0 mm
3·0 mm
10 mV
10 mV
1·0 s
1·0 s
c
Norm. Ringer
3·0 mm
10 mV
1·0 s

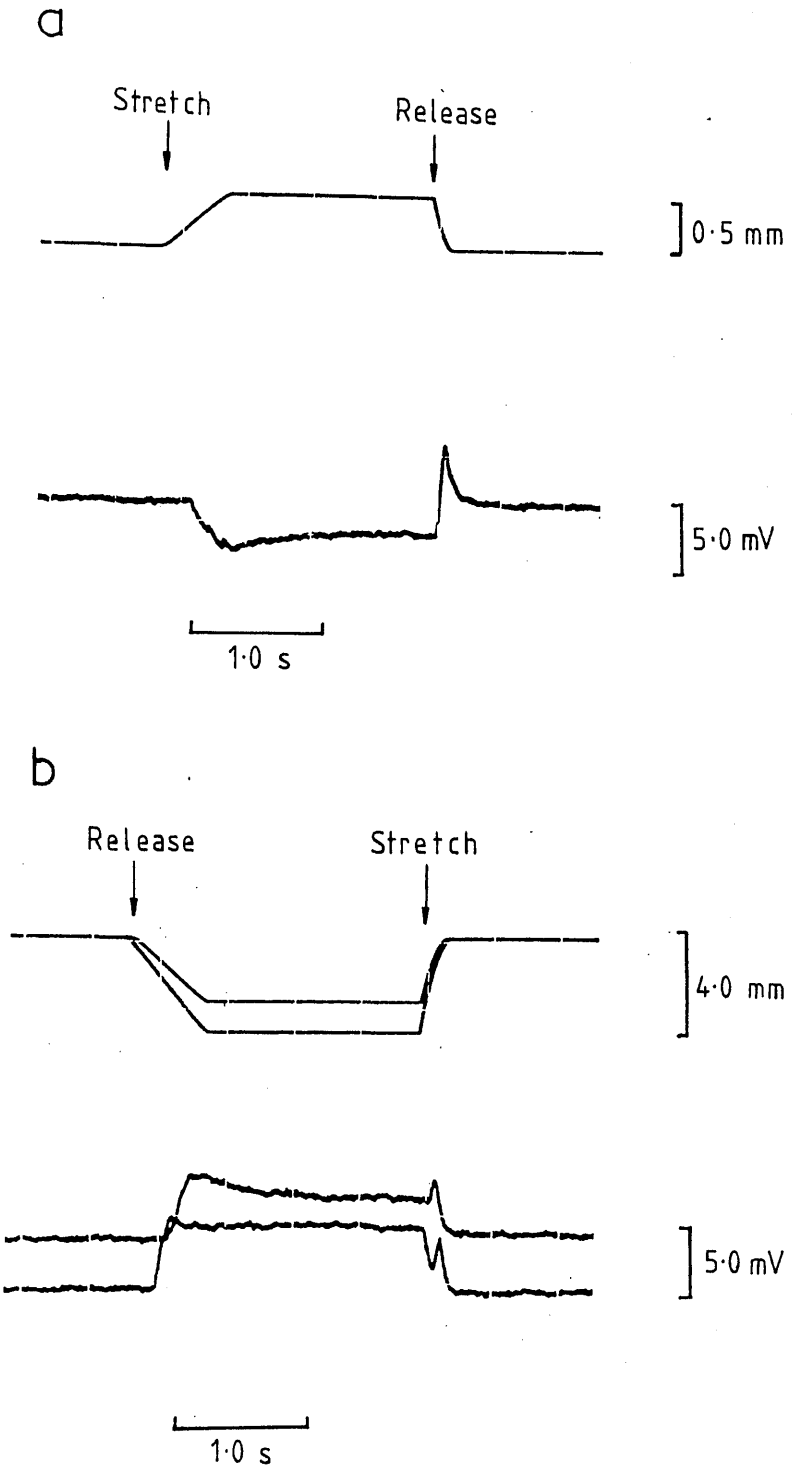


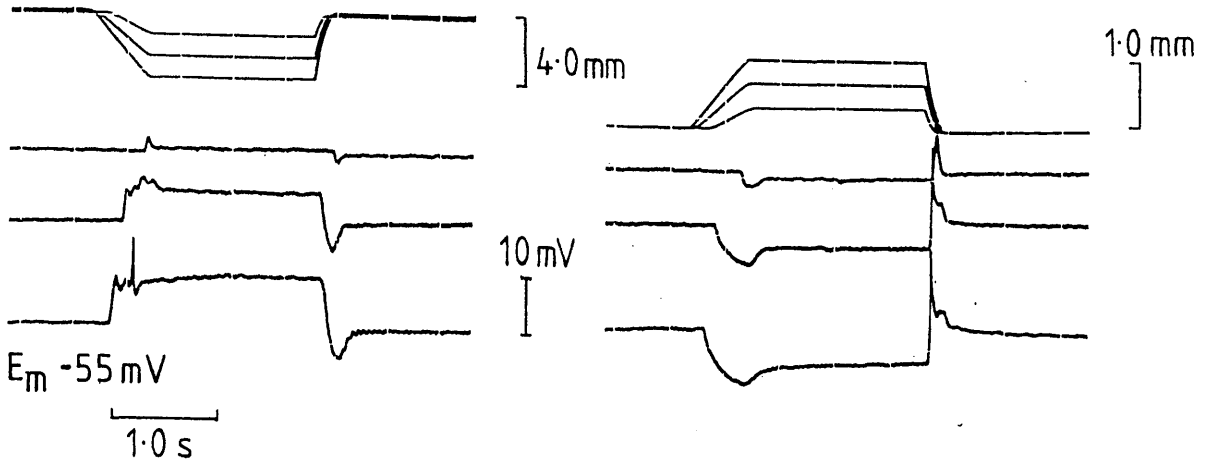
Figure28. Details of the SRN response to imposed ramp stretch and release in the presence of 15 mM Mg^{++}. a, Intraxonal hyperpolarising response of the neurone (lower trace) to an imposed ramp stretch (top trace) of the longitudinal muscle. In the presence of 15 mM Mg^{++} an πundershootπ is apparent in response to the initial lengthening phase of the ramp stretch. b, Intraxonally recorded depolarising responses of the SRN (bottom two traces) to two ramp releases of the longitudinal muscle (top traces, upper intracellular record corresponds to upper ramp release. Length changes performed one after the other, traces superimposed). All synaptic activity is abolished in the high Mg^{++} solution.

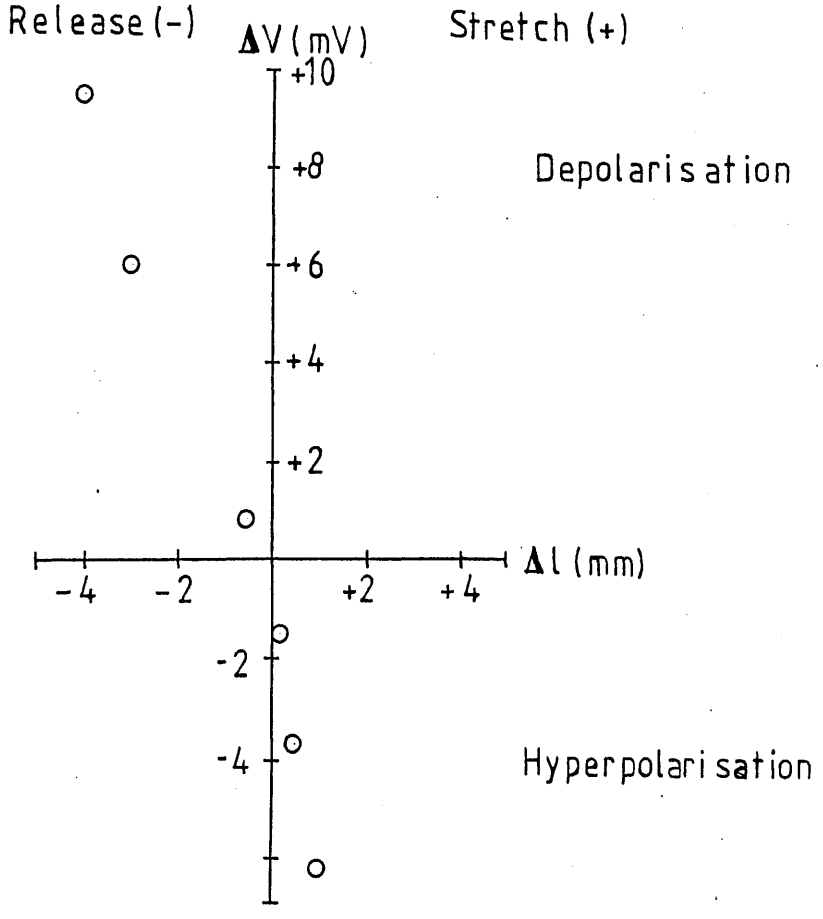
3.7 mm are shown. In both the hyperpolarising and depolarising receptor potentials the two components of the SRN response were still present.

In figure 29 the final amplitude of the static component of the SRN response in the presence of 15 mM Mg^{++} was plotted against longitudinal muscle displacement . Voltage and length values are plotted as displacements from the initial Em of the neurone and the resting length of the longitudinal muscle strip. All values are for a single SRN. The static response of this cell was approximately linear in proportion to the final displacement of the longitudinal muscle. This linear relation was retained over the length range of stretches tested of up to 1 mm and releases of up to 4 mm. The sensitivity of this neurone within the range of ramp releases was 2.3 mV depolarisation/mm. The sensitivity of the cell to a ramp stretch of the longitudinal muscle was 6.2 mV/mm. A marked difference was apparent therefore in the sensitivity between the response of the SRN to ramp releases of the longitudinal muscle and to ramp lengthenings of comparible displacement.

Figure 29 also shows that in high Mg^{++} there is a dynamic component to the response of the membrane potential. This component of the receptor potential was observed in a number of preparations bathed both in 15 mM Mg^{++} (Fig. 29) and normal Ringers fluid (Figs. 27a,c) on the initial lengthening phase of a ramp stretch, or on the re-stretching phase at the end of a ramp release. It appears however that this dynamic component is more pronounced in a high Mg^{++} Ringer

Figure 29. Static response of the SRN in the presence of 15 mM Mg^{++}. The final amplitude of the static response of the SRN, measured prior to the termination of three consecutive imposed ramp releases and three ramp stretches, was plotted against longitudinal muscle displacement. Three SRN depolarising potentials (top left panel, lower three traces) and three hyperpolarising potentials (top right panel, lower three traces) evoked in response to three ramp releases (top left panel,upper traces) and three imposed ramp stretches (top right panel, upper traces) were used to construct the graph shown in the lower panel. Voltage and length values are plotted as displacements from the initial E_m of the neurone (-55 mV) and the resting length of the longitudinal muscle strip. All traces from a single preparation. The static response of this cell was approximately linear in proportion to the final displacement of the longitudinal muscle


4·0 mm
1·0 mm
10 mV
E_m -55 mV
1·0 s


Release (-)
ΔV (mV)
Stretch (+)
+10
+8
+6
+4
+2
Depolarisation
Δl (mm)
-4
-2
+2
+4
-2
-4
Hyperpolarisation

solution.

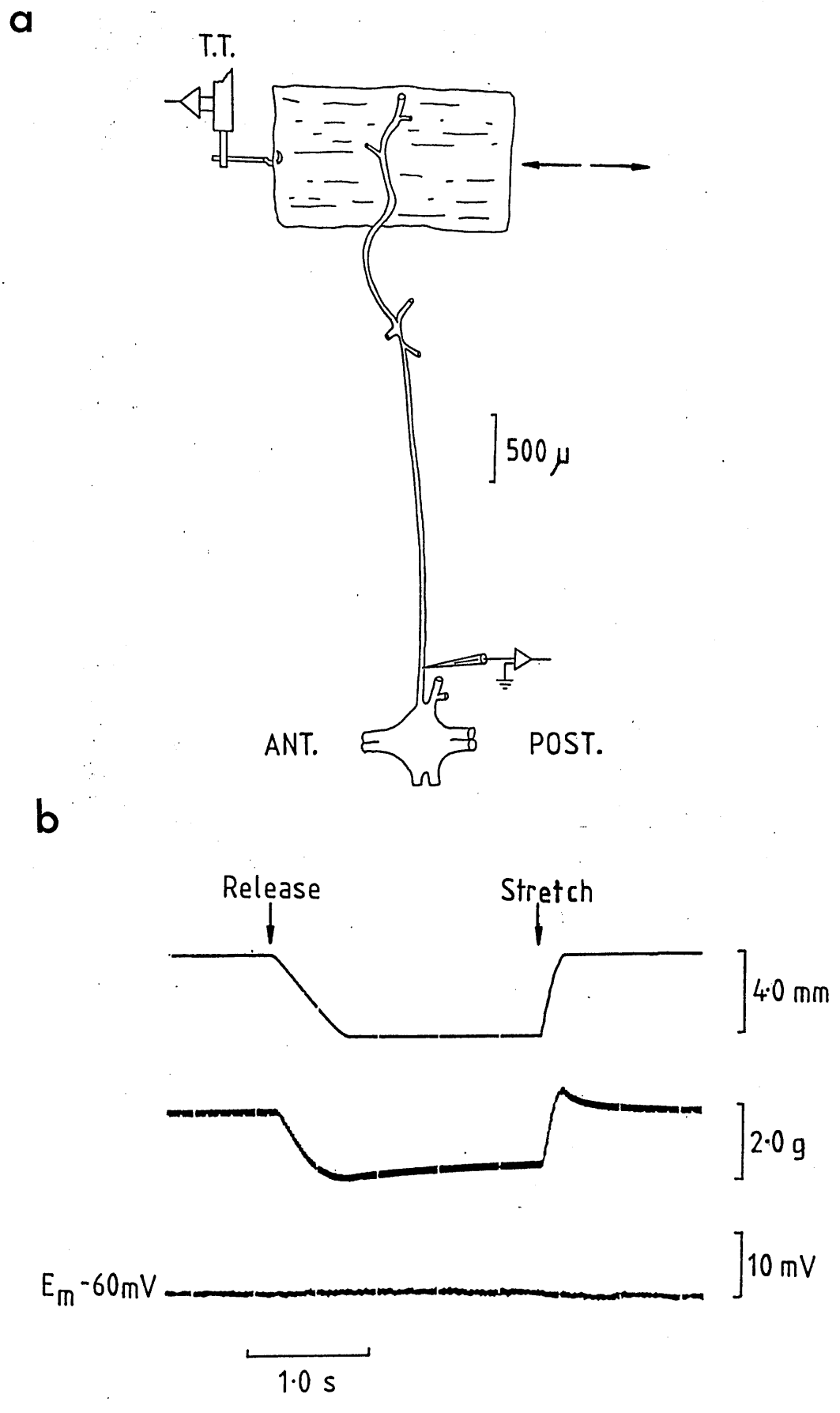
3.4 Simultaneous recording of SRN response and longitudinal muscle tension

In order to investigate the possibility that the SRN may not be responding primarily to the moment to moment length of the longitudinal muscle, but rather to some other component of muscle length, the tension developed within the muscle during a ramp release or ramp stretch was monitored together with the muscle length changes and SRN response.

Figure 30 shows the arrangement used to monitor the tension developed during a ramp stretch and ramp release of the longitudinal muscle whilst simultaneously recording intracellularly from the SRN axon in the anterior root. In such experiments one end of the longitudinal muscle strip associated with the SRN cell body and dendrites was securely attached to the arm of a strain gauge tension transducer clamped to the arm of a Prior micromanipulator (see Methods) whilst the other end was again attached to the moving arm of the muscle puller.

In the segmental ganglion preparation with attached body wall used throughout this study, the posterior nerve root was invariably cut or pinned along with the small piece of longitudinal muscle supporting the intact anterior root. The motor supply to the longitudinal muscle strip associated with the SRN fans was therefore restricted to the anterior root outflow. The bottom panel of figure 30 shows the tension developed (centre trace) in response to a ramp

Figure 30. Ventral longitudinal muscle tension recordings. a, One end of the longitudinal muscle strip associated with the SRN cell body and dendrites was securely attached to the arm of an isometric tension transducer (TT) whilst the other end of the muscle strip was attached to the arm of the muscle puller which allowed movement in both directions (arrows). The response of the SRN was again recorded intracellularly from its axon in the anterior nerve root. b, Tension developed in the longitudinal muscle strip (middle trace, downward deflection: tension drop) in response to an imposed ramp release of the longitudinal muscle (upper trace). Lower trace: intracellular recording from an unidentified axon in the anterior nerve root. The unidentified axon did not respond to the length or tension changes of the longitudinal muscle strip nor was the recording affected by movement artifacts.


a
T.T.
500 μ
ANT.
POST.
b
Release
Stretch
4·0 mm
2·0 g
10 mV
E_m -60mV
1·0 s

release of the longitudinal muscle (upper trace). The drop in passive tension which occured upon release of the longitudinal muscle reached its maximum depression at the termination of the release phase of the ramp. Thereafter, although the longitudinal muscle was held at a constant, shorter length, isometric tension within the fibres gradually increased. Upon the re-stretch phase of the ramp release, a marked overshoot in tension was recorded which rapidly returned to the pre-release resting tension level. The lower trace of this panel shows an intracellular recording taken from an unidentified axon in the anterior nerve root. No response to the ramp release was seen and the recording was unaffected by any possible movement artifacts.

The smooth muscles fibres of the leech longitudinal muscle are an example of one of the types of somatic smooth muscle present in many invertebrate groups. They have been classified as πhelical smoothπ muscle fibres by Hanson and Lowy (1960). It is not known whether the longitudinal muscle of the leech body wall possesses an intrinsic response to stretch by develop ing active tension as does unitary smooth muscle of the mammalian gastro-intestinal tract (Bulbring et al. 1970). In figure 30 a large component of the slow rise in isometric tension which occured after the peak of the initial tension drop may therefore be due to reflex motoneurone activity in response to the length change.

The response of the SRN to a rapid stretch of the longitudinal muscle in which both the muscle length and tension were measured is

shown in figure 31. Panel "a" is the last half of a record from a ramp release showing the final stretch component of the ramp. Panel "b" is the initial lengthening component of a ramp stretch. In both experiments a marked tension overshoot accompanied the rapid extension of the longitudinal muscle. This overshoot of muscle tension was mirrored by an undershoot of the hyperpolarising response of the SRN to an increase in muscle length and resembles the undershoots seen in response to muscle lengthening in figure 29.

Figure 32 illustrates a further feature of the response of the SRN to a ramp release of the longitudinal muscle which was observed in some preparations. The figure shows the depolarising potential responses of two different SRN's to a ramp release of the muscle strip of identical final amplitude in which both length and tension were again monitored. Both SRN's exhibited spontaneous epsp activity at their respective membrane potentials which was also superimposed at a higher frequency upon the release-induced depolarising receptor potentials. For the duration of the static phase of the ramp release, following the initial dynamic peak of the receptor potential, the longitudinal muscle was established at a constant,shortened length. Over this period considerable adaptation of the static plateau of the receptor potential was observed which reflected the accompanying slow rise in longitudinal muscle tension. It appeared therefore that the SRN receptor potential more closely represents the tension within the longitudinal muscle in which its dendrites are buried rather than the length of the muscle itself.

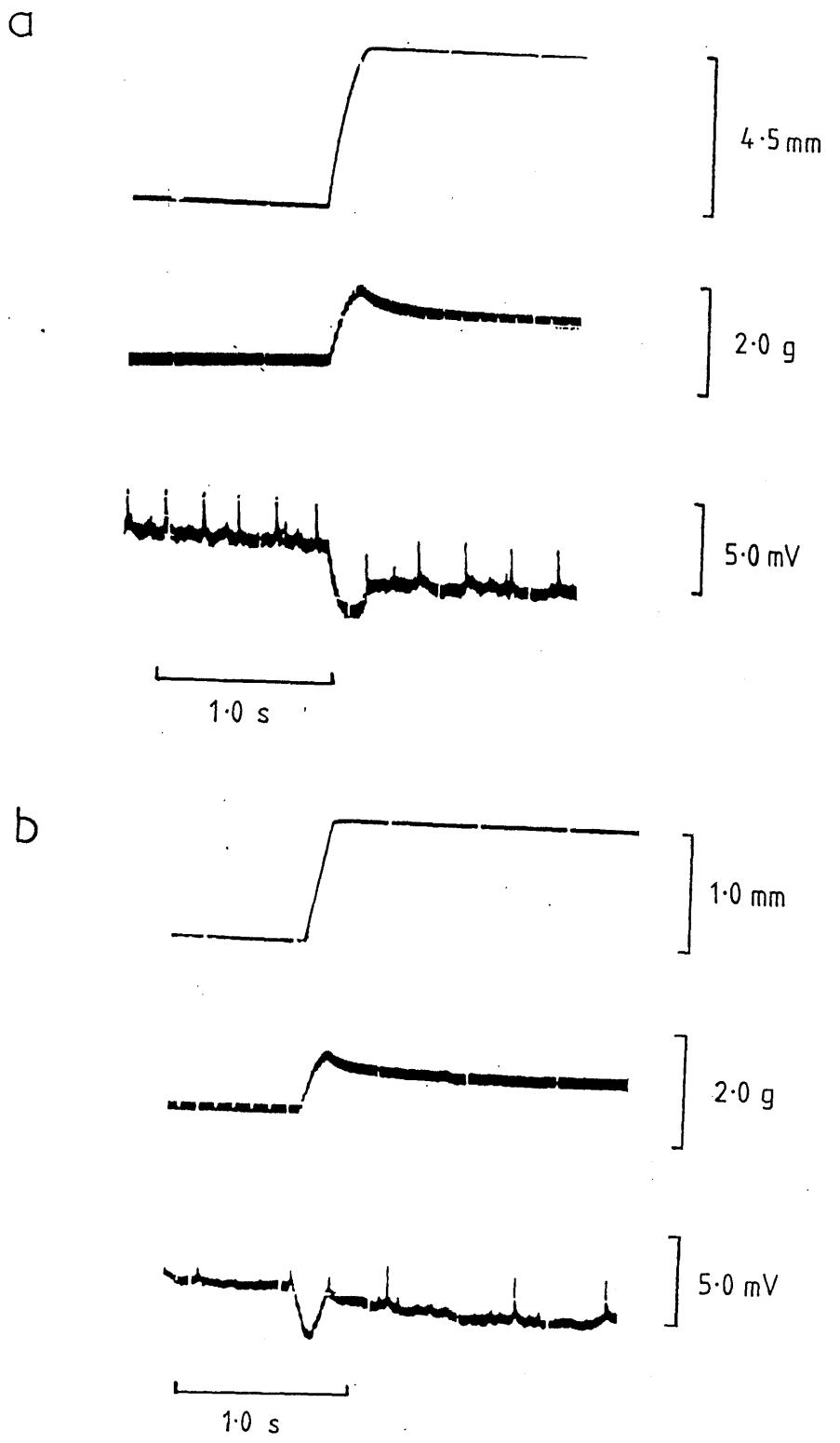


Figure 31. Simultaneous intracellular, tension and length recordings. Intracellular recordings (bottom traces, both panels) from the SRN axon and accompanying tension changes in the longitudinal muscle (middle trace each panel) are shown in response to ramp length changes (top traces each panel). a, Record from a preparation subjected to an imposed ramp release. The final re-stretch component of the ramp with accompanying tension change in the longitudinal muscle and SRN intracellular response is shown. b, The initial lengthening component with accompanying tension and intracellular responses from a different preparation subjected to an imposed ramp stretch of the longitudinal muscle. In both experiments a marked tension overshoot accompanied the rapid extension of the longitudinal muscle. This overshoot in muscle tension was faithfully mirrored by an undershoot in the hyperpolarising response of the SRN.

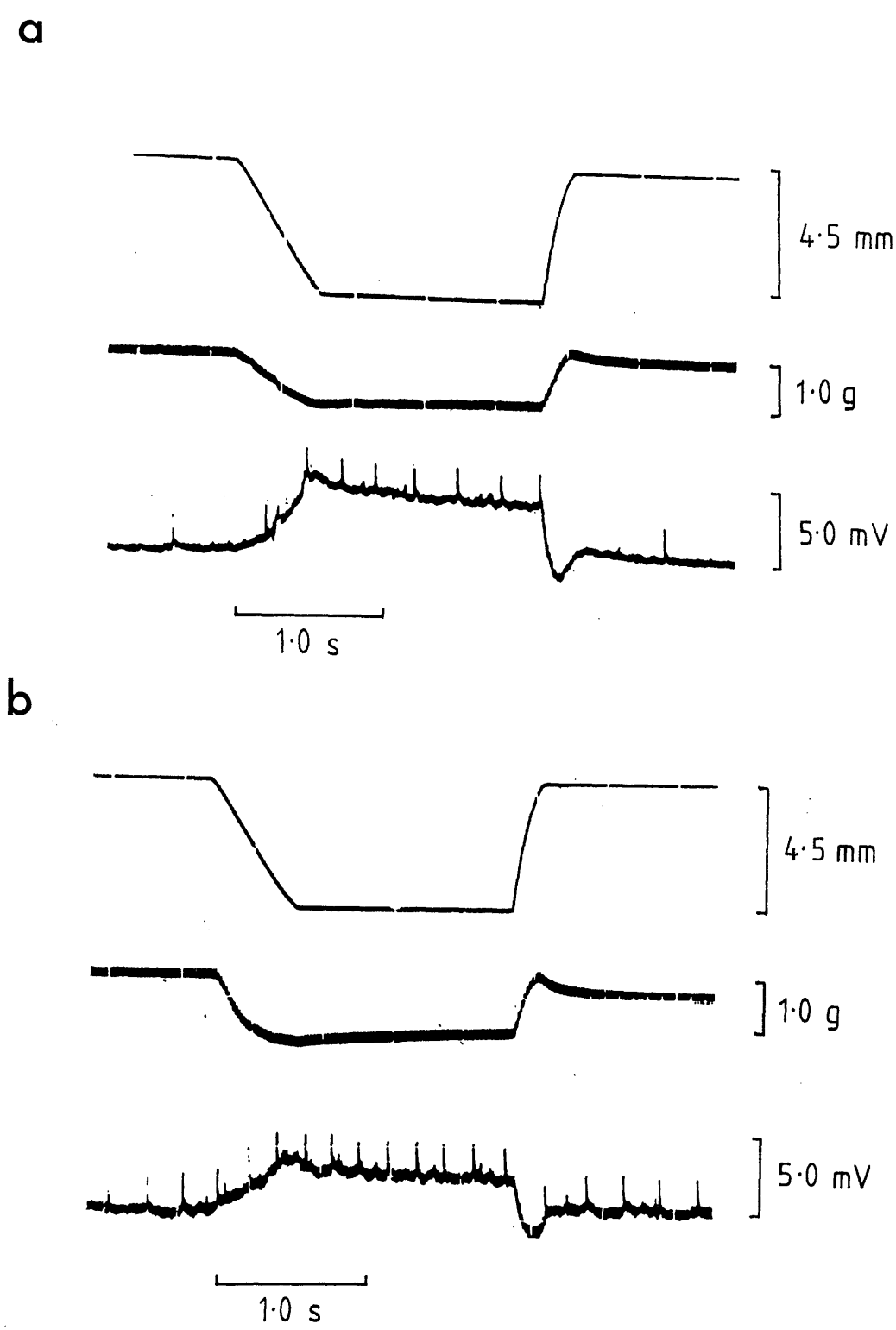


Figure 32. Simultaneous Intracellular, length and tension recordings. Intraxonally recorded SRN responses (lower traces both panels) and accompanying tension changes (middle traces both panels) in response to a single imposed ramp release of the longitudinal muscle (upper traces) from two different preparations. During the static phase of both ramp releases, over which period the muscle was established at a constant, shortened length, considerable adaptation of the static plateau of both receptor potetials is seen which reflects the accompanying slow rise in longitudinal muscle tension over this period.

4.0 SECTION 4.0 SYNAPTICALLY ASSOCIATED CELLS

A common feature of the structure and function of the various stretch receptors previously studied is the existence of a separate efferent supply to the peripheral sensing elements. Thus after the initial observation that two distinct populations of motoneurones with different diameters existed to muscles in the cat (Eccles and Sherrington, 1930), the function of the smaller diameter gamma, or fusimotor fibers as they were called, was demonstrated by Kuffler, Hunt and Quilliam (1951). Stimulation of the gamma efferent fibres which supplied the muscle spindle, resulted in an increase in the spindle afferent discharge without any measurable increase in tension recorded from the muscle tendon. Similarly, Alexandrovicz described, in the invertebrates, efferent axons which innervated the muscle receptor organs in species of the decapod crustacea, (Alexandrovicz, 1951, '52). In this instance not only does the receptor muscle receive an efferent innervation distinct from that supplying the extrafusal muscle fibres, but the terminals of the afferent neurones themselves are innervated by 2 - 3 efferent inhibitory fibres, (Eyzaguire and Kuffler, 1954 ; Alexandrovicz, 1967). In the insect phylum too, the muscle receptor neurone found associated with the segmental longitudinal muscles in a number of species of lepidoptera, receive a separate efferent supply, stimulation of which results in an increase in muscle receptor organ

discharge frequency, (Finlayson and Lowenstein, 1958 ; Weevers, 1966b).

In the leech there does not appear to be a separate receptor muscle. The ventral longitudinal muscle among which the SRN dendrites and cell body lie is known to be innervated in each segment by three of the six motoneurones which supply excitatory innervation to the longitudinal muscles (Stuart, 1970). Two of these motoneurones, the ventral motoneurone (v) and the ventro-lateral motoneurone (vl) innervate more restricted areas of the ventral longitudinal muscle within the segment, but it is not known whether there is a separate motor supply to the bands of ventral longitudinal muscle which insert into the SRN dendrites. The efferent innervation of the leech stretch receptor neurone to be described consists of a direct contact between a centrally located neurone and the stretch receptor dendrites in a manner analogous to the inhibitory efferent innervation of the sensory dendrites of the crustacean abdominal stretch receptor (Kuffler and Eyzaguire, 1955).

4.1 The lateral nociceptive mechanosensory neurone.

In the leech, a large amount of information is available upon the peripheral axon branching patterns and the receptive field organisation of a number of modalities of mechanosensory cells (Nicholls and Baylor, 1968 ; Wai-Yau, 1976 ; Blackshaw, 1981 ; Blackshaw, Nicholls and Parnas, 1982). One such modality, the

nociceptive (N) neurons are primary sensory neurones which respond selectively to noxious mechanical stimuli applied to the skin, (Nicholls and Baylor, 1968). In each midbody ganglion there are four of these cells. Each cell body, situated within the CNS, supplies ipsilaterally a well defined receptive field area on the skin extending from the dorsal midline to the ventral midline. In addition to innervating the deeper layers of the skin, the most laterally placed N cell on each side of the ganglion (N_L) also responds vigorously to mechanical distention of the nephridiopore, (Blackshaw, Nicholls and Parnas, 1982). On visualising the peripheral terminals responsible for the initiation of the N cell sensory discharge by intracellular injection of HRP, it was discovered that the N_L cells made distinctive looped turns across one surface of the dendritic fans of the SRN situated in the body wall, (ref. ibid.). Electron microscope examination of the SRN dendritic fan and HRP-filled N cell terminals showed that pre- and post-synaptic membranes were closely apposed but there were no ultrastructural features characteristic of chemical synapses along the fans. It was impossible therefore from the morphological evidence to draw any conclusions about the directionality of any synaptic interaction between the SRN dendrites and the N_L cell. To this end, paired intracellular recordings were made from the peripheral stretch receptor neurone cell body and the cell body of the ipsilateral N_L cell situated in the segmental ganglion.

The preparation consisted of the body wall and attached segmental ganglion, as used previously for describing the electrical properties of the stretch receptor neurone and as detailed in the

methods section.

Figure 33 illustrates the result of injecting a brief depolarising current pulse into the N_L cell body whilst recording from the stretch receptor neurone cell body some 3-4mm distant in the periphery in normal leech Ringer fluid. Action potentials in the N_L cell body, elicited either upon direct depolarisation of the soma, (1st spike) or occuring spontaneously (2nd spike) resulted in an excitatory post-synaptic potential recorded in the peripheral SRN cell body. Each epsp followed the N_L cell action potential on a 1:1 basis and with a constant latency. In normal leech Ringer, although small (< 1mV), the epsp recorded from the SRN cell body was non the less prominent. Figure 34a shows three consecutive sweeps of an intracellular record from the SRN following intracellular stimulation of the N_L cell, (one action potential only shown), and demonstrates the 1:1 basis of the epsp. This connectivity is also shown in the averaged trace in figure 34b. Figure 34c shows summation of the stretch receptor neurone epsp's following evoked and spontaneous action potentials in the N_L cell. No evidence of a synaptic connection in the reverse direction from the SRN cell body to the N_L cell was seen.

The mechanism of synaptic transmission from the lateral N cell to the peripheral stretch receptor neurone was investigated by i), observing the latency of the epsp in the SRN after the peak of the action potential in the pre-synaptic cell. ii), changing the membrane potential of the post-synaptic cell and iii), bathing the

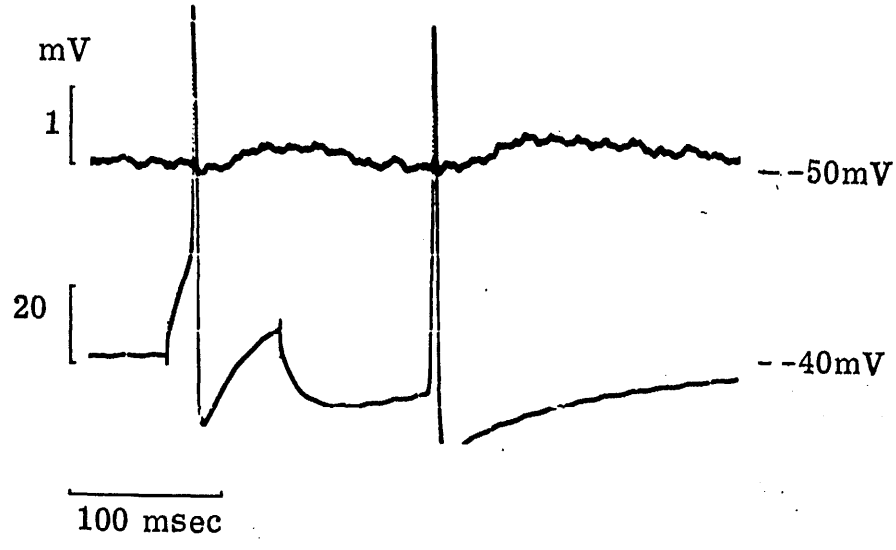
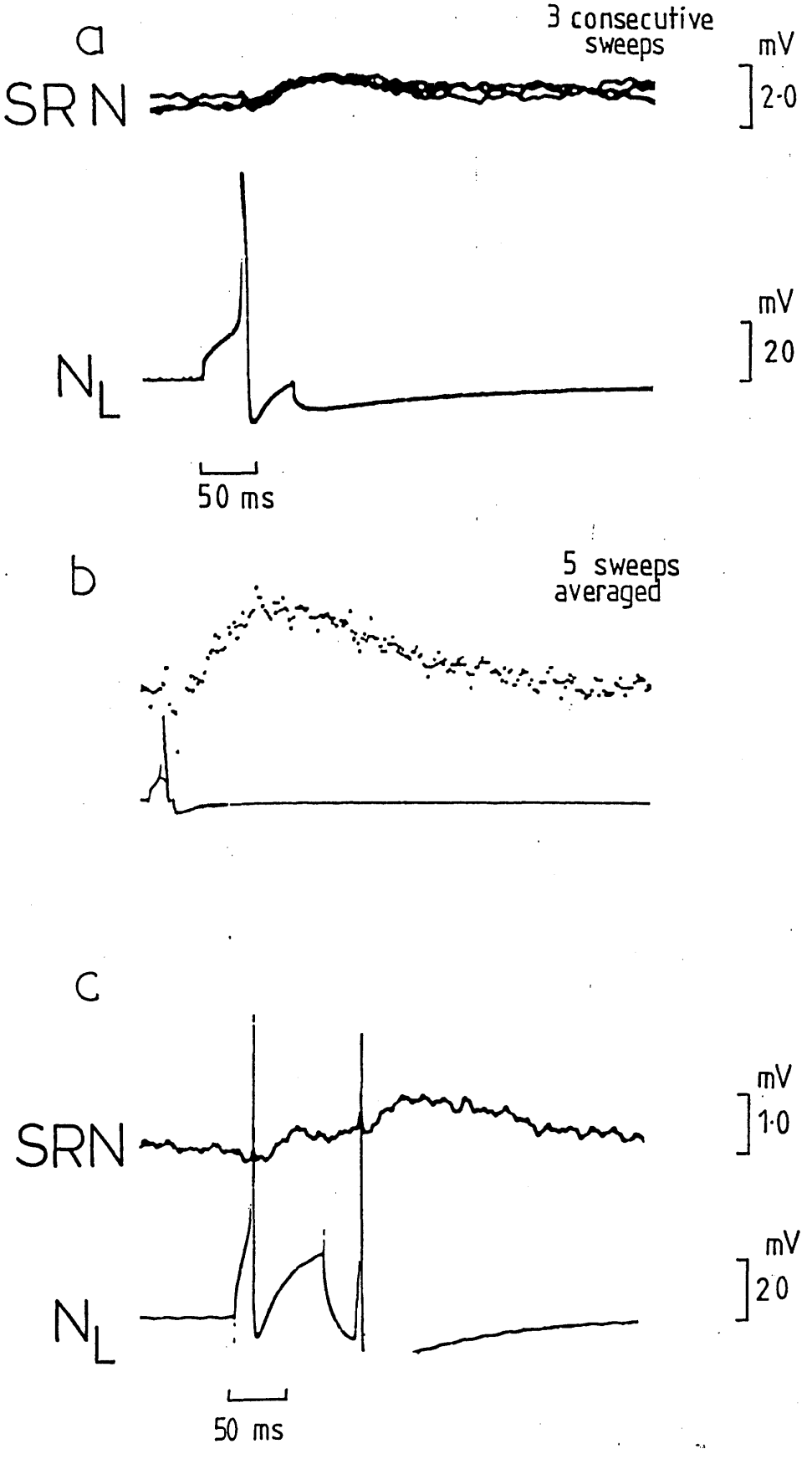


Figure 33. Efferent innervation of the SRN. Paired intracellular recordings were made from the peripheral cell body of the SRN (top trace) and from the centrally located soma of the lateral nociceptive neurone (N_L) (lower trace). Action potentials in the N_L cell body, elicited either by direct depolarisation of the cell body (first spike, current not shown) or arising spontaneously (second spike) resulted in an epsp recorded in the peripheral SRN soma.

Figure 34. Efferent innervation of the SRN. Paired intracellular recordings from the peripheral SRN soma and the N_L cell body. a, Three consecutive sweeps of an intracellular record from the SRN cell body (top trace records superimposed) following direct intracellular stimulation of the N_L cell (lower trace current not shown, one action potential only shown). Each SRN epsp follows the N_L cell action potential on a 1:1 basis. b, The averaged output of the post-synaptic response (upper trace) to 5 pre-synaptic N_L trials (one action potential only shown). c, Summation of single epsp's (top trace) following evoked (first spike current not shown) and spontaneous N_L cell action potentials.


a
3 consecutive sweeps
mV
2·0
SRN
mV
20
NL
50 ms
b
5 sweeps averaged
c
mV
1·0
SRN
mV
20
NL
50 ms

preparation in a high $[Mg^{2+}]$ which is known to block chemical synaptic transmission in the leech, (Baylor and Nicholls, 1969 Stuart, 1970).

Figure 35a illustrates the epsp recorded from the SRN cell body in response to a single, depolarisation evoked, action potential in the N_L cell. The epsp arose with a delay of 18.1 ms after the peak of the N_L cell action potential measured from an expanded trace from the screen of a digital oscilloscope. The delay at this synapse in a different preparation is shown on a faster time base in figure 35b. In this experiment, synaptic potentials were recorded in the stretch receptor neurone cell body in response to single, N_L cell action potentials evoked by direct depolarisation of the N_L cell body. The averaged output of the postsynaptic response to seven trials is shown in figure 35b. The averaged epsp arose with a delay of 19.0 msec. after the peak of the pre-synaptic action potential.

The effect of altering the membrane potential of the post-synaptic cell upon the amplitude of the epsp was investigated. In these experiment the stretch receptor neurone was hyperpolarised by up to 8 mV by injecting 400 ms long current pulses of different amplitudes into the cell body. The epsp which followed the N_L cell spike was recorded during the hyperpolarising pulse. Figure 36 shows one example in which the amplitude of the epsp was increased as the postsynaptic cell was hyperpolarised,as would be expected of a chemically mediated synapse.

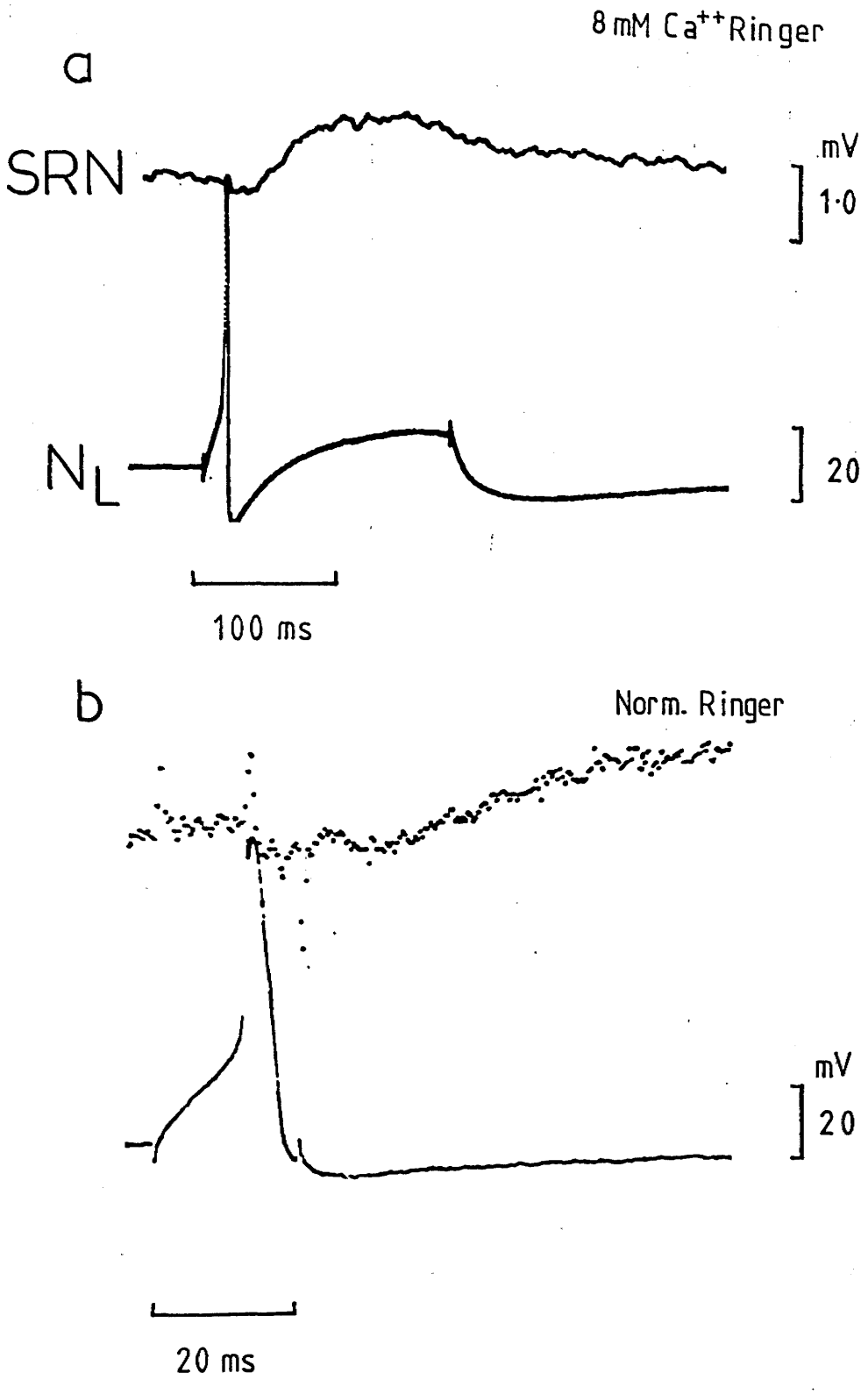


Figure 35. Synaptic Latency. a, An epsp was recorded from the SRN cell body (SRN) in response to a single, depolarisation evoked action potential in the N_L cell (N_L). The epsp arose with a latency of 18.1 ms after the peak of the N_L cell action potential. b, The synaptic delay at this synapse, observed on a faster time base in a different preparation. The averaged output of the post-synaptic response (upper trace) to seven pre-synaptic trials is shown (one pre-synaptic action potential only shown). The averaged epsp arose with a delay of 19.0 ms (arrow).

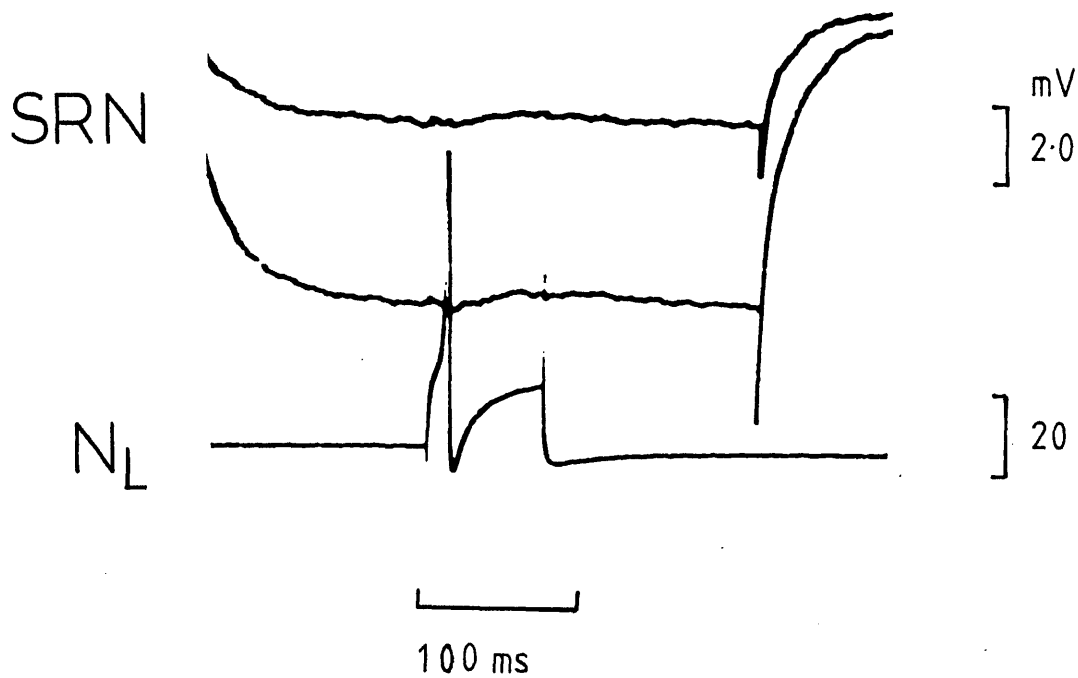


Figure 36. The effect of changing the post-synaptic membrane potential upon the amplitude of the psp. The SRN was directly hyperpolarisaed by injecting square wave hyperpolarising current pulses into the peripheral cell body (400 ms duration). Two superimposed traces (top) show the result of injecting two square wave hyperpolarising current pulses into the soma, the SRN hyperpolarised by approximately 3.0 and 8.0 mV respectively (current not shown). The SRN epsp which followed the N_L cell action potential (lower trace) was reorded during the hyperpolarising current pulse. The amplitude of the epsp is increased as the post-synaptic cell was hyperpolarised.

Figure 37 shows further evidence that the synapse between the N_L cell and the stretch receptor neurone is chemically mediated. In this experiment an SRN epsp recorded in 8 mM Ca^{2+} Ringer following a spike in the N_L cell, was abolished on bathing the preparation in a 15 mM Mg^{2+} solution for 5.0 mins. The SRN epsp subsequently returned over a period of a few minutes when the Ca^{2+} in the bathing solution was increased to 15 mM. Thus the synapse demonstrates the antagonistic action of Mg^{2+} and Ca^{2+} seen at other central chemical synapses in the leech and the vertebrate neuromuscular junction (del Castillo and Engbaek, 1954). In other experiments however, raising the $[Mg^{2+}]$ did not completely abolish the SRN post-synaptic potential. Figure 38 shows an example in which the SRN epsp still persisted after 10 minutes in the presence of 15 mM Mg^{2+}, although the amplitude of the peak of the epsp was reduced. No direct current spread was seen between the N_L cell and the SRN soma on injecting either hyperpolarising or depolarising current into the N_L cell body.

The persistence of a post-synaptic potential in the presence of both 15 mM Mg^{++} and 15 mM Ca^{++} is a useful diagnostic criteria indicative of a monosynaptic connection between two cells (Nicholls and Purves, 1970). Since Ca^{++} and Mg^{++} act synergistically in reducing the excitability of any intervening neurones in a poly-synaptic pathway, but act antagonistically upon transmitter release at the synapse, the likelyhood of observeing a psp in a high Ca^{++}, high Mg^{++} solution is reduced if the pathway envolved is polysynaptic.

Figure 37. Abolition of the psp in high Mg^{++} Ringers solution. A, Paird intracellular recordings from the peripheral SRN soma (top trace) and central N_L cell body (bottom trace) in an 8.0 mM Mg^{++} Ringers solution. A small amplitude epsp was recorded from the SRN soma following a single depolarisation-evoked N_L cell action potential (current not shown). B, Five minutes after perfusion of the preparation with a 15 mM Mg^{++} Ringers solution. The N_L cell action potential-evoked psp is completely abolished in the raised Mg^{++} solution. C, Ten minutes after raising the calcium concentration in the bathing medium. Bathing the preparation in a 15 mM Ca^{++}, 15 mM Mg^{++} Ringers solution reverses the effect of the high Mg^{++} alone. Time scale common to all traces.

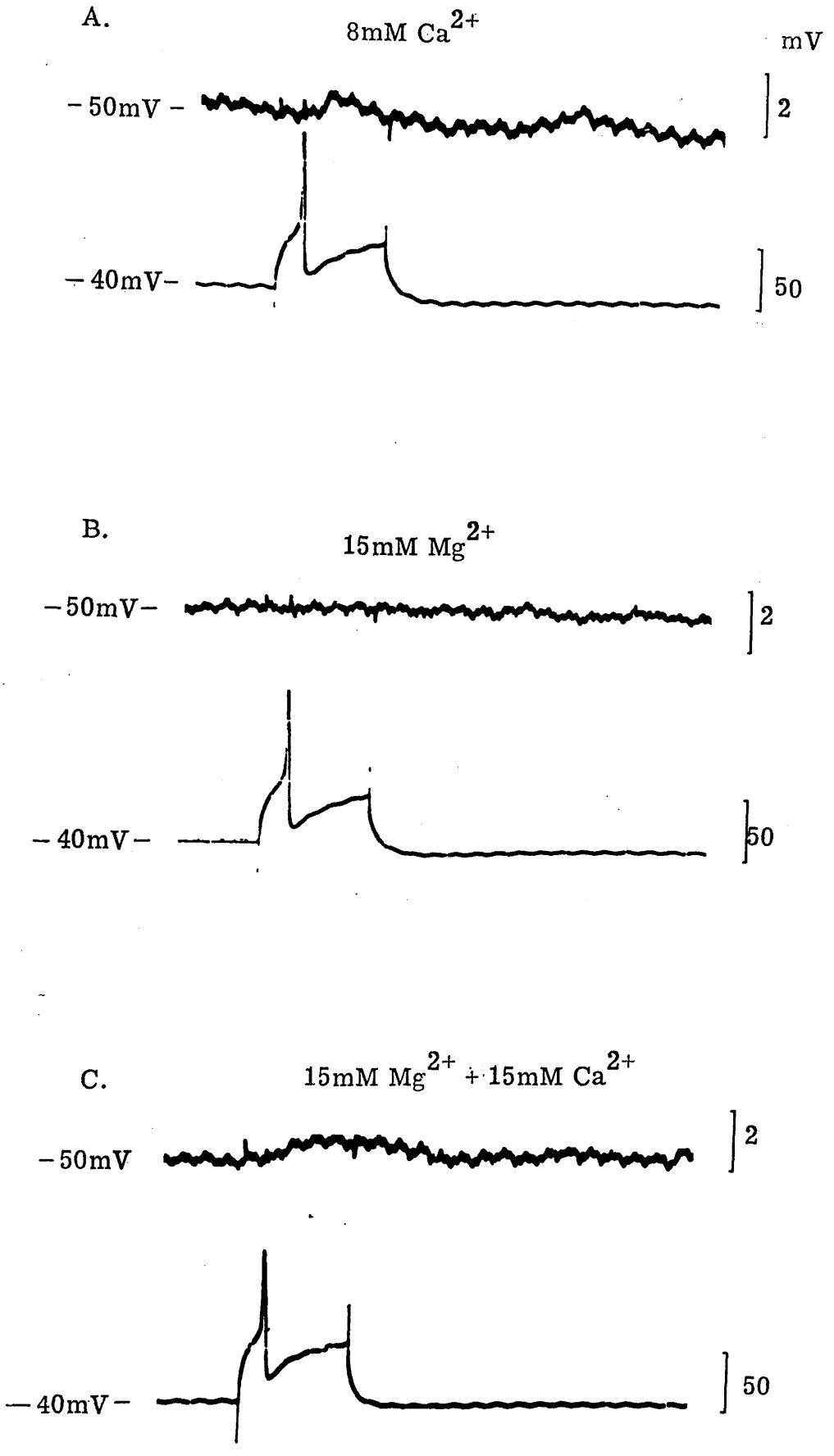
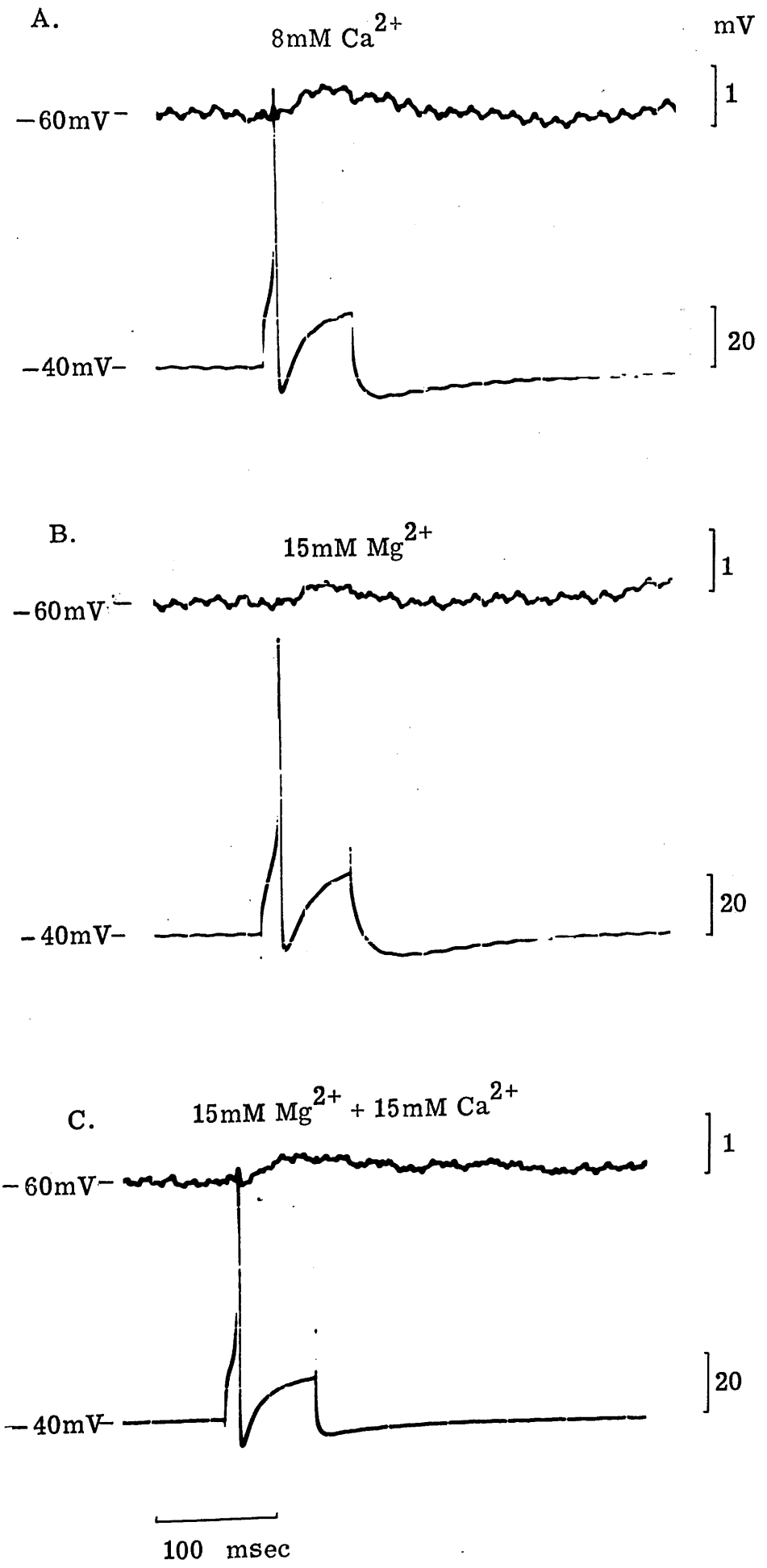

A.
8mM Ca^{2+}
mV
−50mV −
2
−40mV−
50
B.
15mM Mg^{2+}
−50mV−
2
−40mV−
50
C.
15mM Mg^{2+} + 15mM Ca^{2+}
−50mV
2
−40mV−
50
100 msec

Figure 38. Persistence of the psp in a high Mg^{++} Ringers solution. In some experiments, raising the $[Mg^{++}]$ did not completely abolish the post-synaptic potential. A, Paired intracellular recordings from the peripheral SRN soma (top trace) and the central N_L cell body (bottom trace) which shows the normal N_L cell evoked epsp recorded from the SRN soma. B, The N_L cell action potential evoked epsp persisted after 10 minutes exposure to the 15 mM Mg^{++} Ringers solution. C, Persistence of the epsp in a 15 mM Ca^{++}, 15 mM Mg^{++} Ringers solution as before (fig 37). Time scale common to all traces.


A.
8mM Ca^{2+}
mV
−60mV−
1
−40mV−
20
B.
15mM Mg^{2+}
1
−60mV−
−40mV−
20
C.
15mM Mg^{2+} + 15mM Ca^{2+}
1
−60mV−
−40mV−
20
100 msec

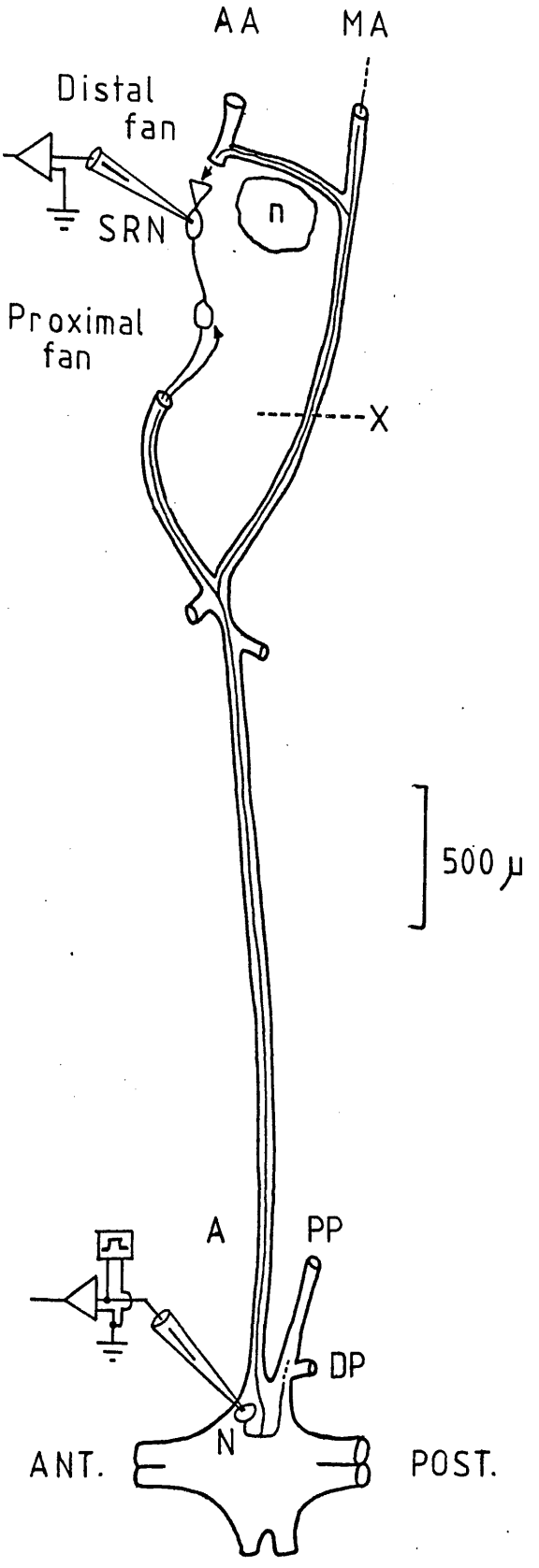
The persistence of the psp in both Ca^{++} and Mg^{++}, suggesting a monosynaptic connection between the two cells, and the long synaptic delay (around 20 ms), raises the question of the location of the N_L to SRN synapse. Morphological evidence from electron microscope studies shows close apposition of N_L cell terminals and the SRN fan-like dendrites (Blackshaw et.al., 1982) but none of the ultrastructural features characterisic of chemical synapses were seen. An alternative possibility is that the two cells synapse within the neuropile of the segmental ganglion and that the cable properties of the SRN allow the psp to be recorded at a distance in the SRN cell body. In the case of a peripheral N_L to SRN synapse, the observed long synaptic delay would consist of two components i), a fixed, constant latency synaptic delay and ii), a conduction delay determined by the time taken for propagation of the N_L cell action potential from its cell body to its coiled terminals upon the peripheral SRN fan which in turn is dependant upon the speed of conduction along the N_L cell axon and its smaller side branches.

The length of the N_L cell axon, traced along the MA root and small side branches between the stretch receptor neurone and the ventral nerve cord in was approximately 5.0 mm. Taking the peripheral N_L cell conduction velocity as 0.6 $msec^{-1}$ or less (Blackshaw et.al. 1982), the conservative figure of 8 ms is obtained for the conduction time of the N_L cell action potential into the periphery. This leaves the ample value of 11 ms for the synaptic latency.

Physiological experiments were performed to determine the location of this excitatory input from the N_L cell to the stretch receptor neurone by selectively cutting certain nerve branches in the periphery. Figure 39 illustrates the branching pattern of the N_L cell axon in the periphery (ref.ibid.) Intracellular injection of Horseradish peroxidase into the N_L cell soma has shown that the main branches of the N_L cell axon run in the main anterior nerve root and its first major anterior branch, (designated nerve roots MA and AAμ Ort, Kristan and Stent, 1974). A side branch of the N_L cell axon within the AA root makes a number of looped turns across the surface of the proximal fan of the stretch receptor neurone before running off to more superficial levels of the dermis. In addition, the N_L cell axon in the MA nerve root distributes a side branch to the AA nerve root distal to the nephridiopore which innervates the distal fan of the stretch receptor neurone. This arrangement whereby afferent and efferent nerve fibres run in different nerve branches for part of their trajectory makes it possible to disrupt part of the peripheral projection of the N_L cell whilst leaving any possible central synaptic connections intact.

Figure 40 shows the psp recorded from the SRN cell body upon intracellular stimulation of the N_L cell soma before and after section of the N_L cell branch in the MA nerve root. Fig. 40a shows the epsp recorded from the SRN cell body in a normal preparation in which both the AA and the MA nerves are intact ; six sweeps are added. In figure 40b the MA nerve was cut at the point marked X in figure 39, hence sectioning the branch of the N_L cell normally supplying the distal stretch receptor neurone fan. Cutting the N_L

Figure 39. Peripheral distribution of the N_L cell branches in the anterior nerve root. Notation of branches as in Ort et al. (1974), a, Anterior ; PP, posterior posterior ; DP, dorsal posterior; AA, anterior anterior ;MA, medial anterior nerve branches. Branches of the N_L cell axon run in the main anterior nerve root (A), the medial anterior nerve root (MA) and the first major branch of the anterior nerve root (AA). A side branch of the N_L cell axon within the AA nerve root contacts the proximal fan of the SRN. The N_L cell axon in the MA nerve root distributes a side branch to the AA nerve root distal to the nephridiopore (n) and contacts the distal fan of the SRN. This anatomical separation of afferent and efferent fibres over part of their course in the periphery makes it possible to disrupt the peripheral projection of the N_L cell to the distal SRN fan by cutting the MA nerve root at X. The SRN axon in the AA and A nerve roots is unaffected by this proceedure.


AA
MA
Distal
fan
SRN
n
Proximal
fan
X
500 μ
A
PP
DP
N
ANT.
POST.

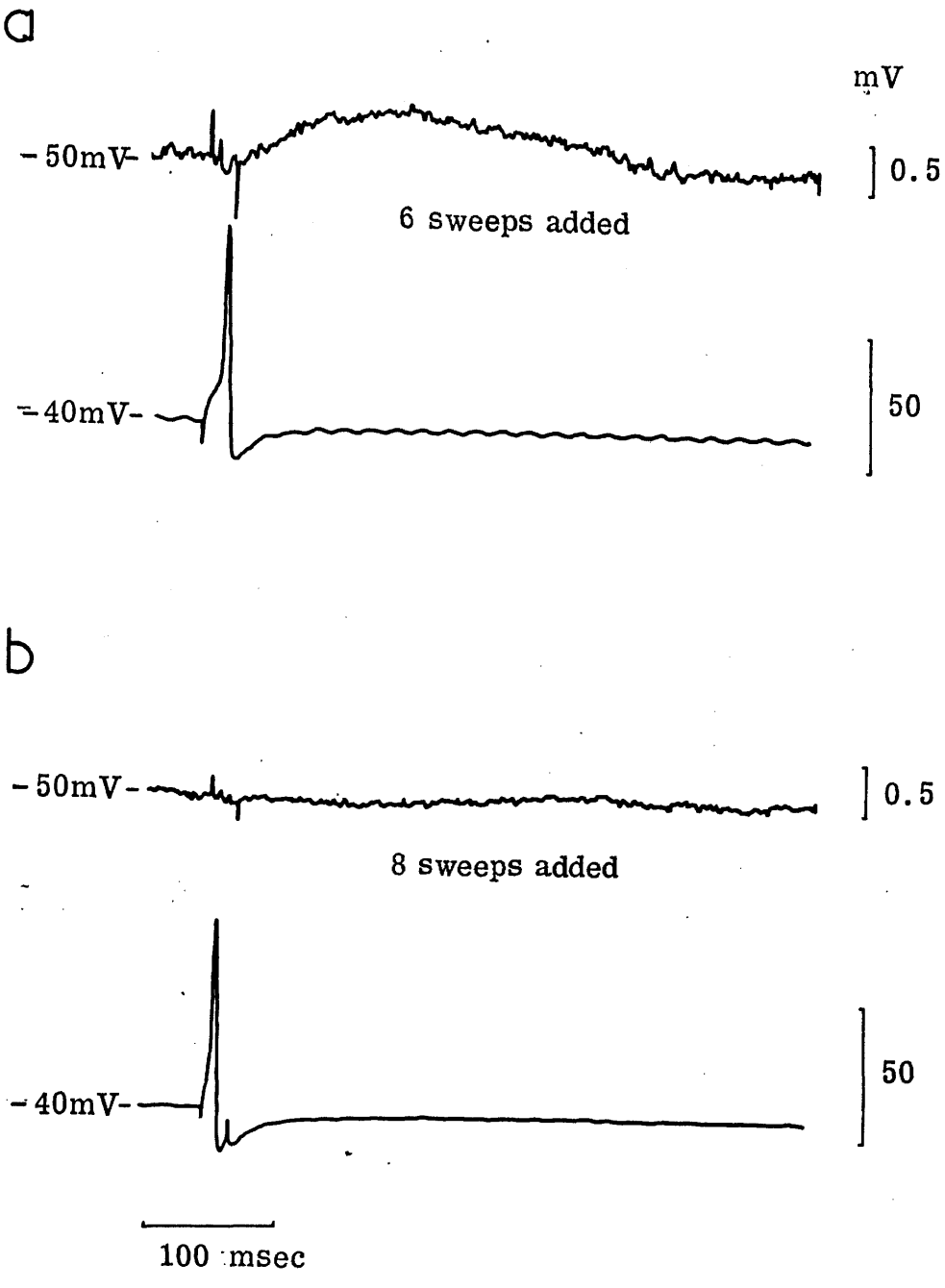
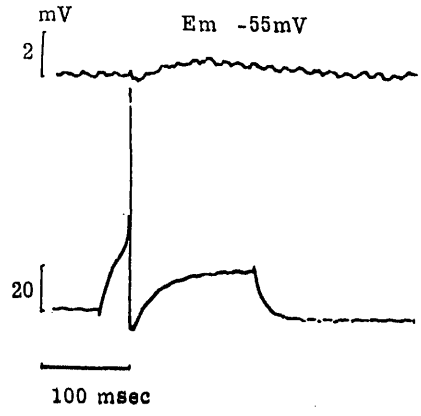


Figure 40. The epsp recorded from the SRN soma upon intracellular stimulation of the N_L cell body before and after section of the N_L cell axon branch in the MA nerve root. a, Paired intracellular recordings from the SRN soma (top trace) and the N_L cell body (bottom trace) in a normal preparation with AA and MA nerves intact. Six post-synaptic responses are added, one pre-synaptic action potential shown omnly. b, Paired intracellular recordings from the two cells in the same preparation after the MA nerve was cut at the point marked X in figure 39. Post-synaptic response (upper trace) 8 sweeps added, one pre-synaptic action potential only shown (lower trace). Cutting the N_L cell axon branch in the MA nerve root abolished the epsp previously recorded in the SRN soma. Time scale common to both panels.

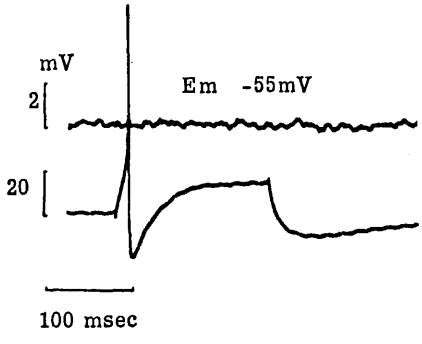
branch in this way to the SRN distal fan abolished the epsp previously recorded from the SRN soma. This suggested that the synapse was located peripherally on the distal fan of the stretch receptor neurone. If the synapse is peripheral cutting the AA root might be expected not to have any effect on the psp recorded from the SRN cell body. However Figure 41 shows that this was not the case. Figure 41a illustrates the normal situation in which the epsp is recorded from the SRN soma with both the AA and the MA nerve roots intact. In the same preparation panel b shows the situation demonstrated in fig. 40. Here, after the initial recording of the psp from the SRN cell body, the recording electrodes were withdrwn from both pre- and postsynaptic cells and the MA root was sectioned at point X . Within 2.0 min. both the SRN and the N_L were re-impaled and the synaptic potential searched for. As in the case of the preparation in fig. 40 the psp was abolished. In another preparation a psp was again recorded from the SRN cell body with both AA and MA nerve roots intact. This time however, the MA nerve was left intact whilst the AA nerve was cut at the point marked Y. Panel c illustrates the result of this proceedure. Surprisingly, disruption of the N_L cell branch to the proximal fan also resulted in the abolition of the epsp from the stretch receptor neurone cell body. This latter proceedure cuts the stretch receptor axon in the AA root, as well as the N_L cell branch to the proximal fan. It appears therefore that the integrity of the post-synaptic cell needs to be maintained in order that the epsp may be recorded.

Figure 41. The SRN epsp elicited upon stimulation of the N_L cell before and after cutting the MA or the AA nerve root. A, Normal situation with SRN epsp (upper trace) elicited in response to a single depolarisation-evoked N_L cell action potential (lower trace), AA, MA nerve roots intact. B, Abolition of the SRN epsp (upper trace) in the same preparation upon cutting the MA nerve root at point X (right hand diagram) In another preparation the AA nerve root was cut at the point marked Y (right hand diagram). This proceedure disrupts both the N_L cell axon branch to the proximal SRN fan as well as the SRN axon itself. The N_L cell axon branch in the MA nerve root to the distal SRN fan remained intact. This proceedure also resulted in the abolition of the epsp from the SRN soma (SRN upper trace, presynaptic N_L cell action potential lower trace).

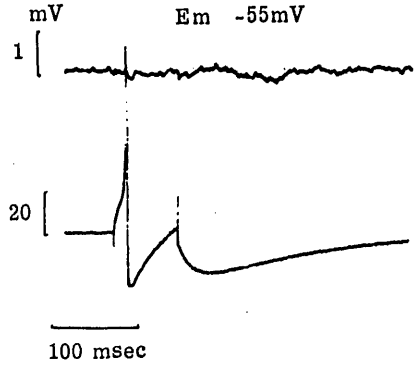
A. Normal

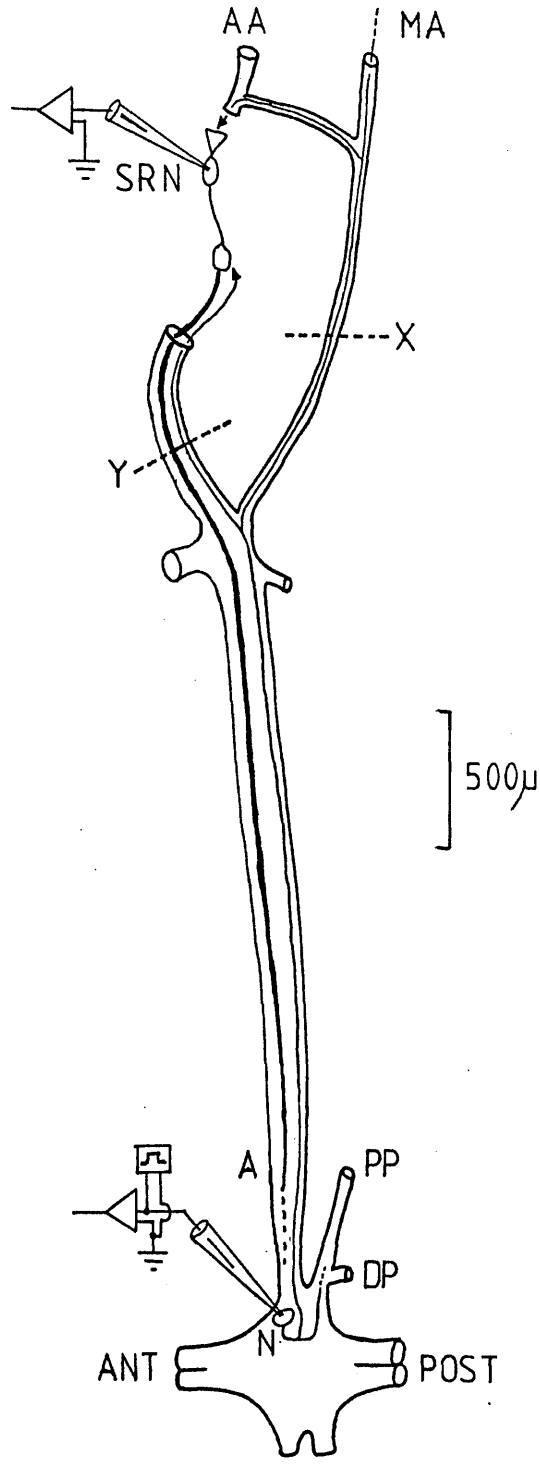


B. Root MA Cut
AA Intact



C. Root AA Cut
MA Intact



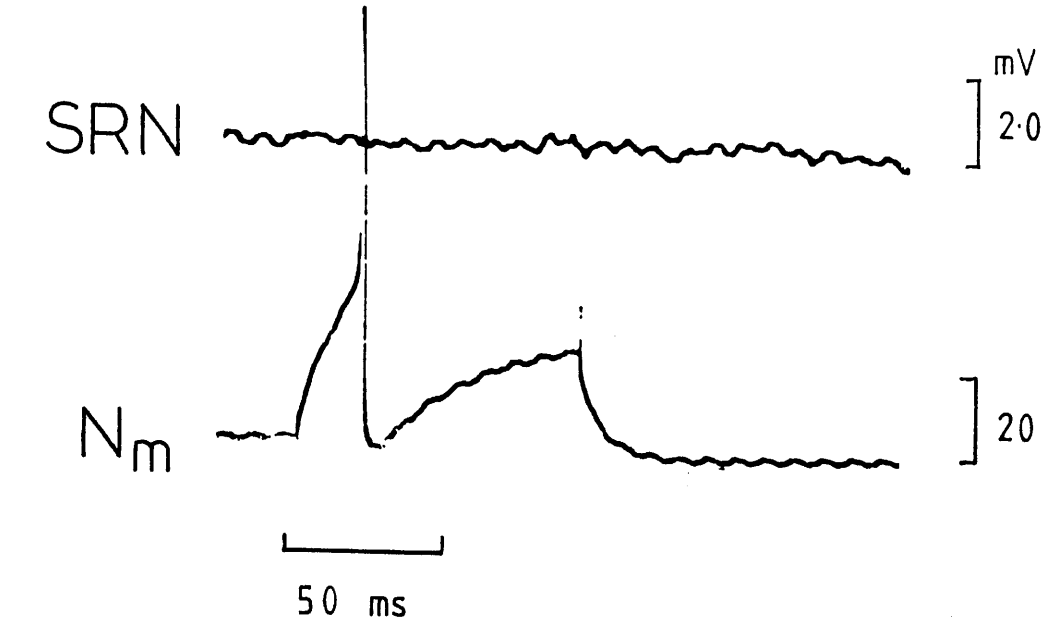


4.2 The medial nociceptive mechanosensory neurone.

The two medially sited N cells within the ganglion (N_m) each have a receptive field which is approximately co-incident with that of the N_L cell on the same side. In addition, the N_m cell also supplies the connective tissue surrounding the viscera, noxious mechanical stimuli applied to which evokes a similar response to noxious stimuli applied to its cutaneous field (Blackshaw, Nicholls and Parnas,1982). Only the N_L cells exhibited the specialised coiled terminals associated with the stretch receptor neurone dendritesμ no morphological association between the N_m cell and the stretch receptor neurone was seen (Blackshaw et.al. 1981). This observation was confirmed electrophysiologically in the present study and is shown in figure 42. No synaptic activity was recorded from the stretch receptor neurone soma in response to single evoked action potentials in the ipsilateral N_m cell body, nor after repeated N_m cell action potentials in which the post-synaptic response was averaged over a 200 ms post-stimulus period.

4.3 The pressure (P) mechanosensory neurones.

The surprising find of an excitatory synaptic connection between two primary sensory neurones from the N_L cell to the SRN, raised the obvious question whether other primary mechanosensory neurones were also synaptically related to the SRN



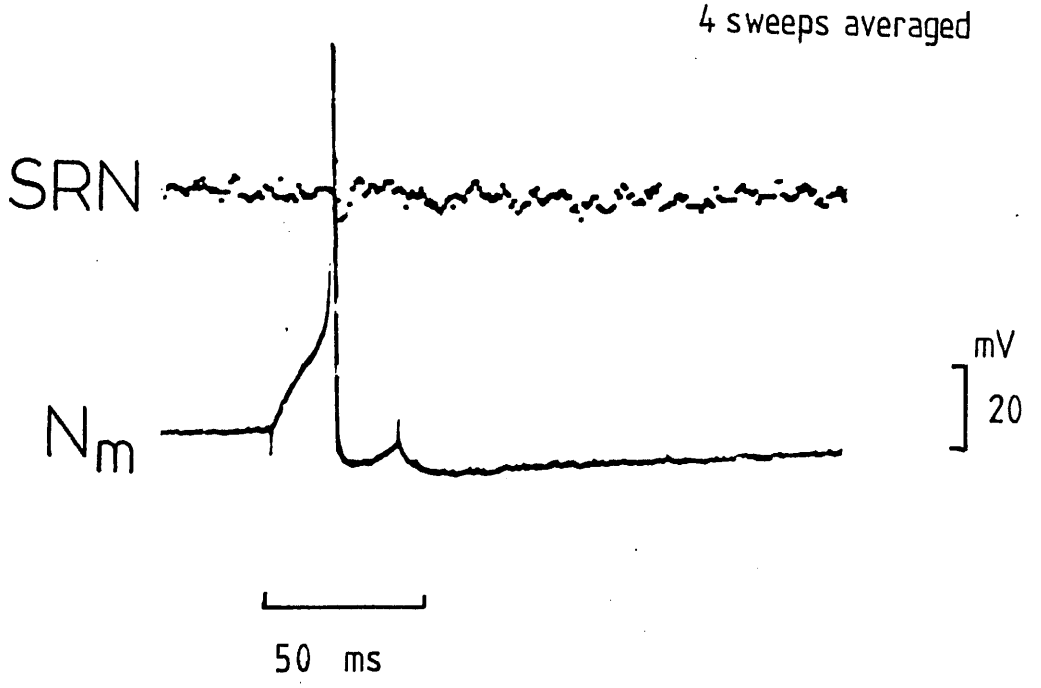


Figure 42. Efferent innervation of the SRN, Paired intracelluar recordings were made from the peripheral SRN soma (SRN, upper traces each panel) and the medial nociceptive neurone (N_M, lower traces each panel). No synaptic activity was recorded from the SRN soma in response to a single, depolarisation-evoked action potential in the ipsilateral N_M cell body (top panel, current trace not shown) nor after repeated N_M cell action potentials in which the post-synaptic response was averaged over a 200 ms post-stimulus period (lower panel, 4 sweeps averaged, one pre-synaptic action potential shown only).

A previous electron microscope study of the fine structure of the SRN dendrites and their associated N_L cell terminals showed numerous profiles of other unidentified axons closely apposed to the surface of the fan lying alongside the HRP-filled N_L cell profiles (Blackshaw et.al.). In the present experiment paired intracellular recordings were made from the peripheral SRN soma and the ipsilateral cell bodies of the lateral (P_L) and medial (P_m) pressure mechanosensory neurons within the ganglion in order to identify electrophysiologically the origin of other processes associated with the peripheral SRN dendrites. The P mechanosensory neurones are themselves primary sensory neurones which respond to deformation of the skin of the leech with a slowly adapting discharge which may last throughout a period of maintained pressure. Their threshold is lower than that required to produce an N cell discharge, (Nicholls and Baylor, 1968) Each segmental ganglion contains two pairs of P mechanosensory neurones all with specific peripheral receptive fields. The most laterally sited P cell (P_L) innervates the ventral quadrant of the ipsilateral body wall by way of both the anterior and posterior nerve roots. The more medially sited of the two P cells (P_m) innervates the dorsal quadrant of the ipsilateral body wall by a single axon branch in the dorsal root of the posterior nerve, (ref. ibid.).

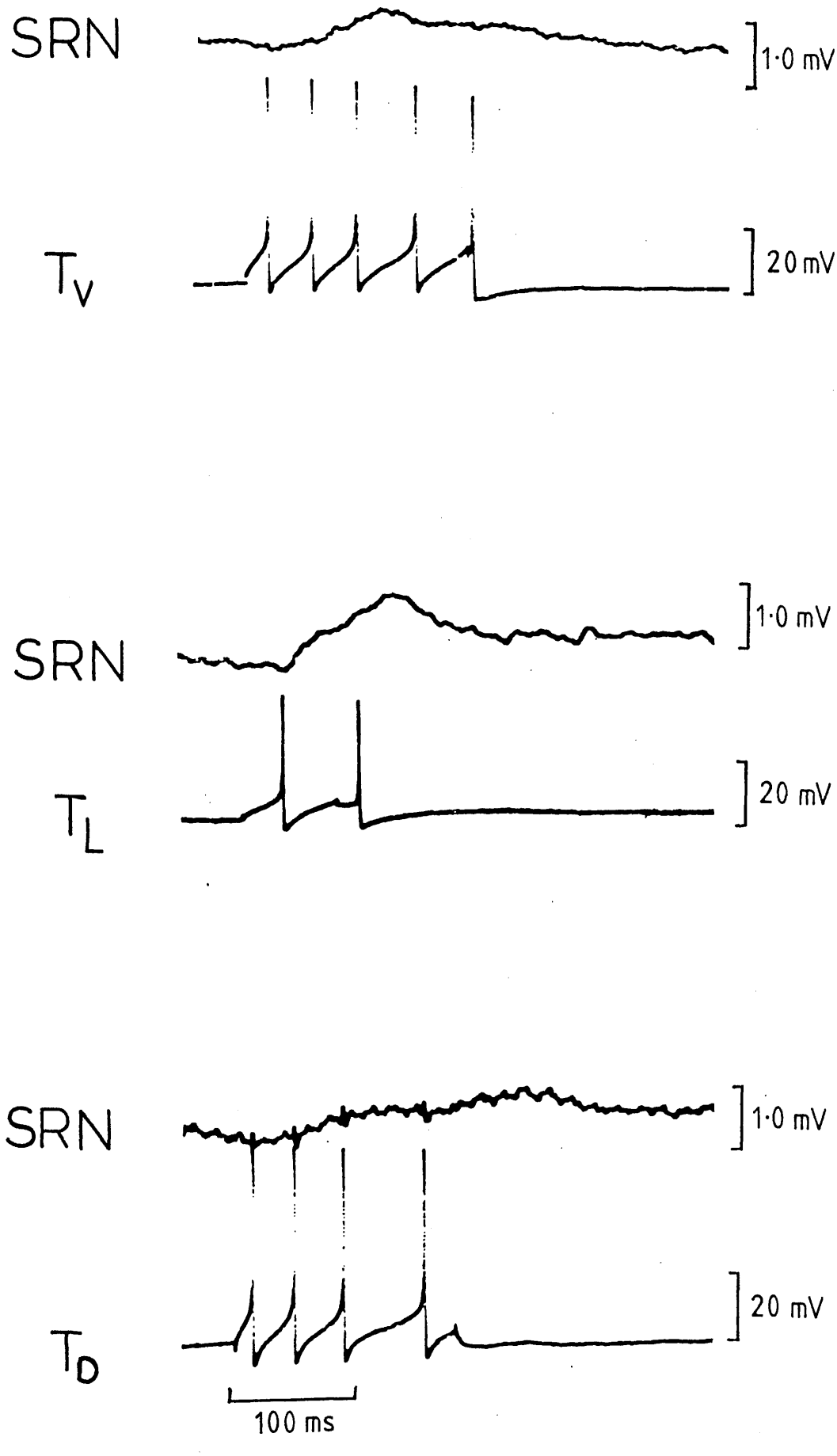
Figure 43 shows records of paired intracellular recordings from the SRN and the ipsilateral P_L cell and from the SRN and the ipsilateral P_m cell. Both recordings were made in 8mM Ca^{++} Ringer solution. In the top panel a train of four action potentials occured in the P_L cell in response to an injected depolarising current

pulse, (not shown). This is followed in the peripheral SRN cell body, at a constant latency and on a 1:1 basis by four epsp's (> 1.0 mV). The epsp's summated to depolarise the SRN soma by approximately 3.0 mV. Both the lateral and the medial pressure mechanosensory neurones were excitatory to the stretch receptor neurone. The bottom panel in figure 43 shows the summation of two epsp's in the SRN soma in response to the first two of three action potentials in the P_m cell body. Note also the failure of the third psp after the last P_m cell action potential. It was not known whether any of the unidentified processes seen apposed to the surface of the stretch receptor neurone fan were indeed P cell terminals. It is unlikely however that the medial P cell contacts the peripheral SRN fan directly. The P_m cell innervates its dorsal receptive field via a single axon branch in the dorsal branch of the posterior nerve root (Nicholls and Baylor, 1968). This anatomical arrangement would necessitate either a direct central connection from the P_m cell to the SRN or a poly-synaptic central connection to a neurone that projects to the SRN fan.

4.4 The touch (T) mechanosensory neurones.

A similar study to that of the P cells was carried out upon the ipsilateral touch (T) mechanosensory neurones. These are primary sensory neurones which respond with a rapidly adapting , high frequency response to light touch of the skin. Six T cells are situated within each segmental ganglion, three on each side. The

Figure 44. All three ipsilateral touch mechanosensory neurones are pre-synaptic to the SRN. Paired intracellular recordings were made from the peripheral SRN soma (SRN) and from the ipsilateral touch mechanosensory neurones which innervate ventral (T_V), lateral (T_L) and dorsal (T_D) skin territories. Action potentials evoked by direct depolarisation in all three ipsilateral touch cells elicited post-synaptic potentials in the SRN soma (current traces not shown). Time scale common to all panels.


SRN
1·0 mV
T_V
20 mV
SRN
1·0 mV
T_L
20 mV
SRN
1·0 mV
T_D
20 mV
100 ms

receptive fields of each T cell have been extensively mapped, (Nicholls and Baylor, 1968 ; Yau, 1976). Each T cell innervates a clearly defined area of ipsilateral lateral, ventral or dorsal skin in its own segment and in adjacent parts of the two neighbouring segments. The most medially situated T cell in the ganglion innervates the lateral third of the ipsilateral body wall and is designated the lateral touch cell, (T_L). The most laterally situated T cell in the segmental ganglion innervates the dorsal third of the body wall, this is the dorsal touch cell (T_D), whilst the middle T cell in the ganglion innervates the ventral third of the ipsilateral body wall and is designated the ventral touch cell (T_V). Both T_V and T_L neurones have axons extending into both the posterior and anterior ipsilateral nerve roots but not the dorsal root, whilst the T_D cell has a single axon branch extending into the dorsal nerve root.

Figure 44 illustrates that action potentials in all three touch cells resulted in excitatory post-synaptic potentials in the soma the stretch receptor neurone. The epsp's were comparable in size to the N_L cell epsp (1-2 mV). Once again failure of synaptic potentials was sometimes observed in the stretch receptor neurone soma after repeated impulses in the T_V cell (eg. top trace fig.44). Similar to the P_m cell, the T_D cell has a single axon extending into the dorsal nerve root which makes it unlikely that the observed SRN epsp is the result of a direct synapse between the T_D cell and the SRN fan in the periphery.

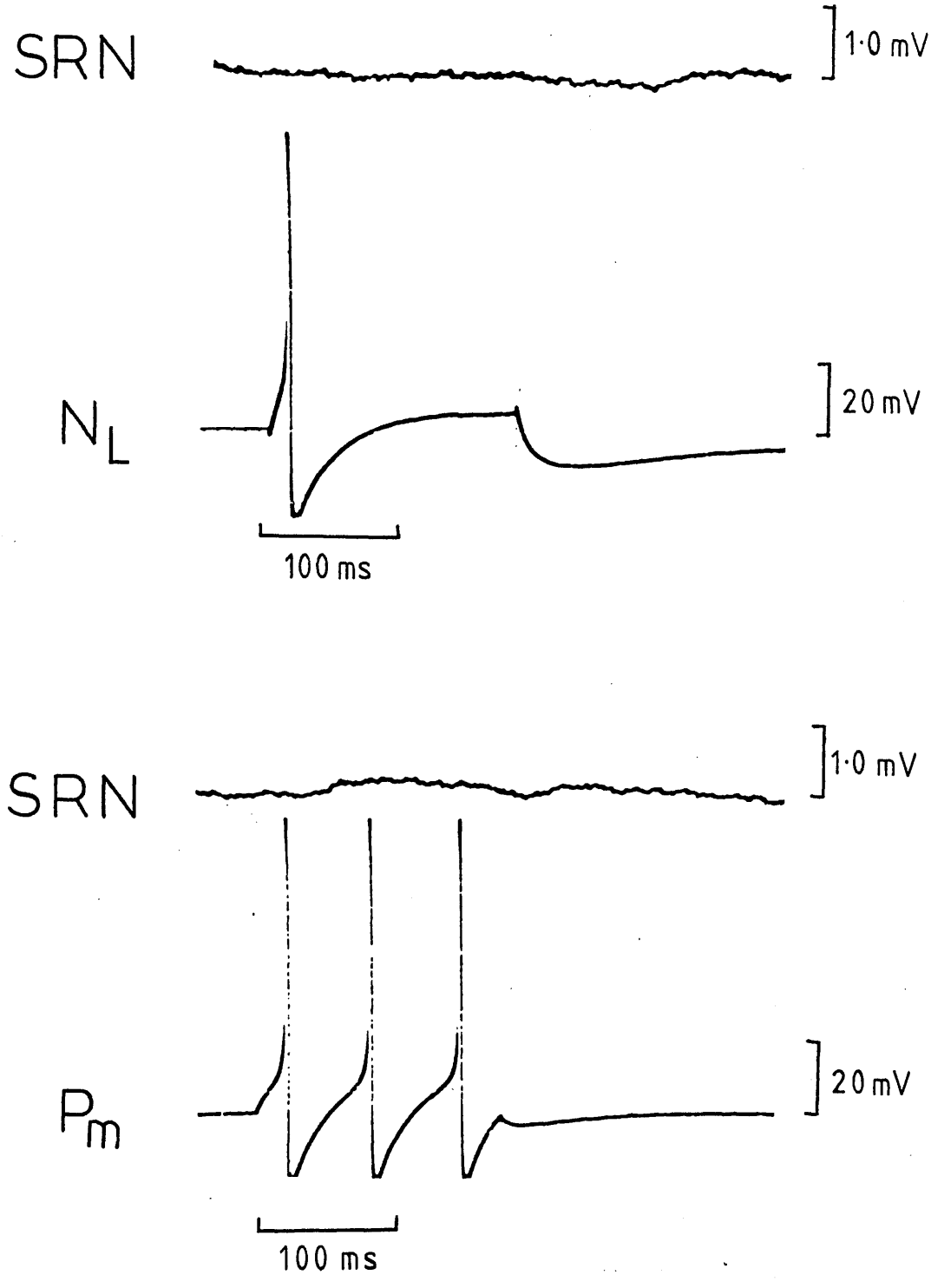


Figure 45. Contralateral mechanosensory neurones are not pre-synaptic to the SRN. Paired intracellular recordings were made from the contralateral lateral nociceptive neurone cell body (N_L) and the SRN (upper panel) and from the SRN and the contralateral medial pressure mechanosensory neurone (P_M, lower panel). An action potential elicited by direct depolarisation of the N_L cell body (current not shown) did not evoke synapyic activity in the SRN. A train of three action potentials elicited by injection of depolarising current (not shown) into the contralateral P_M cell did not evoke synaptic activity in the SRN directly correlated to the pre-synaptic volley.

4.5 Contralateral mechanosensory neurones.

Figure 45 shows the result of paired intracellular recordings between the SRN soma and two contralateral mechanosensory cells. A single action potential evoked by direct depolarisation of the contralateral N_L cell body did not evoke any activity in the stretch receptor neurone. A brief train of action potentials elicited by depolarisation of the contralateral P_m cell body however resulted in a weak depolarisation of the SRN post-synaptic membrane. Since all mechanosensory neurones have strictly ipsilateraly directed axons, it seems most unlikely that this represents a direct synapse upon the contralateral peripheral SRN dendritic fan and is more likely to result from either a central P_m - SRN synapse mediated by central P_M cell branches that sometimes cross the midline of the ganglion (Muller and McMahan, 1976) or a multi-synaptic interaction.

SECTION 5.0 SRN CONTACTS WITH IDENTIFIED SEGMENTAL MOTONEURONES: AFFERENT MODULATION OF MOTOR OUTPUT.

Previous behavioural and physiological observations have presented evidence for the existence of proprioceptive feedback capable of modulating the centrally generated locomotory patterns of the leech, (Gray, Lissmann and Pumphrey, 1938 ; Kristan and Stent, 1976). Until recently however the identity of the afferent or afferents involved was unknown. In the present study, peripheral neurones in close association with the longitudinal muscle of the body wall have been described. They transmit imposed voltage changes decrementally to the CNS, and moreover they respond to changes in body wall length with analogue voltage signals, graded in relation to the final amplitude and velocity of the displacement. An obvious question was whether activity within the SRN can initiate or modulate motoneurone discharge. Accordingly the central connections of the SRN with identified motoneurones to the longitudinal muscle were investigated i), morphologically by paired intracellular injections of the afferent SRN and identified motoneurones and ii),electrophysiologically by a) paired recordings of afferent and motoneurone activity to look for modulation of efferent activity produced by analogue voltage changes within the stretch receptor neurone, and by b), using the method of coherence analysis applied to three motoneurone spike trains and the SRN input.

5.1 Morphology and contacts of identified segmental motoneurones.

In 1970, Ann Stuart described 14 pairs of excitatory and 3 pairs of inhibitory motoneurones which together supply the five different muscle groups in each segment of the leech. Each of these neurones can be recognised in all of the 21 midbody segmental ganglia and each innervates a specific territory of muscle fibres which has a consistent location from segment to segment.

Four bilaterally homologous pairs of motoneurones were identified on the dorsal surface of the ganglion which supply excitatory innervation to part of, or all of, the ventral longitudinal muscle, (see Stuart, 1970, text fig. 6). One of these pairs of cells, the large longitudinal motoneurone (L cell), supplies excitatory innervation to the longitudinal muscle over the entire contralateral segment from the dorsal midline to the ventral midline. The left and right L cells are the largest of the motoneurones and are conspicuously situated at the base of the anterior root in the posterior packet of the segmental ganglion. They are electrically coupled to each other by a non-rectifying electrical synapse, (Stuart, 1970), consequently spiking activity within one cell is mirrored by its partner. As a consequence, symmetrical shortening of the segment is brought about by combined activity within the pair of cells.

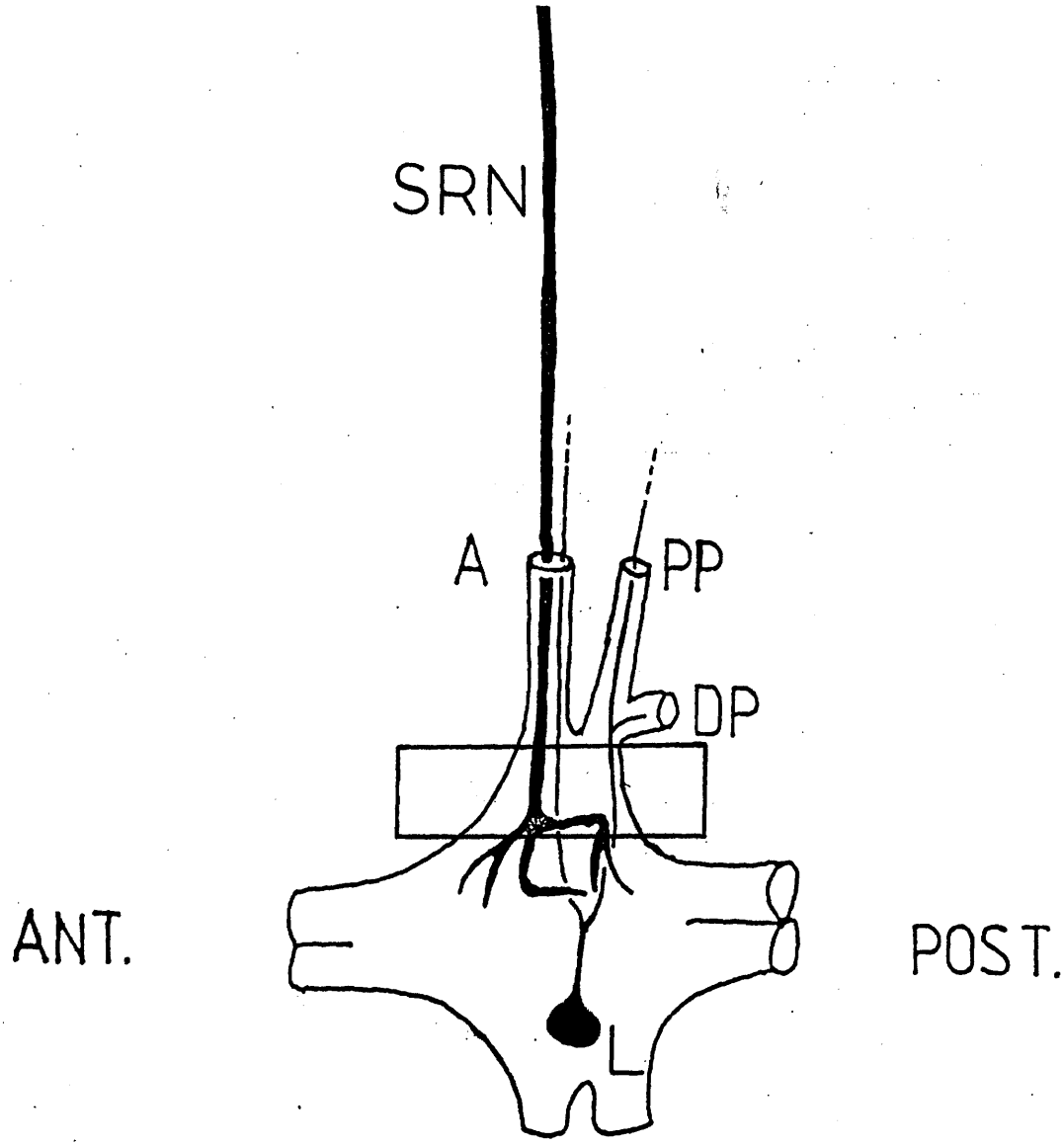
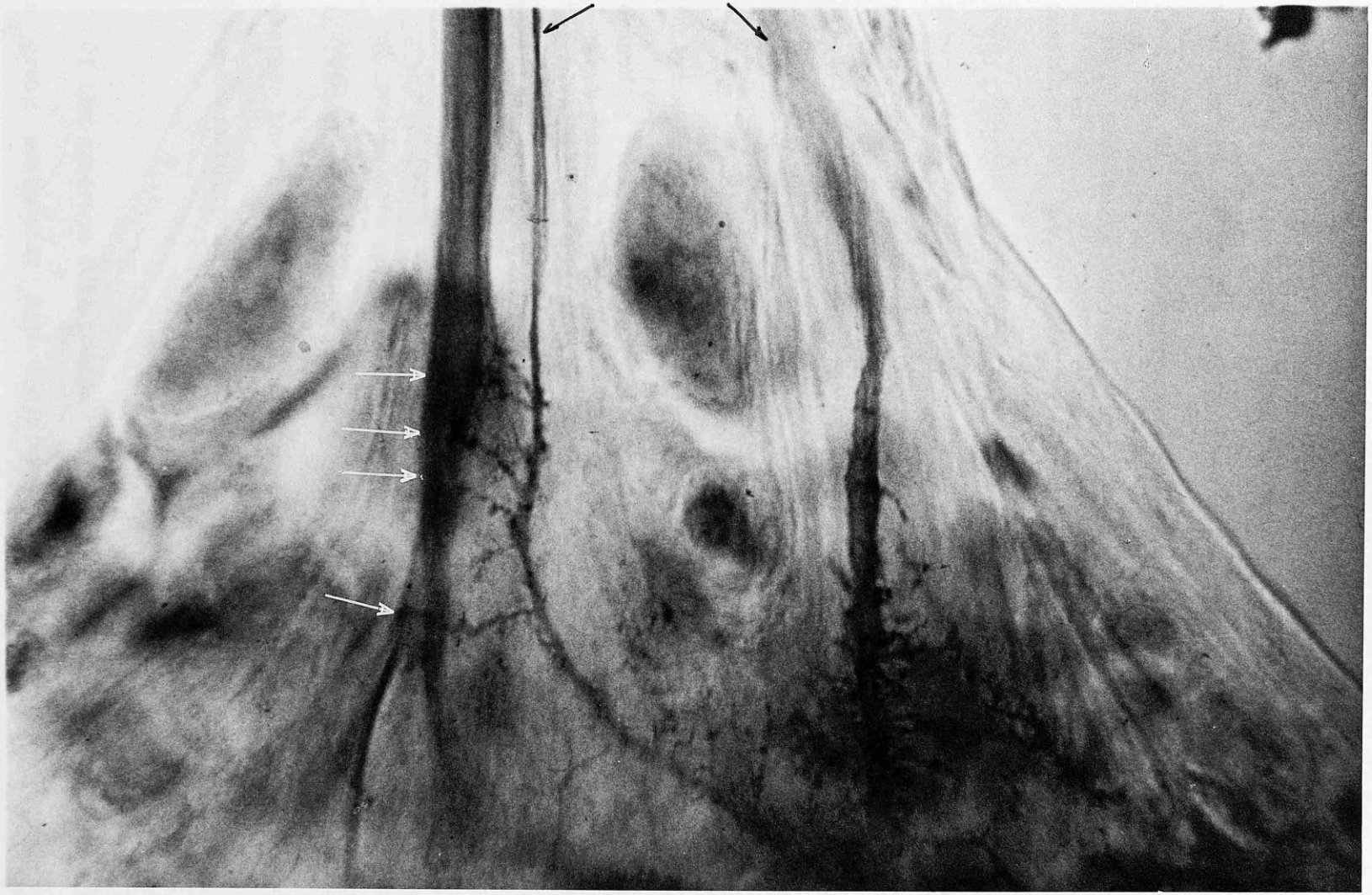


Figure 46. Location of possible synaptic sites between the SRN and and the L motoneurone. Schematic diagram showing the axon projections of the contralateral cell body of the L motoneurone (L) and the peripheral SRN into the anterior and posterior nerve roots of the segmental ganglion. A single axon process arises from the contralateral L cell body and crosses the ganglionoic midline. A larger axon branch then extends into the posterior nerve root and a smaller diameter branch extends into the anterior nerve root. The anterior branch of the L cell and the afferent SRN axon run parallel in the anterior nerve root. The area in the rectangle is shown in greater detail in figure 47.

Figure 47. Part of a whole mount preparation of a segmental ganglion which contains both the central projection of the SRN and the L cell motoneurone arbour. Both cells were injected, via the cell bodies with HRP. Ilustrated is that lateral margin of the neuropile at the base of the anterior and posterior nerve roots illustrated in figure 46. The large diameter (10μm) axon of the SRN enters the segmental ganglion in the anterior nerve root. The anterior efferent axon branch of the L cell runs in the anterior nerve root parrallel to the afferent SRN axon. Numerous fine side branches arise from the anterior branch of the L cell axon and are sequentially studded along a 30μm stretch of the SRN axon (arrows) proir to the branching of the SRN axon in the neuropile.


SRN axon
L cell axons
ANT
POST

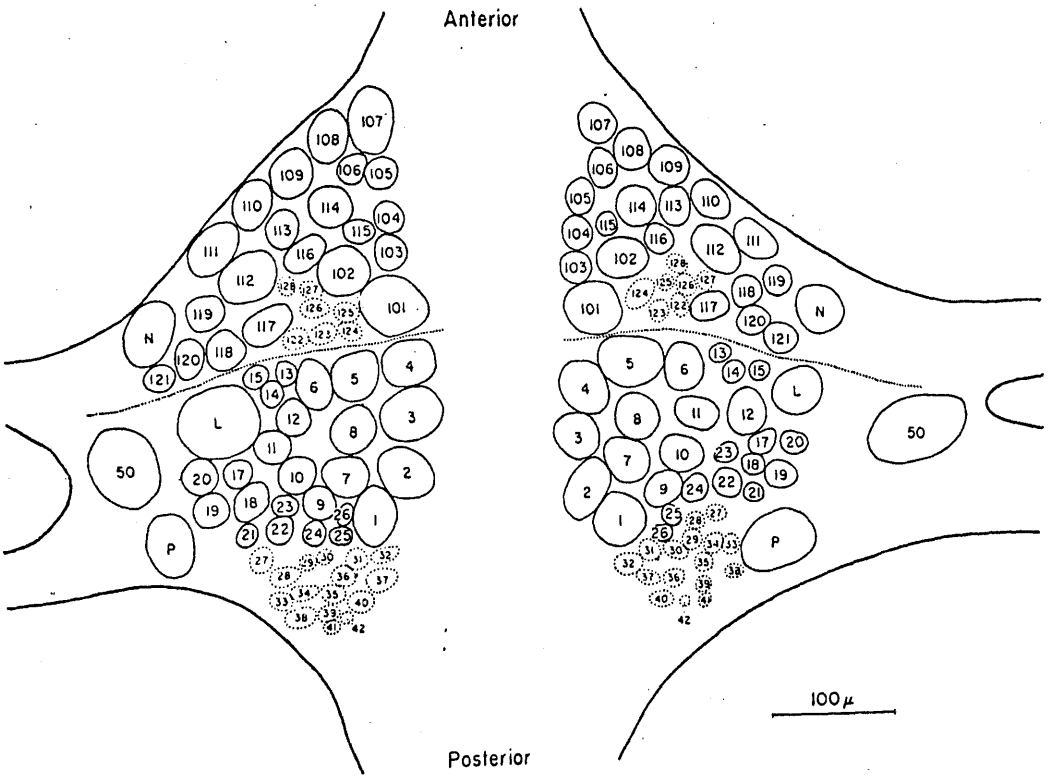
5.2 Identification and contacts of swim-motoneurones

A number of the motoneurones descibed by Stuart (1970), including the majority of those innervating the longitudianl muscle are involved in the swimming rhythm of the leech. Many of these, plus other, previously unidentified, motoneurones have been further characterised and their roles in the swimming rhythm documented, (Ort, Kristan and Stent, 1974). The cell bodies of all the motoneurones described, with activity related to the swimming rhythm, lie on the dorsal aspect of the ganglion. They may be tentatively identified from the position occupied by each cell body in relation to the glial cell packet margins and other cell bodies within the ganglion. A further clue to the identification of individual neurones may be made from their characteristic peripheral branching pattern in the segmental nerves as determined by extracellular electrical mapping of the motoneurone action potential, (ref. ibid.)

A preliminary survey was carried out to determine whether individual motoneurones exhibited characteristic morpohologies and whether they could be identified on this criteria alone, as revealed by intracellular injection of HRP.

Of the numerous cell bodies on the dorsal aspect of the ganglion a group containing both inhibitory and excitatory motoneurones to the longitudinal muscle may be invariably recognised as a cluster of seven cells in the posterior glial packet of the segmental ganglion.

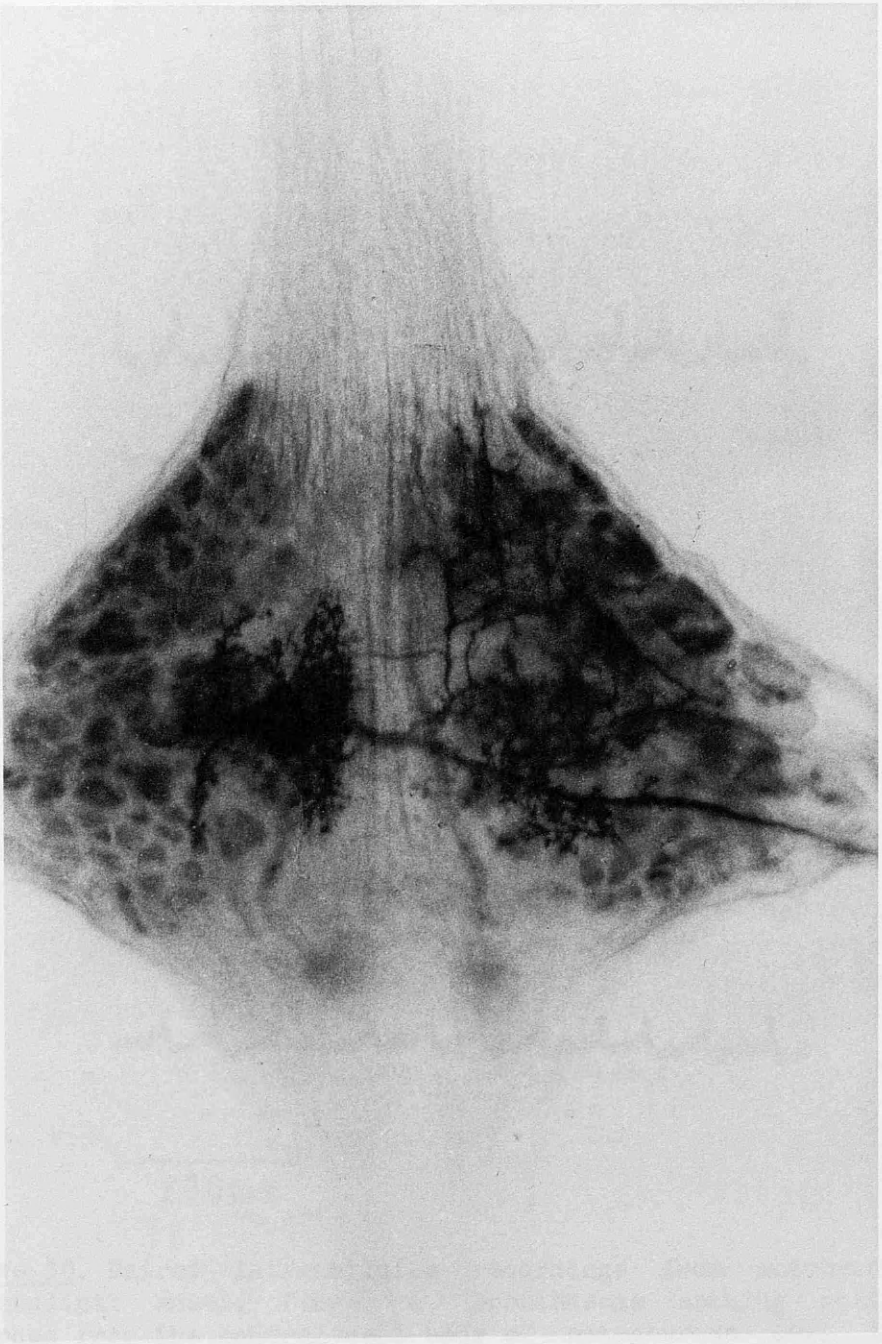
Figure 48. Cell body map of the dorsal aspect of a midbody segmental ganglion showing the numbering system used to define motoneurone cell bodies (from (Ort et al. 1974). Although this numbering system is considered to be provisional, the numbered cells 1-7 described in the text may be invariably recognised as a cluster of seven large neurones lying medially and bilaterally in the posterior glial packet of the ganglion.



These cells are shown in figure 48. This shows the dorsal aspect of a segmental ganglion in which the outlines of the motoneurones have been traced after Methylene Blue staining, (from Muller, Nicholls and Stent, 1981). The group of seven motoneurones, numbered 1 - 7 are arranged characteristically in two rows along the medial edge of the posterior glial packet, their homologous pairs lying in the contralateral half of the ganglion. Three of these cells were injected intracellulary with horesradish peroxidase and their characteristic arborisations described. Confirmation of their identity was made from the region of longitudinal muscle innervated by each motoneurone (Stuart, 1970). Either an exploration of the longitudinal muscle fibres of the segment was made in order to record excitatory junction potentials (ejp's) with a 1:1 relationship to the firing of the motoneurone, or a visual inspection was made of the contracting region of longitudinal muscle upon depolarisation of the motoneurone cell body within the ganglion.

Figure 49 shows a whole mount preparation of a segmental ganglion in which a motoneurone was injected intracellulary with horseradish peroxidase. From the position occupied by the soma on the dorsal side of the ganglion this cell was tentatively identified as an excitatory motoneurone to the dorsomedial longitudinal muscle, (undescribed by Stuart, designated cell 3 by Ort et.al. 1974). This designation was confirmed by paired intracellular recording from the motoneurone cell body and the longitudinal muscle fibres of the body wall. Figure 50a shows in the bottom trace spontaneous spiking activity recorded from the cell 3 soma. The top trace is a

Figure 49. Whole mount preparation of a segmental ganglion in which the cell body of a motoneurone was injected intracellularly with Horseradish peroxidase. From the position occupied by the cell body this cell was tentatively identified as one of the excitatory motoneurones to the dorsomedial longitudinal muscle (designated cell 3 by Ort et al. 1974). A single process arises from the soma to distribute branches in the ipsi and contralateral neuropiles. A single axon extends into the contralateral posterior nerve root. Scale bar 250μm. Anterior connective top of plate.



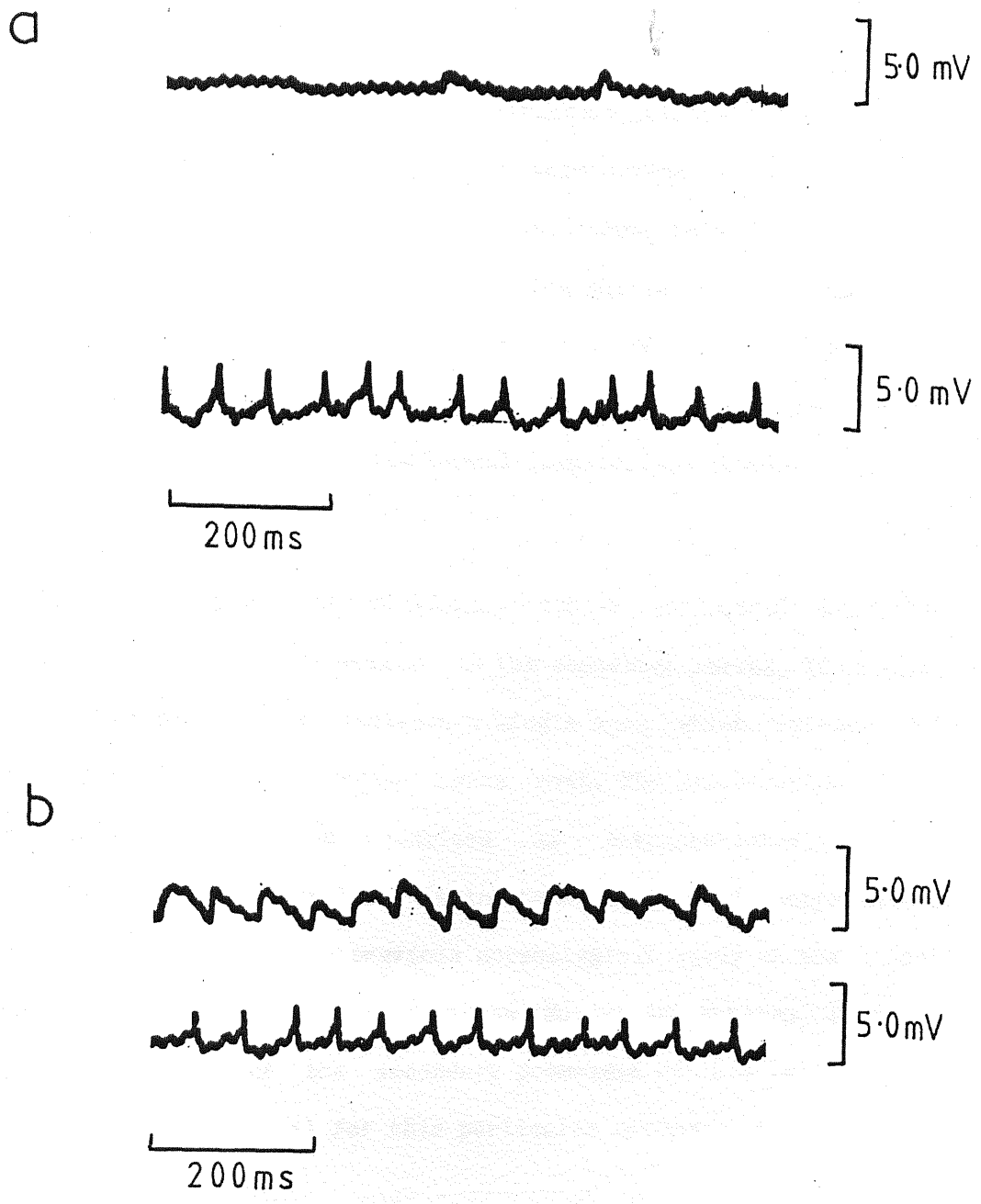


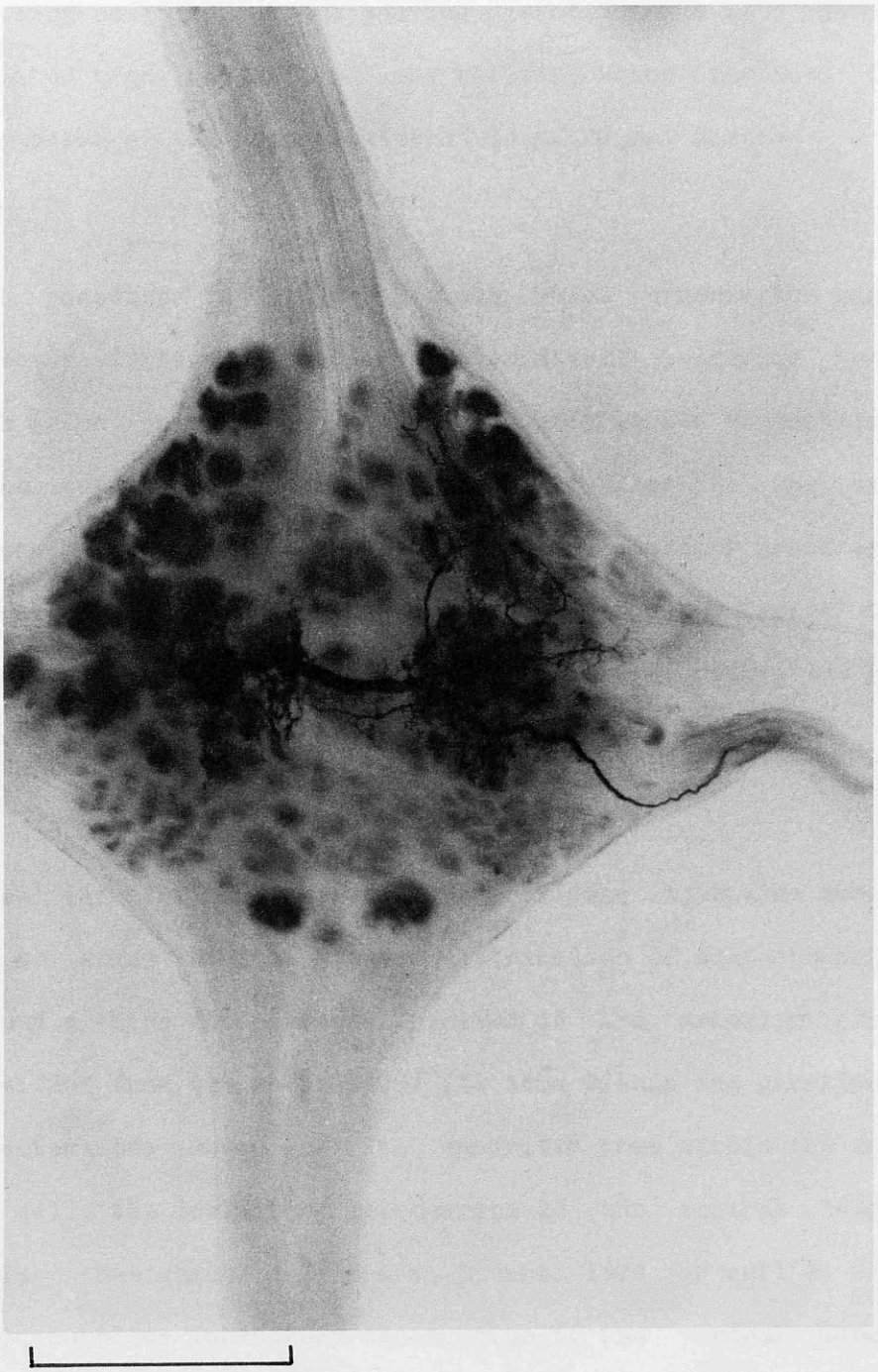
Figure 50. Paired intracellular recordings from motoneurone and longitudinal muscle fibre. a, Spontaneous spiking activity was recorded from the central cell body of motoneurone cell 3 (lower trace) and from a longitudinal muscle fibre in the ventral body wall of the leech (upper trace, E_m -62mV). No correlation was seen between spiking activity in the motoneurone and the synaptic activity recorded from the muscle fibre. b, Simultaneous paired intracellular recordings from the same preparation from motoneurone cell 3 (lower trace) and from a longitudinal muscle fibre lying in the dorsal body wall (upper trace). Excitatory junction potentials were recorded from the muscle fibre following action potentials in the motoneurone soma on a 1:1 basis and with a constant latency. The motoneurone was therefore considered to supply excitatory innervation to the dorsal longitudinal muscle.

simultaneous intracellular recording from a longitudinal muscle fibre from the ventral body wall. No correlation was seen between the activity in the motoneurone and that in the ventral longitudinal muscle. Figure 50b shows a simultaneous intracellular recording, from the same preparation, from motoneurone 3 and from a longitudinal muscle fibre lying in the dorsal body wall. Excitatory junction potentials were recorded in the muscle fibre following on a 1:1 basis and with a constant latency, the impulse activity in the motoneurone cell body. This motoneurone therefore provided excitatory innervation to the dorsal longitudinal muscle.

In accordance with the previously mapped peripheral projection pattern of this motoneurone in the segmental nerves, (Ort et.al. 1974) this motoneurone possesses a single axon which extends into the contralateral posterior nerve root. The arborisation of this motoneurone within the ganglion is characteristic in its distribution of branches within both ipsi- and contralateral neuropiles. As seen in a previous morphological study of the L cell and annulus errector (AE) motoneurone (Muller and McMahan, 1976), it is the pattern of the secondary processes of this cell which is distinctive and typical for this particular motoneurone.

Figure 51 shows a whole mount preparation of a segmental ganglion in which a different motoneurone was injected intracellulary with HRP. From the position occupied from the cell body within the ganglion, this cell was tentatively identified as the ventrolateral motoneurone (vl), (Stuart, 1970), otherwise known as cell 8, (Ort

Figure 51. Whole mount preparation of a segmental ganglion in which the central cell body of a motoneurone was injected with Horseradish peroxidase. From the position occupied by the soma on the dorsal aspect of the ganglion this cell was tentatively identified as the ventrolateral motoneurone (vl, Stuart,1970, cell 8 Ort etal. 1974). Identification of this motoneurone was confirmed by observing contraction of the ventral area of longitudinal muscle upon direct depolarisation of the motoneurone cell body. The secondary branches of this motoneurone show a characteristic and typical branching pattern within the ipsi- and contralateral neuropiles which is different for that shown for cell 3 (figure 49). Anterior connective, top of plate. Scale bar 250µm.

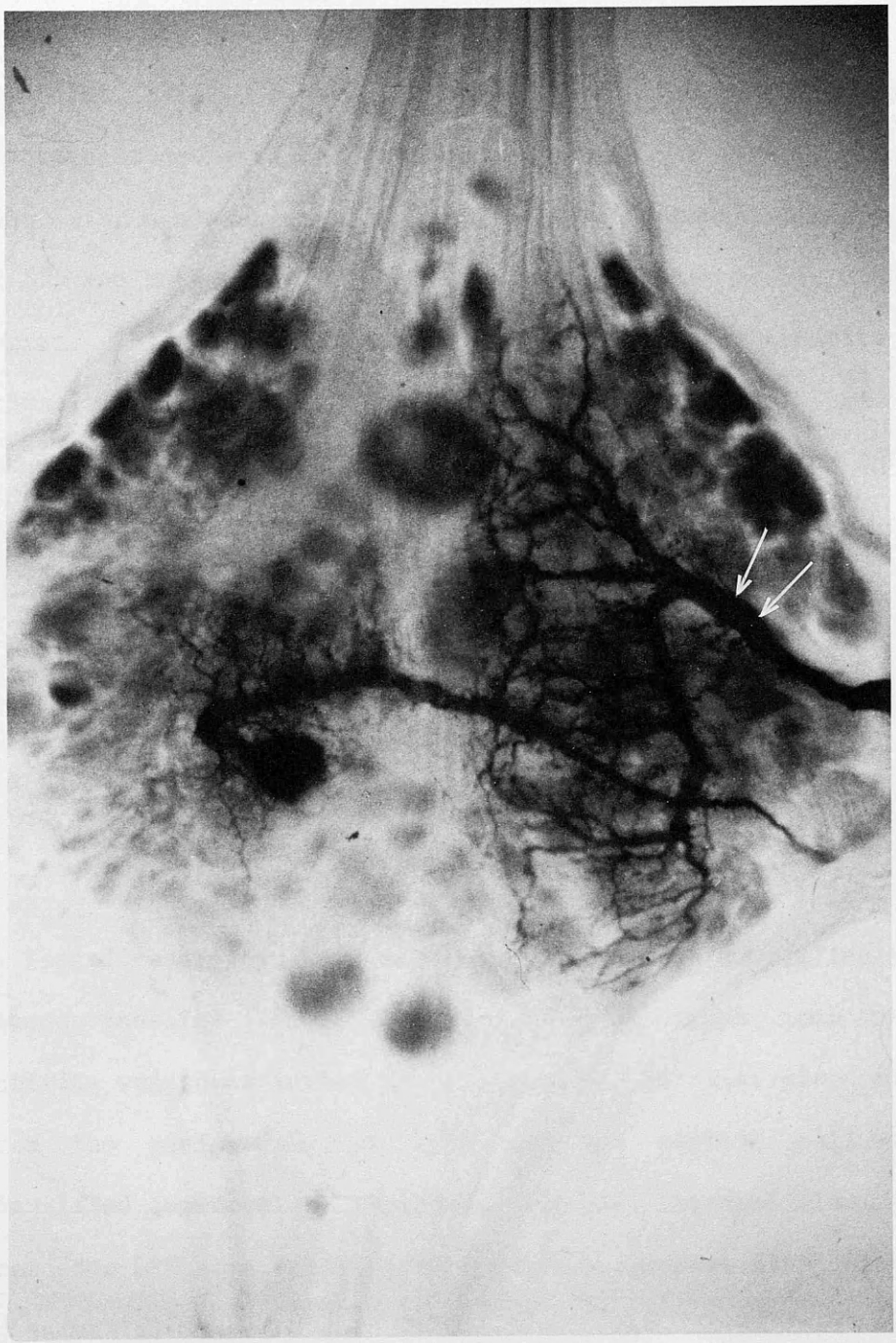


et.al. 1974). It is excitatory to the ventral longitudinal muscle and has a territory extending from the black lateral stripe of the animal to the ventral midline. Depolarisation of this motoneurone by injecting positive current via the microelectrode into the cell body initiated high frequency spiking activity which in turn initiated contraction of the ventral area of longitudinal muscle.

It possesses a single process which crosses the ganglion to project a single axon into the contralateral posterior nerve root which also corresponds to its previously mapped projection pattern in the peripheral segmental nerves. Similar to the excitatory dorsomedial motoneurone (cell 3), the secondary branches of this ventral motoneurone also show a characteristic and typical branching pattern within both the ipsi- and contralateral neuropiles which is different from that of the dorsal motoneurone.

Dual intracellular horseradish peroxidase injections were carried out to study the location and distribution of contacts between the SRN and a third motoneurone, involved in the swimming rhythm and identified from the position of its soma within the ganglion and the characteristic shape of its dendritic tree within the neuropile. This cell, the inhibitory motoneurone to the ventral longitudinal muscle, (designated cell I-l-v, Stuart, 1970 or cell 2, Ort et.al. 1974), supplies inhibitory innervation to the ventral longitudinal muscle in an area extending from the ventral midline to the black lateral stripe of the leech. It is a member of the central swimming oscillator network, (Poon, Friesen and Stent, 1978), i.e. one of the

Figure 52. Distribution of contacts between the SRN and the inhibitory motoneurone to the ventral longitudinal muscle. Whole mount preparation of a segmental ganglion in which the peripheral SRN soma and the central cel body of the inhibitory motoneurone to the ventral longitudinal muscle were injected with Horseradish peroxidase. The large axon of the SRN enters the segmental ganglion in the anterior nerve root and distributes branches within the neuropile ipsilateral to the SRN (see also figure 6). The ventral inhibitory motoneurone (I-1-v, Stuart, 1970) arbourises on both sides of the ganglion in a branching pattern distinct from that shown by either motoneurones 3 or 8 (see figures 49 and 51). Close apposition of the SRN and motoneurone branches occur at points within the neuropile ipsilateral to the SRN. The I-1-v motoneurone contacts the SRN on its axon at the base of the anterior nerve root (arrows) with a succession of fine processes. Anterior connective, top of plate. Scale bar 250μm.



intersegmental chain of neurones connected via reciprocal excitatory connections, which are capable of generating an endogenous fictive swimming rhythm. It is also considered to play a role in the mediation of proprioceptive input to the swimming rhythm (Kristan and Stent, 1976). Figure 52 shows that the ventral inhibitory motoneurone contacts the SRN on its axon at the base of the anterior root with a succession of fine processes (arrows). Close apposition of SRN and motoneurone branches occured at other points within this region. This ventral inhibitory motoneurone also exhibits the most extensive arborisation within the neuropile of any of the swimming motoneurones examined. The dendritic trees of both the SRN and the ventral inhibitory motoneurone show extensive overlap over the greater part of the neuropile ipsilateral to the SRN.

5.3 Paired recordings: SRN and identified segmental motoneurones

Paired recordings were made from the SRN and identified segmental motoneurones.The effect of SRN afferent input upon motoneurone activity was investigated by i), paired intracellular recordings from the peripheral SRN soma and the central cell bodies of identified segmental motoneurones. And ii), intracellular recording from the SRN soma whilst monitoring the extracellular activity from a segmental nerve root of a previously identified segmental motoneurone.

It has been shown that in response to changes in body wall length or tension of the leech the membrane potential of the SRN undergoes either a depolarisation or a hyperpolarisation depending upon the direction of the longitudinal displacement (Section 3.3). Similar analogue voltage changes were injected into the SRN cell body via the recording microelectrode to mimic the response of the SRN to changes in longitudinal muscle tension.

D.C. hyperpolarising or depolarising current pulses of between 0.5 and 1.0 nA in amplitude and 2.0 s - 0.5 min. in duration were injected into the peripheral SRN cell body which were sufficient to elicit voltage deflections in the cell body of between 10 and 20 mV.

The electrotonic length (L = fibre length/λ) of the SRN axon, a measure of the axon length in terms of units of its space constant (given the previously calculated value of λ=2.5-4.0 mm and the distance between the peripheral cell body and segmental ganglion as approximately 3-4 mm) ranged between 1.2 and 1.0. That is, the central synaptic region of the SRN may be as little as one length constant away from the peripheral SRN soma. At this distance therefore, the electrotonic potential will fall to 1/e of that recorded at the site of current injection. Potential changes therefore of between 3.7 and 7.4 mV were calculated to occur at the central synaptic zone of the SRN. It has been shown previously (section 3.2) that stretch-induced voltage deflections of similar amplitude may be reorded from the SRN axon near the ganglion.

Figure 53 shows paired intracellular recordings from the peripheral SRN soma and from the central cell body of the contralateral L motoneurone. In common with other motoneurones in an isolated ganglion preparation attached to segment of body wall, the L cell (bottom trace in each panel) showed spontaneous high frequency activity. In figure 53a a depolarising DC current was injected via the microelectrode into the SRN cell body sufficient to depolarise the SRN E_m by 18 mV and held for a duration of 7.0 sec. No apparent change in the E_m or action potential frequency of the L cell occured in response to SRN depolarisation. In fig. 53b an initial hyperpolarisation of the SRN E_m by 15 mV was followed by a further 10 mV hyperpolarising DC current and two subsequent depolarising DC current shifts to return the SRN to its initial E_m of -50 mV. Such changes in SRN E_m over a period of 10 sec did not produce any obvious change in the E_m or firing rate of the L cell.

In further experiments extracellular motoneurone activity was recorded from the segmental nerve roots and the effect of SRN intracellular depolarisation or hyperpolarisation investigated.

Of the previously identified segmental motoneurones involved in the swimming rhythm (section 5.2), the excitatory motoneurone to the dorsal longitudinal muscle (cell 3) extends an axon into the dorsal branch of the posterior nerve root (branch DP). The activity of motoneurone cell 3 in the dorsal nerve root was identified by a simultaneous extracellular recording from the dorsal root and an intracellular recording from the centrally situated cell body

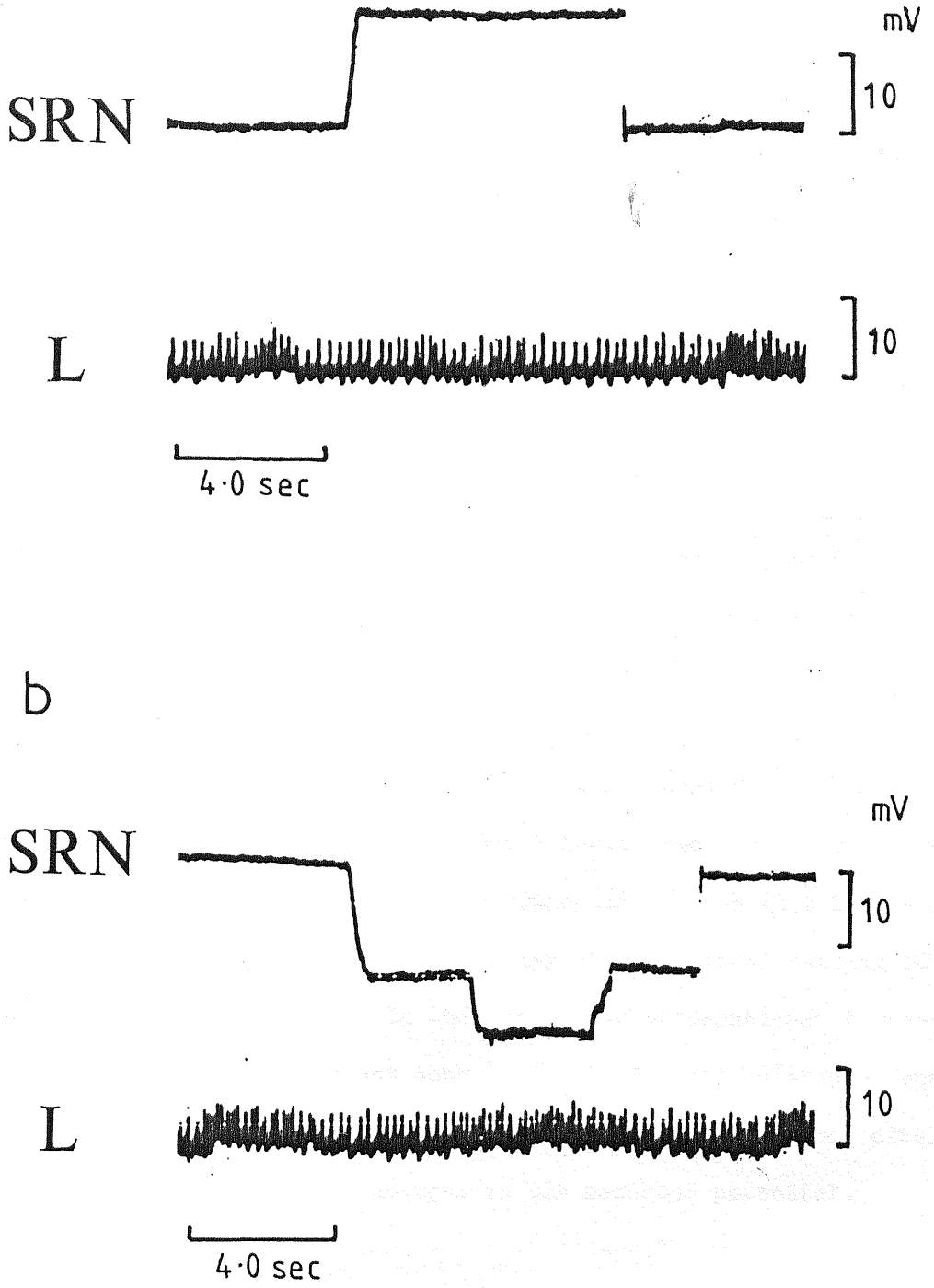


Figure 53. The effect of changes in SRN E_m upon intracellular recorded L motoneurone activity. a, Paired intracellular recordings were made from the peripheral SRN soma (SRN) and from the centrally situated cell body of the contralateral L motoneurone (L). Depolarising DC current (not shown) was injected via the microelectrode into the SRN soma, sufficient to depolarise the SRN E_m by approximately 18 mV and held for a duration of 7.0 seconds. No apparent change in E_m or spontaneous action potential frequency of the L cell was seen related to the SRN depolarisation. b, Continuation of the paired intracellular recordings from the preparation in a. An initial hyperpolarisation of the SRN bt 15 mV by injection of direct current into the SRN soma was followed by a further 10 mV hyperpolarising potential and two subsequent depolarising DC current shifts to return the SRN E_m to its initial E_m of -50 mV (SRN upper trace, current not shown). These changes in SRN E_m over a period of approximately 10 seconds did not evoke any obvoius change in E_m or firing frequency of the contralateral L cell (L, lower trace).

(figure 54). As shown previously the cell 3 extracellular spike was the largest recordable motoneurone spike in the DP nerve (Ort et.al. 1974). Such conspicuous extracellular activity therefore provided a convenient indicator of dorsal longitudinal muscle activity and a correlation was consequently sought between ventral SRN activity and that of cell 3.

The lower trace in figure 54b shows on a slower time base, a continuation of the extracellular record of cell 3 from figure 54a. The upper trace in this figure shows an intracellular record from the peripheral SRN cell body. From an initial E_m of -46 mV the SRN was hyperpolarised by approximately 20 mV for a period of 8.0 sec. The mean frequency of the extracellularly recorded motoneurone discharge over the period of the SRN hyperpolarisation (2.2 Hz) was lower than the mean discharge frequency at the initial resting SRN membrane potential (3.4 Hz). In this and other preparations however such an observation was not consistent and indistinguishable from moment to moment alterations in motonerone discharge frequency often observed in the abscence of changes in SRN membrane potential.

No obvious or consistent change was observed therefore, in either the intracellular or the extracellularly recorded motoneurone discharge frequency in response to hyperpolarisation or depolarisation of the SRN. In the present study, long hyperpolarising or depolarising DC current was injected into the peripheral SRN soma to mimic the changes in SRN membrane potential shown to occur when recording from the axon upon stretch of ventral

longitudinal muscle. The evoked changes in E_m of the SRN soma were considered to be of sufficient amplitude and the space constant of sufficient magnitude such that adequate changes in the membrane potential of the central presynaptic terminals of the SRN would occur and hence influence the rate of SRN transmitter release.

Since the intracellular recording point in the motoneurone cell bodies was at a considerable distance and therefore electrotonically remote from the presumed site of synaptic contact between the two cells, it is unlikely that individual ipsp or epsp synaptic events would be observed in response to SRN pre-synaptic input. The apparent inability however of the presynaptic SRN activity to influence extracellular motoneurone activity recorded from the nerve roots points perhaps to a more subtle relationship between stretch receptor activity and motoneurone response and the possible inadequacy of conventional analysis techniques under such circumstances. In order to overcome these difficulties a statistical analysis was carried out in collaboration with Dr's Rosenberg, Murray-Smith and Halliday. The degree of coherence of several motoneurone spike trains was assessed and the level of their dependence both upon each other and the SRN afferent input was determined. Preliminary results from this work are presented as Appendix 1.

APPENDIX I

Suggestions for further work: Partial coherence analysis the linear dependence of extracellular motoneurone activity amd $SRNE_m$

This work was carried out in collaboration with Dr's Rosenberg, Murray-Smith and D. Halliday. Coherence analysis, developoed by Brillinger et al. (1976) for tracing synaptic connections between neurones has been extended by Dr's Rosenberg and Murray-Smith to identify interactions between neuronal spike trains and analysis was performed with D. Halliday.

Statistical analysis of the extracellularly recorded motoneurone activity in response to SRN afferent input was carried out using methods of Brillinger, Bryant and Segundo (1976). In essence, the degree of coherence of activity between several motoneurone spike trains was assessed. Their coherence was used to give a measure of their dependence upon each other both in the presence or absence of the SRN afferent input.The coherence is a useful measure of the degree of relationship between any two spike trains A and B. A coherence of zero results if the two spike trains are independant. Using such a technique the degree of coherence between combinations of any pair of spike trains from motoneurones M_1 M_2 or M_3 in both

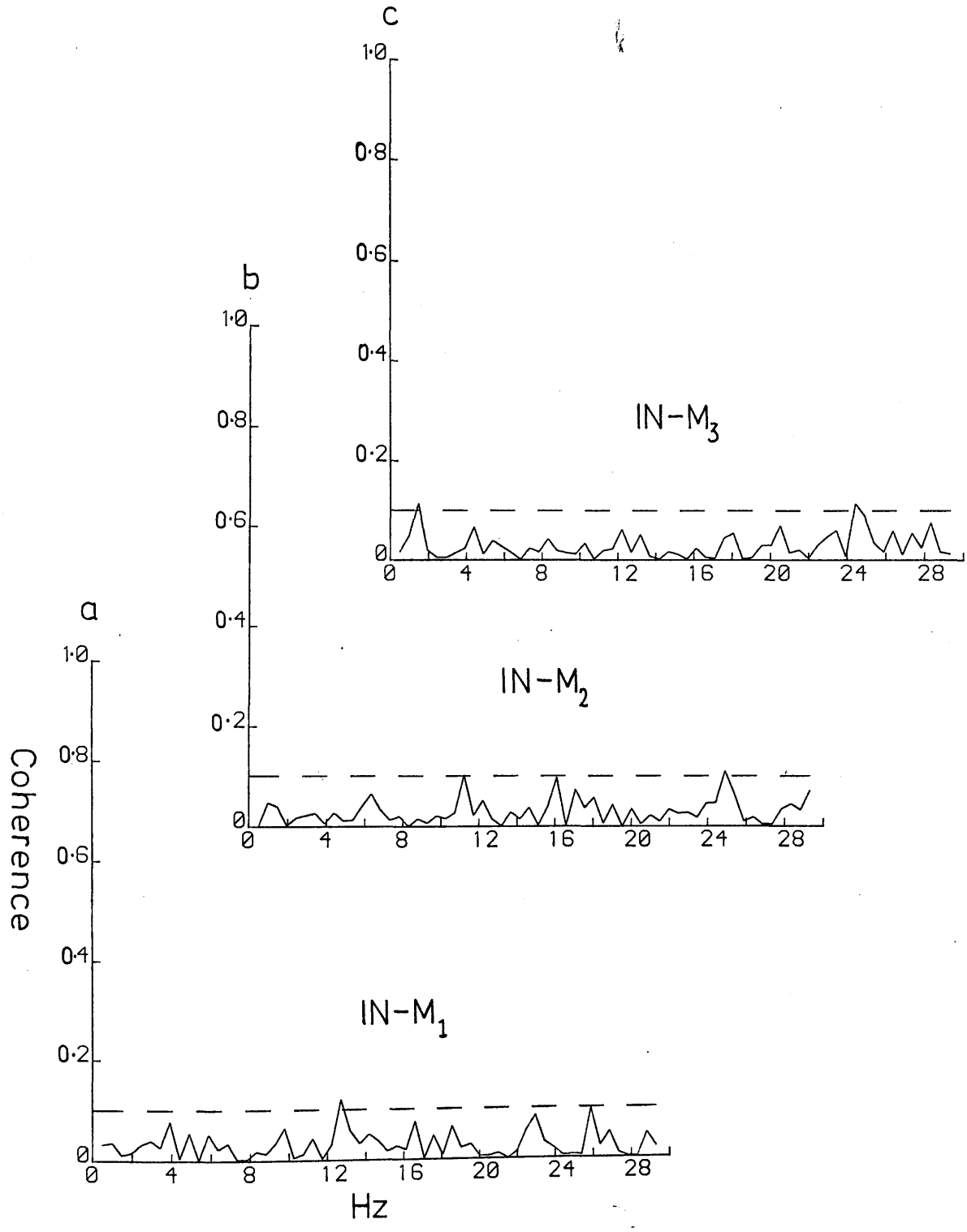
the presence and the absence of the SRN input IN may be calculated.

The statistical analysis is most easily carried out using a random pattern of afferent activity in the stretch receptor neurone. This was achieved by using the occurance in time of the particles generated by a low intensity gamma source. The output from a Geiger counter, placed adjacent to the gamma particle source, was used to trigger a sequence of depolarising or hyperpolarising square wave current pulses from an isolated stimulator. Each Geiger counter pulse generated a hyperpolarising or depolarising square current pulse of between 1.0 and 2.0 nA amplitude and 40 ms duration was injected into the SRN which resulted in either a positive or negative voltage deflection of the SRN E_m of between 20 and 30 mV.

The medial anterior nerve root (MA) was dissected from the body wall and sectionned distal to its branching point with the anterior anterior nerve root (AA, see fig.1). Spontaneous extracellular activity from several motoneurone units was subsequently monitored from the MA nerve root. In a particular experiment, three individual motoneurone spike trains, which differed in amplitude and frequency, were recorded on magnetic tape both prior to, and during random SRN input. These four activity trains (motoneurone spike trains M_1, M_2 and M_3 and the random input, IN) were used for coherence analysis.

Figure 55 shows the degree of coherence over a 30 second period between the random 40 ms. duration, 20 mV amplitude depolarising SRN pulses (IN) and each of the three individual spike trains M_1, M_2 and

Figure 55. The degree of coherence of activity between the SRN input and individual motoneurone spike trains. Each graph, a-c represents on the ordinate, the degree of coherence between input and output, ranging between 0 and 1. A coherence of zero results if the two activity trains are independant of each other. On the abscissae is plotted motoneurone spike frequeny (Hz). A 95% level of confidence above which a significal degree of coherence occurs is given by the dashed lines. Random, 40 ms duration, 20 mV amplitude depolarising SRN pulses (IN) were applied and the coherence of activity between this afferent input and each of three spontaneous motoneurone spike trains (M_1, M_2 and M_3) assesed. Over the range of motoneurone spiking frequencies observed (0-30 Hz) no significant peaks of coherence arose for any of the three motoneurones. The coherence of activity therefore betwen the SRN and any of the three individual motoneurones is not significant.


c
b
a
1·0
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0
IN-M3
IN-M2
IN-M1
0
4
8
12
16
20
24
28
Coherence
Hz

M_3. Each graph represents, on the ordinate, the degree of coherence from 0 to 1. The 95% level of confidence above which a significant degree of coherence occurs is given by the dashed line. On the abscissa is the motoneurone spike train frequency. It may be seen that at no motoneurone spiking frequency is there a significant degree of coherence between the SRN input and any of the three individual motoneurone spike trains. This reflects the lack of correlation between imposed changes in SRN E_m and the activity of individual motoneurones, recorded either intra- or extracellularly, (section 5.3). We may assume from this coherence data that it is unlikely that a direct connection exists from the SRN to the motoneurones whose activity was recorded in this experiment.

Figure 5.6 shows the coherence of activity measured between pairs of each of the three individual motoneurones during a 30 sec. control period in the absence of the SRN input (IN). No significant degree of coherence between any of the motoneurone spike trains is observed at any motoneurone spiking frequency. We may assume that in the absence of afferent SRN input spontaneous activity is present within each motoneurone, but that co-ordinated activity between individual units is absent. What then happens to the degree of coherence between motoneurone units in the presence of a random SRN input? Figure 5.7 shows the degree of coupling between the three motoneurone spike trains in the presence of the randomly applied SRN input. The data show the degree of coherence which exists between each pair of motoneurone units studied. Coherence of activity appears only over the lower motoneurone spiking frequency range (1-4 Hz). Moreover, the coherence appears to be greatest between M_3 and

Figure 56. The coherence of activity between mtoneurones in the absence of SRN input. Each graph, a-c represents the coherence (0-1, ordinates) at each motoneurone spiking frequency (0-30 Hz, abscissae). a, The degree of coherence of activity was measured between motneurone spike trains M_2 and M_3 during a 30 second control period in the absence of SRN input. A small degree of coherent activity between these two spontaneous motoneurone spike trains may be noted at a firing frequency of approximately 16 Hz. b, The degree of coherence of activity between spontaneous activity in motoneurones M_1 and M_3 over a 30 second period in the absecnce of SRN input. Low coherence of activity between spike trains at 9.0 Hz. c, The degree of coherence of activity between motoneurone spike trains M_1 and M_2 over a 30 second period in the absence of SRN input. Low degree of coherence over 17 and 22 Hz frequency range. It may be assumed that in the absence of SRN activity, spontaneous motoneurone activity is present but that no co-ordinated activity exists between individual units.

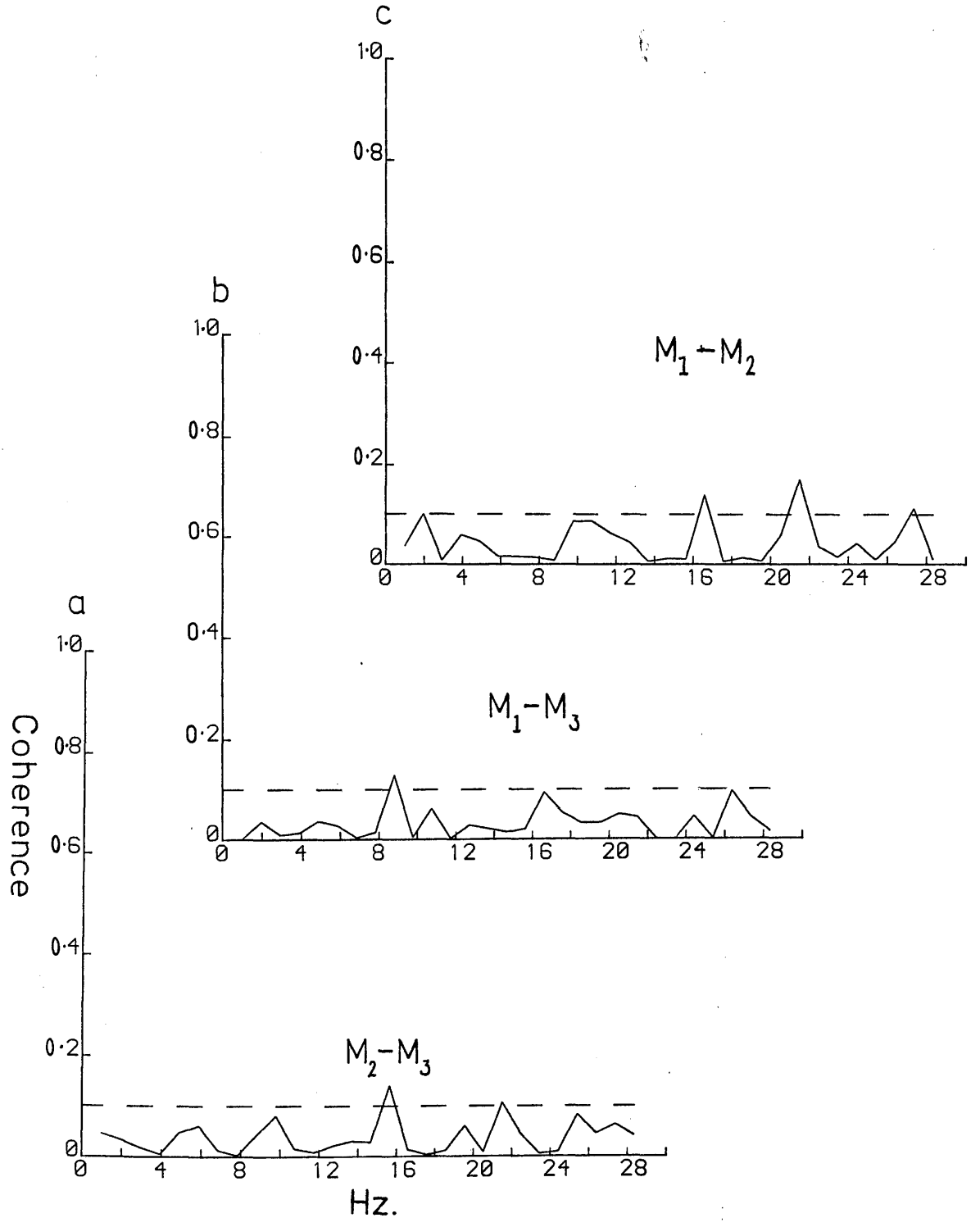
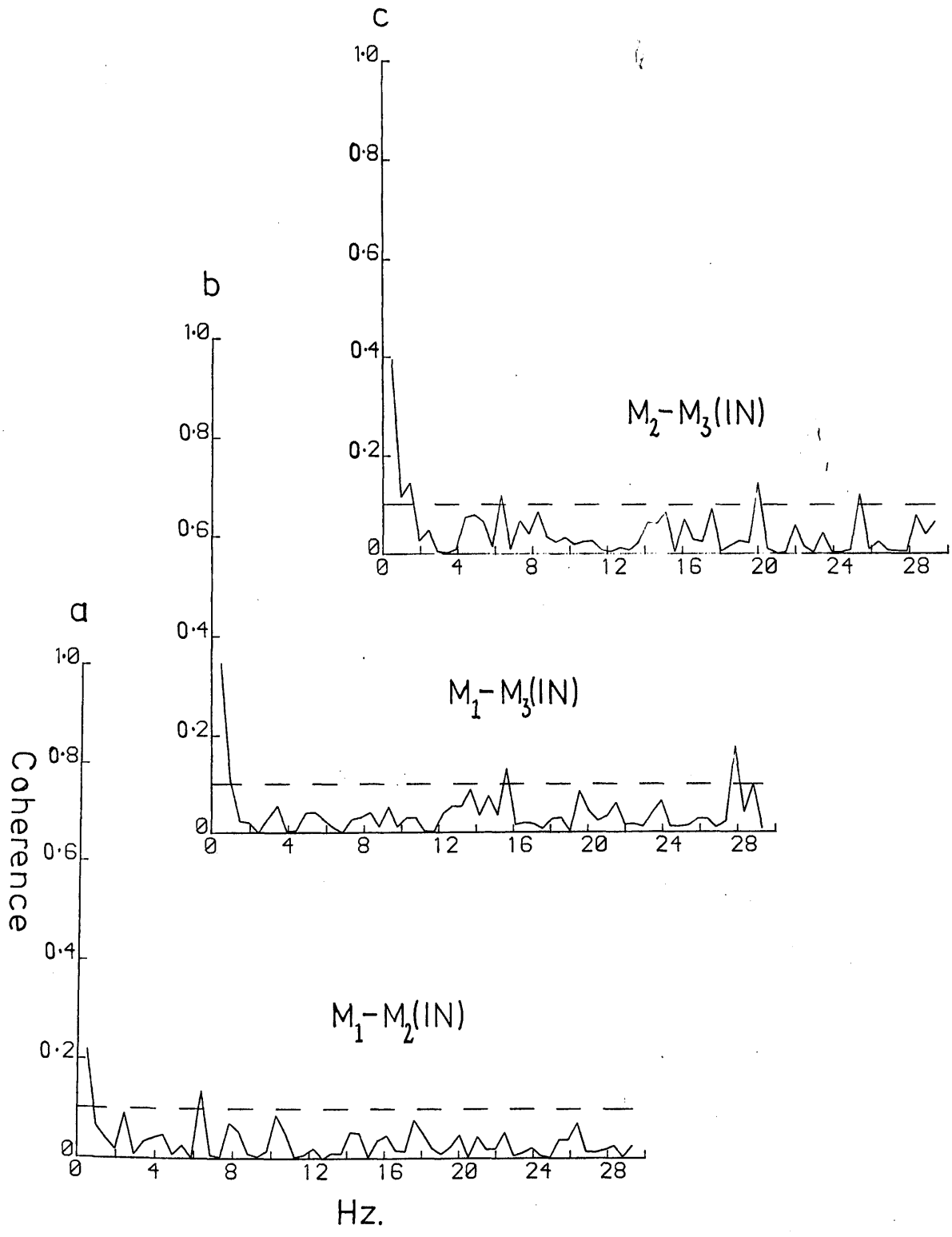

c
1·0
0·8
0·6
0·4
0·2
0
$M_1 - M_2$
0 4 8 12 16 20 24 28
b
1·0
0·8
0·6
0·4
0·2
0
$M_1 - M_3$
0 4 8 12 16 20 24 28
a
1·0
0·8
0·6
0·4
0·2
0
Coherence
$M_2 - M_3$
0 4 8 12 16 20 24 28
Hz.

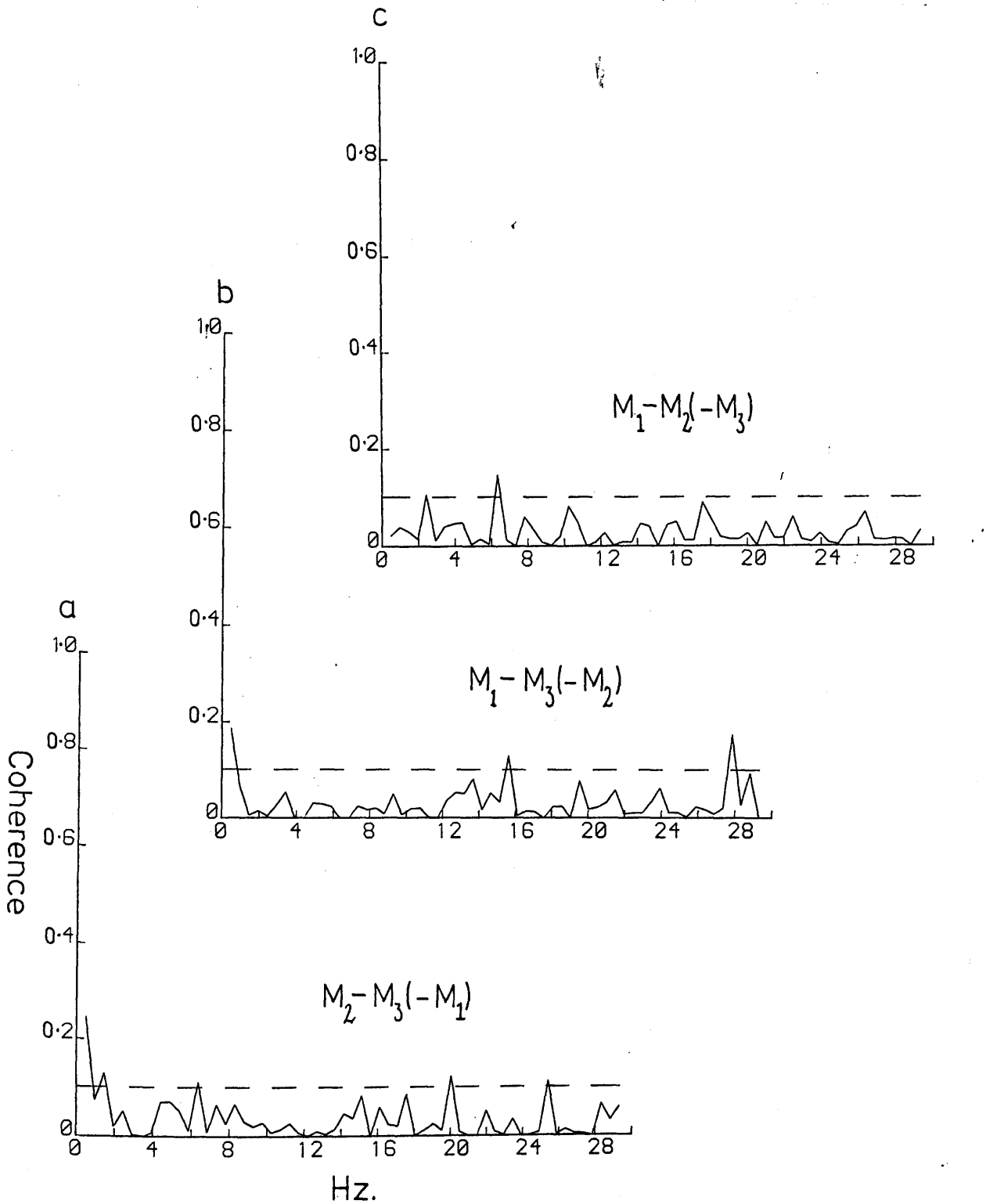
Figure 57. The coherence of activity between motoneurones in the presence of the SRN afferent input. a, the degree of coherence of activity between motoneurone spike trains M_1 and M_2 was determined over a 30 second period in the presence of a randomly applied, 40 ms duration, 20 mV amplitude square wave depolarising SRN pulse train (IN). In the presence of an SRN afferent signal and below a spiking frequency of 2 Hz the degree of coherence of activity between the two motoneurone units became statistically significant. b, Similarly, the degree of coherence of activity between motoneurone spike trains M_1 and M_3 was determined over a 30 second period in the presence of the SRN input. With the SRN inpout present a peak arose in the graph at a motoneurone spiking frequency of below 2 Hz indicating a significant degree of coherence between the two motoneurone activities at these low frequencies of firing.. A smaller peak indicated significant coherence of activity between the units at a frequency of approximately 28 Hz. c, The greatest degree of coherence between motoneurone spike trains in the presence of the SRN input was observed betwen motoneurone units M_2 and M_3 at a spiking frequency of below 2 Hz.


c
b
a
1·0
0·8
0·6
0·4
0·2
0
0
4
8
12
16
20
24
28
M2 – M3 (IN)
M1 – M3 (IN)
M1 – M2 (IN)
Coherence
Hz.

M_1 and between M_3 and M_2. The coherence between the activities of M_1 and M_2 is considerably weaker than between any of these two cells and M_3. This suggests that the coupling influence of the SRN input upon motoneurone activity may be mediated through activity within M_3.

This effect of M_3 may be further investigated by the technique of partial coherence analysis. Under such circumstances the degree of coherence between any two motoneurone spike trains in the presence of the input may be determined but with the influence of the activity of one of the three motoneurones statistically removed. The results of such a proceedure are shown in figure 58. This shows (fig 58a) the degree of coherence between spike trains M_2 and M_3 in the presence of IN but without spike train M_1 present, in such circumstances the coherence between spike trains at low frequencies remains significant. The degree of coherence also remains statistically significant between trains M_1 and M_3 in the presence of IN but the absence of M_2 (fig. 58b). In contrast figure 58c shows that when the influence of M_3 is subtracted out, the degree of coherence between M_1 and M_2 in the presence of IN is considerably reduced. It appears therefore that the coupling effect of the SRN input upon the three motoneurone spike trains is driven through one of these units, M_3, although as has been shown previously, the M_3 spike train and SRN input show no direct coherence of activity.

Figure 58. Partial coherence of activity between motoneurones in the presence of the SRN input. a, The degree of coherence of activity was determined between motoneurone spike trains M_2 and M_3 over a 30 second period in the presence of the SRN input but with the influence of motoneurone spike train M_1 statistically removed. In such circumstances the degree of coherence between the two units remained significant at frequencies below 2 Hz. b, The degree of coherence of activity between motoneurone spike trains M_1 and M_3 in the presence of the SRN input but with the influence of the activity of motoneurone spike train M_2 statistically removed. Again, coherence of activity remained significant below 2 Hz and at 28 Hz. c, In contrast upon statistically removing the influence of motoneurone spike train M_3, the degree of coherence of activity between motoneurone spike trains M_1 and M_2 in the presence of the SRN input is considerably reduced. This suggests that the coupling effect of the SRN input upon the three motoneurone spike trains is driven through motoneurone unit M_3.


c
1·0
0·8
0·6
0·4
0·2
0
$M_1 - M_2(-M_3)$
0 4 8 12 16 20 24 28
b
1·0
0·8
0·6
0·4
0·2
0
$M_1 - M_3(-M_2)$
0 4 8 12 16 20 24 28
a
1·0
0·8
0·6
0·4
0·2
0
Coherence
$M_2 - M_3(-M_1)$
0 4 8 12 16 20 24 28
Hz.

DISCUSSION

The present study has given the first description of an identified neurone in a soft-bodied invertebrate which responds to changes in length of the body wall muscles. The existence of sensory neurones capable of detecting stretch of the body wall of annelids had been previously inferred from the results of both behavioural (Gray and Lissman, 1938) and physiological experiments (Kristan and Stent, 1976; Kristan and Callbrese, 1976). Theoretically, their presence was invoked as part of proprioceptive feedback which could influence the centrally generated swimming rhythm (Kristan and Stent, 1976). It was only recently however that a suitable candidate family of peripheral neurones was discovered in the leech with a morphology highly suggestive of proprioceptive function (Blackshaw, 1981 ; Blackshaw et.al. 1982).

Before the end of the nineteenth century many beautiful and detailed light microscopic descriptions of the leech nervous system were available, (Retzius, 1891 ; Rohde, 1891). The nineteenth century studies described the general arrangement of the ventral nerve cord of the leech and showed that large numbers of axons of various diameters enter and leave the segmental ganglia. These studies were extended by the contemporary light and electron microscopic work of Coggeshall, Macagno and colleagues, (Coggeshall and Fawcett, 1964 ; Wilkinson and Coggeshall,1975 ;Macagno, 1980). The detailed work of Coggeshall (Wilkinson and Coggeshall, 1975) concentrated mainly on the discrepancy he noticed between the large number of small axons (20,000 +) associated with each of the ganglia and the far smaller number of cell bodies found within each ganglion, (about 400, Macagno, 1980). Little attention was paid to the small numbers of

large diameter axons in the peripheral nerve roots. Of the 1% of fibres in the nerve roots above 1.0 micron in diameter several of these were said to be "prominent" but the size and location of these large fibres was "not distinctive enough to allow these fibres to be individually identified from one animal to the next". Retzius (1891) had also illustrated large fibres, presumed sensory, entering the segmental ganglion and dividing in a T-shaped manner. It is only recently that the large diameter axons were shown to belong to peripheral neurones whose distinctive terminals are associated with the body wall muscle (Blackshaw et al 1982).

1.0 Morphology

1.1 No separate receptor muscle

In the leech there does not appear to be a special receptor muscle independant from the main mass of longitudinal muscle fibres. The proximal and distal dendrites of the SRN lie in series with the longitudinal muscle fibres of the body wall each associated with separate bundles of longitudinal muscle fibres. This is in contrast to most other muscle receptor organs hitherto reported (Kuhne, 1863 Alexandrovicz, 1951, 1952a, 1952b, 1956, 1958 ; Alexandrovicz and Whitear, 1957 ; Pilgrim, 1960 ; Finlayson and Lowenstein, 1955 Osborne and Finlayson, 1962). Even in those insects which have stretch sensitive neurones which are not associated with a

specialised receptor muscle, the stretch sensitive elements are generally found in association with connective tissue strands, slung between points of articulation of the exoskeleton, as for example in the dragonfly larva, the bee and the cockroach, (Finlayson and Lowenstein, 1955).

The possibilty remains however that the longitudinal muscle fibre bundles associated with the SRN dendrites may have some degree of separate motor innervation from the rest of the longitudinal muscle fibres. The area of ventral longitudinal muscle which encompasses those fibres associated with the SRN dendrites is known to be innervated by four excitatory and one inhibitory motoneurone (Stuart, 1970 ; Ort et.al 1974) whose territories widely overlap. Over the region where the territories of the motoneurones overlap therefore, individual muscle fibres can receive innervation from up to four excitatory and one inhibitory motoneurone. The degree of individual control or amount of separate innervation the longitudinal muscle associated with the SRN dendrites may receive remains to be investigated.

1.2 Central morphology

The projection of the SRN into the CNS, revealed by the injection of HRP and LY into the peripheral cell body, showed that as with other identified sensory cells of specific modality in the leech, the central arborisation of the SRN has a distinctive morphology

recognisable from ganglion to ganglion and from animal to animal. That neurones of specific modality have a characteristic morphology is a property common to other invertebrates (Frazer et.al. 1967). This is also true of many mammalian primary sensory fibres, such as the flame-like central arbours of hair follicle afferents in lamina III of the cat spinal cord (Brown et.al. 1977), and the characteristic inverted V-shaped outline of Ia afferent collaterals through laminae VI, VII and IX (Brown and Fyffe, 1978).

The central branching of the SRN extends solely within its own segmental ganglion, no branches were seen extending across the midline or into either of the neighbouring connectives. This is in direct contrast to other primary sensory neurones in the leech which distribute processes in the connectives to nearby ganglia (Yau, 1976), and are therefore intersegmental. Such intersegmental distribution is also the case with many vertebrate sensory neurones, for example, cat muscle spindle afferents enter the spinal cord and divide to distribute collaterals rostrally and caudally over several segments (Brown and Fyffe, 1978). That proprioceptive information in annelids is distributed in a widespread and meaningful way has been shown in physiological experiments in both the earthworm and the leech (Gray at al. 1938 ; Kristan and Stent, 1976). The distribution of afferent information from the SRN to neighbouring segments is therefore presumably dependant upon second order neurones.

2.0 Electrical Properties

2.1 Membrane potential

The membrane potential of the SRN varied from preparation to preparation. This variation in E_m was not thought to represent any damage or penetration artifact of the cell but to reflect the dependance of SRN E_m upon ventral longitudinal muscle length. This is clearly demonstrated in fig. 27, results section 3.3. Here step changes in longitudinal muscle length produce concomitant changes in the SRN E_m recorded from the axon. In order to visualise and impale the SRN cell body in the periphery it was necessary to apply a cetrain amount of stretch to the longitudinal muscle which supported the SRN dendrites and cell body. Under these circumstances the recorded transmembrane potential was approximately -50mV to -60mV. This is in contrast to the values of between -30mV to -40mV obtained when recordings were made from the axon when no stretch was applied to the longitudinal muscle.

2.2 Action potential

An overshooting action potential could be evoked from the SRN upon passing 3-4 nA of depolarising current into the peripheral cell body. An AP could not however be elicited upon injecting current of either polarity into the large SRN axon. An asymmetrical

distribution of ion channels along the neurone is therefore apparent. Contrary to the "classical" picture of the neurone, the voltage dependant channels responsible for the action potential are absent from the SRN axon.

An action potential elicited from the soma upon injection of depolarising current is transmitted passively along the axon (fig. 11). Its amplitude and width are distorted in relation to the space and time constants of the membrane. The sub-threshold responses of the cell are similarly transmitted along the axon in relation to the membrane electrical constants. However the sub-threshold and action potential are not attenuated to the same degree (fig. 11). As might be expected from the period necessary for complete charging of the membrane capacitance, which in the case of the SRN whose time constant is large (25 ms) may be considerable, for a signal of brief duration the effective space constant is shortened. This is in good qualitative agreement with the theoretical result expected for passive propagation of a transient response along a cable (Jack, Noble and Tsien, 1975). In the present case the amplitude of the all-or-nothing signal recorded from the axon had fallen to 25% that recorded some 4mm away in the cell body. For sub-threshold currents on the other hand, spread of the steady-state potential along the axon is affected only by the space constant of the cell and not by the membrane capacitance. Hence at the same distance along the axon the sub-threshold potential had fallen to only 36% of that recorded in the cell body. Such observations are in good agreement with the differences seen by Mirolli (1979) in the passive propagation along the axon of the fast and slow components of the response of Scylla S

fibres to a strong depolarising current.

Like the centrally situated T, P and N cutaneous mechanosensory neurones each of which has a characteristic shape of action potential (Nicholls and Baylor, 1968), the SRN somatic action potential produced in response to a square wave depolarising current pulse is distinctive and characteristic of the cell. In response to a long depolarising current pulse a single AP was most frequently observed followed by an isoelectric plateau potential maintained for the duration of the current pulse. In the other leech primary sensory neurones an after-hyperpolarisation (a.h.p.), thought to be generated by the delayed (outward) rectifying K current, is often pronounced following an action potential. No evidence of an after-hyperpolarisation (a.h.p) was observed in the SRN. On occasion in response to a large (>10 nA) depolarising current pulse, repetetive activity could be evoked from the SRN soma. This was characterised by a marked reduction in the amplitudes of the second and subsequent spikes compared to the first and a gradual increase in the interspike intervals (fig. 9). A similar firing pattern is observed in neo-natal rat motoneurones when the cells are activated with long depolarising current pulses and tonic firing initiated (Fulton and Walton, 1986). In these circumstances the authors suggested that the decay in AP amplitude resulted from the current shunt produced by the conductance underlying the a.h.p of the AP. This was not the situation in the present case however where an a.h.p following the somatic SRN action potential was absent. Instead the slower rise times and reduced amplitudes of the second and subsequent action potentials points largely to a slow inactivation

of the channels responsible for the action potential. The reliability with which an anodal break spike was elicited from the SRN soma presumably reflects the de-inactivation of fast channels responsible for the spike potential upon membrane hyperpolarisation.

2.3 TTX insensitivity.

The insensitivity of the action potential to micromolar amounts of TTX raises the question of the identity of the ionic species responsible for the SRN somatic action potential. In other preparations where TTX insensitivity of the action potential has been shown, calcium rather than sodium ions in fact carry the current (Geduldig and Junge, 1968). In many crustacean and molluscan neurones the action potentials are mediated by calcium rather than sodium (ref. ibid.), and it is now well established that Ca action potentials occur in the dendrites and somata of a variety of vertebrate central neurones (Llinas and Sugimori, 1980 ; Llinas and Yarom, 1981 ; Fulton and Walton, 1981). In the leech it has been shown that the amplitude of the overshooting action potentials from the cutaneous mechanosensory neurones depend on external sodium concentration (Nicholls and Kuffler, 1964), as in the squid giant axon (Hodgkin and Katz,1949). Quantitative differences occur however between the leech and the classical squid preparations in the relationship of the action potential amplitude and external Na concentration. In the squid nerve, replacement of seawater with increasing amounts of isotonic dextrose solution results in a

progressive decline in the amplitude of the action potential such that in a solution containing 20% seawater the action potential fell to 24% of that recorded in 100% seawater. In the leech mechanosensory cells however, the spike size fell to only 63% of its original size when external Na was replaced to 10% and was completely abolished in 0% Na. Kleinhaus and Prichard (1976) have shown that the maximum rate of depolarisation of the AP of the leech Retzius cell is also directly dependant upon the external Na concentration and that this AP is remarkably insensitive to TTX. The sensitivity of the rate of depolarisation of the AP to TTX was far lower than to changes in external Na. The dependance of the action potentials of the leech T,P and N cells upon external Na was also confirmed by Kleinhaus and Prichard (1983). The sensitivity of the AP of the mechanosensory neurones to 50 and 15 micromolar TTX was far higher however than that shown for the AP of the Retzius cell. The principle change noticed was a decline in the maximum rate of depolarisation of the T, P and N AP's which was reduced in a dose dependant manner. These results are in contrast with the observations made in the present study where no effect was seen of TTX upon the AP's of either of the mechanosensory neurones or the SRN (fig 10). In the present study a lower TTX concentration was used (0.3 micromolar) which may be at the lower end of the curve of the reported dose-dependancy of the AP's for TTX. Higher concentrations were not tested. Recently it has been shown that a 0.03 micromolar TTX concentration will reduce the normally high resting Na conductance present in the Retzius cell but not the Na conductance present during spike electrogenesis (Beleslin, 1985). Hence it appears that absolute TTX concentration is critical.

Whether or not the SRN action potential exhibits the same Na dependancy as that shown by the other leech neurones remains to be investigated. However the phenomena exhibited in the leech, of Na channels which give rise to spiking activity and which are insensitive to TTX poisoning is not in itself a surprising fact ; many such examples are known to exist. These include the Na channels present in the tunicate egg cell membrane (Hagiwara and Jaffe, 1979) ; a large part (approx. 40%) of the Na current responsible for the pure Na spike in tissue cultured adult mammalian dorsal root ganglion neurones (Fukuda and Kameyama, 1980) ; and a TTX resistant Na component of the action potential has also been shown in other primary sensory neurones of the adult cat (Gallego, 1983). TTX insensitive Na channels have been reported in denervated rat EDL muscle fibres (Redfern and Thesleff, 1971). This TTX insensitivity develops within 2-3 days of denervation and may be reversed upon re-inervation of the muscle. In denervated muscle fibres 10 micromolar TTX failed to abolish the regenerative response, although the rate of rise was reduced and the amplitude reduced by approx. 15mV. In denervated muscle there is little doubt that the TTX resistant currents are carried by Na since they are abolished by the replacement of external Na with an isotonic mixture of calcium and sucrose (Redfern and Thesleff, 1971). The authors suggested that this resistance to the blocking effect of TTX reflected the appearance in the membrane of a second type of Na channel, either from de-novo synthesis or from modification of existing channels. Pappone (1980) subsequently showed that two types of sodium channel existed in denervated skeletal muscle which differed in their slower kinetics and reduced sensitivity to TTX.

The depolarisation evoked AP of the SRN is similar to that elicited from the T fibre of the crustacean Thoraco-coxal muacle receptor organ upon intracellular injection of a large depolarising current pulse (Roberts and Bush, 1971 ; Blight and Llinas, 1980). Above a certain threshold of depolarisation the crustacean T fibre exhibits a transient spike-like event superimposed upon the passive membrane response which is graded according to the current intensity. The ionic basis of this T fibre spike component recorded peripherally from the afferent fibres of Carcinus was investigated by Roberts and Bush (1971). They showed that a 0.3 micromolar TTX concentration reduced the depolarisation transient by approximately 50%. In contrast however it was found in another crustacean, Callinectes that micromolar concentrations of TTX which blocked the motoneurone spiking in the animal, had little or no effect upon the T fibre transient spike which was recorded from the proximal synaptic zone of the T fibre (Blight and Llinas, 1980). It must be stressed here that the experiments of Blight and Llinas were concerned only with the proximal segment of the crustacean T fibre and it appears that its membrane properties differ significantly from those of the peripheral fibre nearer the receptor ending. Thus Blight and Llinas (1980) showed that perfusion of the preparation with saline in which calcium ions had been replaced with cadmium caused a significant reduction in the amplitude of the depolarisation transient recorded from the proximal T fibre segment. The most probable explanation offered was that the T fibre depolarisation transient represented an increased membrane conductance primarily for calcium, although the possibility remained that the movement of sodium ions could also provide a significant

contribution to the spike. Similar TTX insensitive transients have been described for non-spiking secretory cells in the lobster and slug (Graubard, 1978 ;Kater, 1977). The evidence from the crustacean T fibre suggests therefore that voltage dependent sodium and calcium channels are non-uniformly distributed along the length of the fibres. The peripheral part of the neurone near to the receptor ending is characterised by its sodium dependent responses to stretch and a depolarisation transient which is suseptible tp TTX poisoning (Roberts and Bush, 1971). The proximal region of the T fibre meanwhile exhibits a depolarisation transient which most likely represents an increased membrane conductance primarily for calcium (Blight and Llinas, 1980). Both the central graded calcium spike and the peripheral graded sodium spike may have roles connected with the response properties and transmitter characteristics of the crustacean receptors. In the leech the peripheral TTX insensitive spike is a localised event which may play an important role in the response of the cell to phasic stimuli or in overcoming some of the limitations imposed by the lack of a regenerative impulse.

2.4 Other membrane characterisics

The values calculated in the present study for the membrane constants of the SRN are in good agreement with the properties required for the passive propagation of slow graded changes in membrane potential over long distances.

The estimate of the length constant in these fibres (approx. 4.0 mm) was based on the decrement of steady state potential between two points along the SRN axon following the injection of a hyperpolarising current pulse into the SRN soma. Calculation of the length constant was however complicated by the presence of the proximal fan between the point of current injection (cell body) and the second recording point near the ganglion (see fig. 14). The large surface area of the fan and the additional large area of membrane provided by the mushroom-like outgrowths sitting on its surface undoubtedly acted as a large current $\pi \sin k\pi$ to reduce the apparent measured length constant and it is likely that the value for the axon length constant is an underestimate. The value for the length constant obtained for the ventral SRN indicates that the central synaptic zone of the cell may be as little as 1 space constant away from the peripheral transducing region. A comparable value of 4.9 mm was obtained for the length constant of the photoreceptor cell of the lateral ocellar eye of the barnacle (Shaw, 1972). These large diameter axons (10-20 microns) behave as passive

cables and transmit small analogue voltage changes over distances of approximately 11 mm to the CNS in response to illumination of the ocellus. Both values for leech SRN and barnacle photoreceptor are small however when compared to the value of 5.8 cm calculated by Mirolli (1979) for the space constant of the S fibre of Scylla. Undoubtedly one of the components contributing to the very large Scylla length constant is their large diameter (60-100 microns). However the length constant values of the SRN, barnacle photoreceptors and Scylla S fibres are all still relatively large when compared to the values for the giant fibres of the cephalopods. The axons of the squid Sepia, which are as large as 200 microns in diameter, have a length constant of up to 1.0 cm (Weidman, 1951). The length constant of the axons of the crayfish spiking stretch receptor neurone, 18 microns in diameter, was estimated at only 0.85 mm (Mellon and Kaars, 1974).

A further adaptation of the SRN, barnacle photoreceptor and crustacean coxal receptors for the transmission of graded signals is the high specific membrane resistance (R_m) of their axons. The value of R_m calculated for the SRN from the infinite cable model and assuming current flow in both directions from the point of injection was 22.0 Kohm cm^2. This value is smaller than those calculated for Scylla (Mirolli, 1979) which ranged between 250.0 Kohm and 760.0 Kohm cm^2 depending on the value used for specific axoplasmic resistance, R_i. The value of R_m for the SRN is considerably higher however than those of previously studied spiking neurones, eg. 5.0 Kohm cm^2 for a 30 micron diameter crab leg nerve fibre (Hodgkin and Rushton, 1946), and 9.0 Kohm cm^2 for squid giant axons (Weidman,

1951). In the barnacle, whose photoreceptor axon are long and relatively thin (7.8-10.9 mm by 10-20 microns) loss of signal amplitude is minimised by a somewhat higher axon specific membrane resistance. An estimate of 170,000 ohm cm^2 was given by Shaw (1972), using a value for internal axoplasmic resistance, R_i, of 270 ohm cm.

2.5 Distal axon properties.

It was characteristic of recordings made in the axon to observe small amplitude transient events superimposed upon the recorded SRN transmembrane potential. Three possibilities existed for the origin of such activity (i), somatic action potentials initiated in the periphery and transmitted decrementally to the axon recording point (ii), dendritic spiking originating in the central arbour of the SRN (iii), synaptic activity. Simultaneous recording of activity within the SRN soma and axon showed that spontaneous spiking activity was never recorded from the SRN soma even when the transients were present in the axon. The events were not therefore thought to be somatic spikes transmitted decrementally along the axon. The possibility remains however that the events recorded were due to dendritic spiking activity generated within the central arbour of the SRN. Both Na and Ca evoked spiking activity has been reported from the dendritic trees of a number of neuronal types (Wong, Prince and Basbaum, 1979 ; Llinas, Sugimori and Walton, 1977 ; Murase and Randic, 1983). Observation of the transient activity however at high sweep speeds (fig. 15) indicates features very

characteristic of post-synaptic activity, namely, summation of psp's was often observed (cf. fig. 15b), and they had a fast rise to peak and slow exponential decay. The events were also blocked by Mg^{++} concentrations known to block central chemical synapses in the leech (Baylor and Nicholls, 1969). In concluding that the spontaneous small amplitude transient activity was synaptic in nature, the question of the identity of the pre-synaptic element(s) remains. Evidence was presented (figs. 17a, 17b), that the frequency of the psp activity, recorded from the SRN axon, was dependent upon the level of depolarisation of the SRN. Figure 17a shows that at the E_m recorded of -40mV the psp activity recorded from the SRN axon was infrequent, but increased in frequency (and characteristically decreased in amplitude) as the SRN E_m was moved to more depolarised levels. A possible explanation of this phenomenon is that the activity of unidentified neurone(s), presynaptic to the SRN, is controlled by the level of depolarisation of the SRN terminal itself. If regions of the SRN terminal or the axon itself were reciprocally pre-synaptic to the unidentified pre-synaptic elements, then the activity of such pre-synaptic terminals could be effectively influenced by the SRN E_m.

Alternatively, it is now well established that the integrative properties of many neurones are strongly affected by the electrical properties of their soma-dendritic membranes. For example contrasting electrophysiological properties have been observed between the somatic and dendritic regions of mammalian cerebellar Purkinje cells (Llinas and Sugimori, 1980a,b). Evidence from mammalian inferior olivary cells has also shown that changes in

their resting membrane potential level may result in the de-inactivation of ionic conductances which are otherwise inactive at the resting E_m level and hence result in a completely different pattern of electrical activity upon synaptic or electrical stimulation of the cell (Llinas and Yarom, 1981). The possibility in the present study that depolarisation of the SRN E_m activates a high threshold conductance, normally inactive at resting membrane potential levels and responsible for the mediation of the post-synaptic potentials, remains to be tested.

3.0 Response characteristics

As mentioned ealier (Literature review) previous studies have described spiking activity in response to stretch of the body wall in various soft- bodied invertebrates (Gray and Lissman, 1938 ;Gray, Lissman and Pumphrey, 1938; Laverack, 1969; Smith and Page, 1974; Kristan and Stent, 1976; Drewes and Fourtner, 1976). Few of these early studies however made suitable distinction between afferent input which arose from cutaneous mechanoreceptors, activated upon body wall distortion, or that input which arose purely as a result of changes in length of underlying muscle and which was therefore purely proprioceptive in content. In none of the previous studies was the activity or morphology of a specific receptor type described. Laverack (1969) recorded slowly adapting spiking activity from the segmental nerve roots of the leech Hirudo in response to sinusoidal indentation of the body wall. He showed that this mechanoreceptive information was transmitted along the fast through-conducting pathway present in Faivres nerve of the leech, the S cell (Frank, Jansen and Rinvik, 1975). This pathway is known to mediate the rapid body shortening which follows a mechanical stimulus to the body wall (Magni and Pellegrino, 1978). Stretch receptors thought to lie in the ganglionic sheath have been reported to also excite this fast conducting pathway (Smith and Page, 1974). Smith and Page were unable to evoke S cell discharge upon light tactile stimulation of the skin or by direct intracellular

activation of any of the T,P or N cells present in the CNS of Haemopis. A series of sequential dissection experiments then led the investigators to conclude that "tactile stimulation of the ganglionic sheath readily elicited large fibre responses". These ganglionic sheath receptors were considered sensitive enough to respond to tactile simulation of the skin of an intact segment. Stretch-sensitive receptors lying in close association with the CNS have however also been reported in the abdominal connectives of the crayfish (Kennedy et.al. 1966), and in vertebrates in the lateral edge of the white matter in the lamprey spinal cord, the so-called edge cells (Grillner, Williams and Lagerback, 1984).

That the S-fibre system and the T mechanoreceptor cells are synaptically linked (albeit indirectly) and that the former does receive mechanosensory information from the T cells has since been documented (Muller and Scott, 1981; Bagnoli et.al., 1975; Magni and Pellegrino, 1978). It is also now known that upon natural stimulation of the T cell, electrical transmission through an intervening interneurone to the S cell may be reduced when impulse conduction is blocked presynaptically at certain branch points of the T cell, (Yau, 1976; Muller and Scott, 1981). Conduction block may therefore explain the failure of Smith and Page to elicit an S cell discharge in response to T cell excitation.

In the earthworm, convincing evidence for the existence of stretch sensitve units in the body wall has been obtained (Drewes and Fourtner, 1976). In this study, phasic spiking activity was

recorded from the segmental nerve roots in response to low amplitude siusoidal stretching of the body wall. This activity was shown to be distinct from that recorded from the same nerve roots but evoked upon activation of tactile sensitive units by light touch or brushing of the cuticle.

3.1 Decremental analogue signalling.

Graded, decrementally conducted signals are not new, many primary sensory neurones in both vertebrates and invertebrates are known to transmit afferent information in a non-impulsive, decrementally conducted manner (Ripley, Bush and Roberts, 1968; Werblin and Dowling, 1969; Shaw,1972; Hudspeth and Corey, 1977), and many synaptic interactions are now known to occur betwen central neurones without the generation of all-or-nothing impulses (Dowling and Boycott, 1966 Ralston, 1968a.b.; King, 1976; Hudspeth and Corey, 1977). In the leech the reciprocal inhibitory synaptic interactions between the excitatory and inhibitory motoneurones to the longitudinal muscle are now also known to be mediated primarily by the graded depolarisation of the presynaptic terminal without the necessity for an action potential (Granzow et.al. 1985). Of the previously described decrementally conducting primary afferent neurones however, only one other group i.e. the crustacean thoracic-coxal muscle receptor organ is proprioceptive in function. The leech SRN shares many of its response features with the S and T fibres of the crustacean TCMRO. The response of all of these primary

afferent fibres to a ramp stretch stimulus consists of a decrementally conducted DC potential which is maintained for the duration of the ramp length change and is proportional to the final amplitude of that ramp length change. All three receptors show both phasic and tonic characteristics and hence all cells signal both rate of displacement as well as the absolute change of length.

The "spiky" component of the response of the SRN to an increase in longitudinal muscle length which was generally superimposed upon the phasic "hump" and distinct from the spontaneous synaptic activity (figs. 24,25,and 26), is thought to be the decrementally conducted cell body spike. This spike was only present during the phasic portion of the response and the possible functional significance of this transient will be considered later (Discussion, section4.0). This active response of the SRN is probably similar to the variable "spiky" alpha component of the crustacean T-fibre response to stretch and in its response to injection of depolarising current described by Bush and colleagues (Roberts and Bush,1971 Bush, 1981).

The presence of two distinct sensory neurones in the crustacean TCMRO complex, the S and T fibre, has allowed their independant morphological and fuctional specialisation. The T fibre shows a greater dynamic sensitivity whilst the S fibre shows a greater static response to a ramp length change. It is known that there are other peripheral neurones innervating the ventral longitudinal muscle of the leech body wall (Blackshaw et al. 1982). It is not

known however whether any of these other neurones are specialised to signal particular components of the stretch stimulus. The leech ventral SRN studied is however capable of signalling at least two components present in a ramp stretch or ramp release stimulus, i) a static analogue response of the SRN E_m dependent upon the final magnitude of the ramp length change and ii) a dynamic response in which the rate of change of the SRN E_m is dependent upon the rate of change of length of the longitudinal muscle, (fig. 26a,b). In the insect also, a single stretch receptor neurone signals three different components present in the stretch stimulus (Weevers, 1966a). In the leech a limited number of identified centrally located mechanosensory neurones are known to be responsible for conveying most, if not all, mechanosensory information to the CNS (Nicholls and Baylor,1968) Similarly a limited number of motoneurones are known to supply both exciatory and inhibitory innervation to the body wall (Stuart, 1970). In such a system therefore in which a limited number of channels are available, it is of considerable advantage that a single afferent neurone, responsive to stretch of the body wall, is capable of signalling a number of components present in the stretch stimulus.

3.2 Biphasic response of the SRN

One of the most distinctive features of the SRN response is its biphasic nature. Furthermore the moment to moment transmembrane potential recorded from the SRN depends on the length of the

longitudinal muscle in which the dendrites of the receptor neurone are buried. The polarity of the membrane potential change produced by distortion of the body wall is dependent upon the direction of the length change. The response of the SRN to an imposed stretch therefore consists of a hyperpolarising potential whilst the response to an imposed release is a depolarising potential. It seems from a close examination of figures from the work of Bush (Bush, 1981), that the S fibre of the crustacean TCMRO also shows similar biphasic response characteristics. Such biphasic responses of the S fibre are of opposite polarity however, stretch of the receptor muscle results in a depolarising potential in the S fibre. The biphasic response of the leech SRN is similar to that observed in vertebrate hair cells in response to direct stimulation of the stereocilia (Hudspeth and Corey, 1977).

How is the decrementally transmitted receptor potential generated in the SRN ? Evidence exists from the crustacean non-impulsive mechanoreceptors that sensory transduction occurs as a result of increased ionic conductance across the membrane of the stressed dendritic terminals (Mirolli, 1979). Also the input resistance of S and T fibres is decreased by stretch (Mirolli, 1979 ; Bush, 1981). Correlated morphological work has shown that changes in shape and dimensions occur in the fine (>1.0μm) terminal dendritic fingers of the crustacean S fibres in response to stretch (Krauhs and Mirolli, 1975). In the acoustico-lateralis system deflection of the sensory hair bundle of the vertebrate hair cell in the excitatory direction is associated with a conduction increase, whilst movement in the opposite direction results in a conduction decrease and subsequent

hyperpolarisation of the hair cell E_m (Hudspeth and Corey, 1977).The input resistance of the SRN was not monitored during longitudinal muscle stretch. The variation however found in SRN input resistance from one preparation to the next, (14.2 Meg Ohm - 26.9 Meg Ohm) may well be indicative of the varying degrees of stretch under which the neurone was held during each experiment and hence the underlying variation in membrane conductance.

4.0 Transmission at non-spiking synapses

Considerable interest has also been focused upon the mechanism of synaptic transmission at non-spiking tonic synapses. A detailed study of the transmitter release and subsequent post-synaptic activity at such a tonic sensory-motor synapse has recently been carried out for the crustacean T fibre to promotor motoneurone synapse (Blight and Llinas, 1980). For the normal functioning of this synapse the pre-synaptic membrane potential is held above threshold for transmitter release. A resting discharge then exists in the tonic motoneurone units. Small changes in membrane potential produced by muscle stretch or relaxation therefore strongly modulate the firing of the tonic motoneurones. Whether the leech SRN-motoneurone synapses function in a similar way is unknown. The necessity however in both crustacea and soft-bodied annelids of constantly signalling body wall shape and posture may well point to a common and invariant mechanism.

5.0 The response of the SRN in relation to natural movement of the leech

In the leech as in many soft-bodied animals which possess a hydrostatic skeleton there is no well defined resting length of the body wall. The muscles act in conjunction with the hydrostaitic skeleton to bring about changes in shape and to perform its various locomotory activities. The leech has a highly extensible body wall capable of considerable three-dimensional shape changes and it has a number of locomotory behaviours (see Sawyer in Muller, Nicholls and Stent, 1981). In addition it undergoes a characteristic "shortening reflex" in response to activation of cutaneous sensory receptors. One gait of the animal, that of crawling envolves anti-phasic waves of contraction of the longitudinal and circular muscles which produce alternate extension and contraction of the body. The other main locomotory activity, that of swimming, is achieved by the leech undulating its extended and flattened body in a dorso-ventral direction, forming a wave which travels backward along the animal. This is accomplished by the antiphasic contraction of the dorsal and ventral longitudinal muscle of successive segements of the body wall. "Reflex shortening" occurs upon simultaneous tonic contraction of the entire longitudinal musculature, which changes the shape of the animal from a long thin cylinder to a squat elipsoid. The changes in longitudinal muscle length which occur during activity may be considerable. In a resting posture the body length of an adult animal weighing 2-3 g may vary from 5 to 10 cm. When swimming the body can extend up to twice this length. Changes of up to 200% may therefore occur in the length of the longitudinal muscle upon

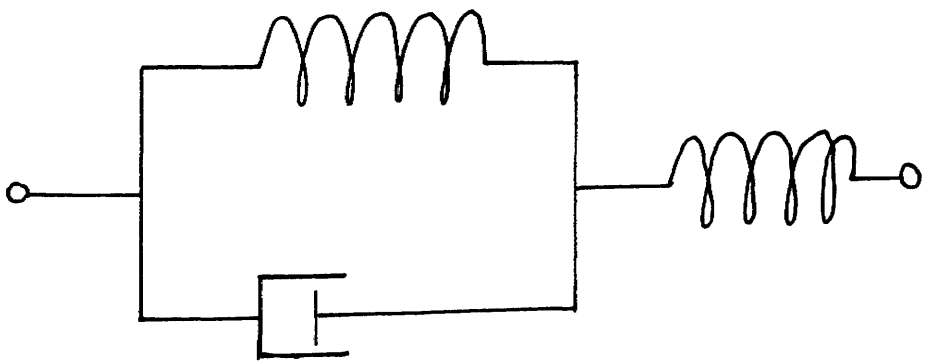
changing from one mode of activity to the next.

During the swimming wave when the body is held flattened and elongated the longitudinal muscles of each segment shorten during the contractile phase by about 20% of their length in the distended phase (Kristan et.al. 1974a). Assuming therefore a segmental length of approximately 5.0 mm during the swimming rhythm, changes in length of the dorsal and ventral longitudinal muscle of approximately 1.0 mm would be expected to occur. When these same muscles undergo a shortening reflex, the longitudinal muscle may contract by as much as 70% of the resting segmental length (Kristan et.al. 1974a). In a segment which was 5.0 mm long for example a length change of 3.5 mm may be expected during a shortening reflex.

The length changes imposed upon the longitudinal muscle in the present study ranged between 0.4 and 4.5 mm, that is, between 8.0% and 90.0% of the πrestingπ segmental length of the animal and were therefore considered to approximate those changes seen in intact animals during natural locomotory activities. Over this range, the sensitivity of the SRN response to ramp length changes appeared to be linear. However a drop in sensitivity was frequently noticed at either extreme of extension. This saturation of the response often occured in one direction of muscle movement before the other, indicating that the longitudinal muscle was already nearing its extreme of extension in that particular direction prior to the imposed ramp stretches or releases.

5.1 Length or tension receptor?

The tension changes recorded from the ventral longitudinal muscle during the imposed ramp length changes showed small overshooting tension transients at both the beginning and at the end of the ramp stretch stimulus (fig 30).The size of the tension transients depended on the velocity of the stretch or release, being larger at higher velocities. This behaviour of the leech longitudinal muscle may be adequately explained by the dynamic model put forward by B.H.C.Matthews (1933) and P.B.C.Matthews (1964) to explain spindle afferent adaptation in terms of the visco-elastic properties of the muscle spindle fibres (see below)



Visco elastic model of the receptor muscle. A component consisting of elasticity (a spring) and viscosity (dash pot) in parallel are connected in series to a second component of elasticity. The dendritic terminals are attached to the elasticity component at the rigth hand side.

If a sudden stretch is applied to the muscle, the instantaneous rise in tension is large, (determined only by the right hand elastic element). This is followed by a gradual decline in tension as the viscosity element (the dash pot) is elongated. A final tension level is ultimately reached at the new resting length, determined by the two elasticities in series.

The second feature of the muscle tension response to a ramp shortening is the slow gradual "creep" or increase of tension towards the resting baseline level, during the constant, shortened length phase of the ramp, (fig.30). It is possible that this slow rise in isometric tension may be due to reflex motoneurone activity in response to the ramp shortening. Although the posterior root was invariably cut in these experiments, the motor outflow through the AA anterior nerve root might be sufficient to account for such reflex muscle activity.

The receptor potential response of the SRN closely reflected the tension changes recorded from the ventral longitudinal muscle, albeit in an inverse manner, such that the SRN responded to a decrease in longitudinal muscle tension with a depolarising receptor potential and vice-versa. For example in fig.32 during rapid extension of the longitudinal muscle length the SRN E_m closely followed the tension transient.

Measurements made from the S and T fibres of the custacean TCMRO also show that the tension changes recorded from the receptor muscle fibre during imposed length changes closely reflect the S fibre receptor potential (Bush and Godden, 1974). Moreover, the initial transients commonly seen in both the S and T fibre dynamic responses to a ramp stretch may be correlated with the initial level and rate of rise of tension within the receptor muscle.

The relation of tension changes to early adaptation and steady-state discharge has been studied in other muscle receptors and afferent fibre systems. Brown (1967) showed a good correlation between receptor muscle tension and frequency of discharge of the slowly adapting crayfish abdominal stretch receptor. By observing the adaptation of the generator potential from such a slowly adapting crayfish stretch receptor under conditions of length and tension clamp, it was suggested that approximately 70% of the generator adaptation can be explained by the simple visco-elastic properties of the receptor muscle (Nakajima and Onodera, 1969). The decline in the generator potential seen under length-clamp conditions was not observed under conditions of tension clamp, suggesting that the receptor potential more closely follows receptor muscle tension than its absolute length. The decline in tension of the receptor muscle following sudden muscle stretch hence partly explains the adaptation response.

In vertebrates the dependence of the muscle spindle generator potential upon intrafusal muscle tension is more complex. Although

it has been shown that the generator potential response of the muscle spindle afferents closely follows the tension changes produced by stretch (Husmark and Ottoson, 1971), the generator potential obtained under tension clamp conditions does not follow the steplike tension changes produced in the receptor muscles. The generator potential response also probably shows some dependance upon the rate of change of tension of the receptor muscle. Since the investigators only recorded the "muscle spindle generator potential" which is a summed response from all afferent endings is not possible to show that the sensitivity of the generator potential to the rate of change of tension is the responsibility of a particular spindle afferent or that some do indeed faithfully follow changes in muscle spindle tension. It has been shown however (Boyd, 1976) that the frequency of discharge of some group II spindle afferents does appear to closely reflect tension in the muscle spindle.

Receptor organs which respond to muscle tension are classically considered to lie exclusively "in series", eg, Golgi tendon organs (Matthews,1972). "In series" tension receptors have also been described in the longitudinal muscle of the duodenal muscularis externa in vertebrates (Iggo,1955 ; Cottrell and Iggo, 1984). The exact location of a particular receptor is thought to be very important in determining its response. For example it has been suggested that in the ferret stomach the same receptor type may respond to tension in one location and to length in another (Andrews, Grundy and Scratcherd, 1980). It appears however that a receptor may also respond to changes in muscle tension even though it lies in parallel with the contracting muscle fibres. Thus the S

fibre of the crustacean TCMRO innervates the flanking strands of the receptor muscle and yet its response characteristics are strongly influenced upon passive distension by the tension within the receptor muscle (Bush and Godden, 1974).

5.2 SRN activity and the hydrostatic skeleton

In order to determine more precisely the role of the SRN in signalling changes in longitudinal muscle tension it is necessary to consider more closely the dynamics of distension of the leech body walls both during natural movement and during imposed length changes. A soft bodied animal such as the leech lacks any fixed points of articulation against which its muscles can act. The ventral and dorsal longitudinal muscles of a particular segment do not act as direct antagonists. The force exerted on the hydrostatic skeleton of the animal, by, say, the contraction of the dorsal longitudinal muscles of a particular segment, is transmitted to the dorsal and ventral body walls of many other segments. Hence the concurrent distension of the ventral body wall is due mainly to the action of the longitudinal force of the hydrostatic skeleton acting upon the relaxing ventral longitudinal muscle.

When an isolated piece of longitudinal muscle is passively stretched as during the ramp stretch stimuli used in the present

study, tension within that muscle strip increases, evoking the characteristic hyperpolarising potential from the SRN. Vice versa, when the muscle strip is shortened during an imposed ramp release, tension within the muscle drops and a depolarising receptor potential is evoked within the SRN. During natural locomotory behaviour such as swimming however, active contraction of the ventral longitudinal muscle which shortens the ventral body wall, results in an increase in tension of the shortened ventral muscle. Meanwhile extension of the dorsal longitudinal muscle commanded by activity within the dorsal inhibitory motoneurones results in a decrease in tension. During locomotory behaviour such as swimming therefore it is the balance of activity between the inhibitory and excitatory motoneurones to the longitudinal muscle which allows the hydrostatic skeleton to confer a particular configuration or posture upon the leech body wall. In the case of the swimming rhythm, one may imagine that there is a residual tone in the longitudinal muscle produced by endogenous activity from excitatory motoneurones. The function of the dorsal and ventral inhibitory motoneurones would then be to reduce this residual dorsal or ventral muscle tone. The inhibitory motoneurones to the dorsal longitudinal muscle for example would then command the specific distension of the dorsal longitudinal muscle, mediated by the hydrostatic skeleton, which occurs during the concommitant contraction and subsequent increase in tone of the ventral longitudinal muscle of that particular segment. From the point of view of the SRN therefore, extension of the dorsal longitudinal muscle would be monitored as a drop in muscle tension with a subsequent depolarisation of the E_m of the putative dorsal stretch receptor. Simultaneous contraction of the

ventral longitudinal muscle will register as an increase in tension with subsequent hyperpolarisation of the ventral SRN.

For any particular contraction-distension cycle therefore of the ventral longitudinal muscle of a particular segment during the swimming rhythm, a matching hyperpolarisation-depolarisation rhythm will be transmitted by the ventral SRN to the central nervous system as the tension changes from the ventral body wall are closely monitored. We see therefore that although under the artificial conditions of passive longitudinal muscle stretch, the increased tension generated within the muscle results in a hyperpolarising SRN receptor potential, under conditions of natural locomotion, extension of the longitudinal muscle is associated with a decrease in tension and a consequent depolarising SRN response.

6.0 The advantages and disadvantages of graded non-spiking afferent signalling

One of the more serious disadvantages of decremental conduction of signals over large distances, appart from the obvious bulky size of the axons, is a consequence of the need for a relatively high membrane resistance. This means that the time constant of the axon will also be large, hence electrical changes occuring at one end of the fibre will take a long time to be effective at the other. This has a further limiting effect upon the range of frequencies which can be reliably transmitted by the axon. These limitations may be overcome to some extent by the provision, possibly applicable in the case of the SRN, of some degree of voltage dependent response in the neurone. Such voltage dependent responses in an otherwise passive cell (the spiky component of the T fibre response), were considered by Bush (1981) to be of value in "sharpening up" the rising phase of the depolarising receptor potential. Similarly , the depolarisation transient of the SRN may have a similar function in the phasic, velocity sensitive portion of the response of the SRN already noted, (see fig. 24). The role of the graded calcium spikes recorded from the synaptic region of the T fibre in Callinectes was also considered important by Blight and Llinas (1980), for they showed that the depolarisation transient, based on a sudden influx of calcium ions, was important in speeding up the post-synaptic response and shortening the post-synaptic reflex latency.

An obvious advantage for the use of non-spiking transmission is that it is not necessary to first encode the input signal into a digital signal followed by subsequent decoding to give a graded post-synaptic response, with considerable scope for the loss or distortion of information at each step.

A theoretical approach has been used to show that when only a single spiking input unit is available discontinuities occur in the output signal (Pearson, 1976). In order to obtain a smoothly graded output signal a large number of spiking inputs are necessary. Such is the important function of the large numbers of afferent neurones commonly present in many proprioreceptor systems. Non-impulsive transmission is favoured if only, as in the case of the leech, a limited number of input channels are available. In such a situation only non-impulsive tranmission has the ability to drive the post-synaptic cells smoothly through a wide range of frequencies.

7.0 Synaptically associated cells

As previously described (see literature review), efferent innervation of the muscle receptor organ is an integral part and universal feature among all of the receptor complexes of the various animal groups hitherto studied. The complexity and degree of specialisation of such efferent control elements varies greatly. Hence the mammalian muscle spindle is supplied by two groups of efferent fibres which innervate specific intrafusal fibres. Activity

within one of these efferent groups, the dynamic gamma axons, results in an increased dynamic sensitivity of the muscle spindle afferents, whilst activity within the static gamma axons endows an increased positional sensitivity upon the spindle afferent fibres. In contrast a single population of nerve fibres supplies excitatory motor innervation to the single receptor muscle of the insect muscle receptor organ.

In the examples discussed above, efferent modulation of the afferent discharge is mediated through motor control of the receptor muscle. In other muscle receptors however, direct inhibitory innervation of the stretch receptor terminals themselves has been shown (Kuffler and Eyzaguire, 1955 ; Alexandrovicz, 1967 ; Jansen, Nja, Ormstad and Walloe, 1971). In these cases modulation of the stretch-induced receptor potential serves to directly influence impulse initiation in the afferent neurone.

In the present study no specialised receptor muscles in association with the SRN have been observed. Electron micrographs have revealed the presence of neuro-muscular junctional specialisations between the longitudinal muscle fibres associated with the SRN and unknown nervous elements, (S. Blackshaw, pers. communication). However the detailed innervation of the muscle fibres associated with the SRN dendrites remains unknown. Efferent innervation of the SRN however does occur (results section 4.0). Surprisingly some of the efferent innervation is supplied by identified cells which themselves are primary mechano- sensory

neurones. This represents the interesting situation of a synaptic interaction between two primary sensory neurones of different modalities.

Baylor and Nicholls (1969) showed that synaptic potentials could be recorded from leech T cells as a result of activity within another modality of mechanoreceptor, the P cell. These synaptic interactions were excitatory as well as inhibitory. Synaptic connections also exist between P and N mechanosensory neurones here however the responses were always depolarising.

The existence of an interaction between primary afferent neurones in the mammalian dorsal horn has also been known for over 50 years. Thus the dorsal root reflex (DRR) is an antidromic volley of action potentials which can be recorded from a spinal dorsal root following the stimulation of an adjacent dorsal root. Intraxonal recordings have shown that the reflex is triggered by a rapid depolarisation of the terminal regions of the afferent fibres (Eccles and Krnjevic, 1959). This primary afferent depolarisation (PAD) is in part mediated by a specfic neuronal pathway, in which the transmitter GABA is released onto the terminals of the primary afferent fibres (Jankowska et al. 1981 ; Bagust et al. 1982). An interaction between different modalities of afferent fibre in the generation of the DRR has been well documented. Afferent volleys in the cutaneous superficial peroneal and sural nerves evoked DRR's into the afferents in the nerves of the flexor digitorum longus and flexor hallucis longus muscles (Eccles et al. 1961). It has been proposed

that the neuronal pathway involved is probably a trisynaptic (2 interneurone) pathway (Jankowska et al. 1981), and would not therefore represent a direct synaptic connection between primary afferent neurones of different modalities as appears in the leech. The depolarisation of a primary afferent input to the mammalian CNS is thought to provide a means by which certain afferent pathways may centrally modify the activity of other afferent inputs (Eccles et al. 1962).

Although interactions occur therefore between the primary afferent fibres of both the leech and mammal the locations of these interactions differ. Thus PAD is one facet of the reflex inhibitory activity present within the mammalian spinal cord. On the other hand the type of synaptic interaction observed between the SRN and the leech N mechanosensory cell represents the peripheral modulation of a receptor potential similar to that seen in the crayfish stretch receptor.

Of the synaptic associations described between the SRN and the mechanosensory neurones in this study, the synapse between the lateral N cell (N_L) and the SRN will be described first. Evidence that the N_L-SRN synapse is monosynaptic is that the SRN epsp follows the N_L cell action potential on a 1:1 basis and with a constant latency. Following intracellular stimulation of the N_L cell the SRN epsp persists in the presence of both 15 mM Mg^{2+} and 15 mM Ca^{2+}. This also suggests a monosynaptic connection between the two cells. As has been mentioned (results section 4.1), since Ca^{2+} and Mg^{2+} act

synergistically in reducing the excitability of any intervening neurones in poly-synaptic pathways (but act antagonistically upon transmitter release at a direct synapse) the likelihood of observing a psp in a high Mg^{2+}, high Ca^{2+} solution is reduced if more than two neurones are involved in the pathway (Nicholls and Purves, 1970).

Electron micrographs of the N_L coils lying on the peripheral SRN dendrites revealed close apposition of the N_L cell terminals and SRN fan but none of the ultrastructural features characteristic of a classical chemical synapse (Blackshaw et al. 1982). This clearly raised the possibility that the synapse may be electrical in nature. In physiological experiments a long delay of 18.0-19.0 ms was recorded between the peak of the N_L cell action potential and the onset of the SRN epsp. If the synaptic connection between the N_L cell terminals and SRN fan is assumed to occur in the periphery and the time deducted for propagation of the N_L cell action potential along its axon and terminal branches, (assuming a conduction velocity of 0.6 $msec^{-1}$) a peripheral synaptic delay of 11-12 ms is obtained. This calculated synaptic delay is long when compared with the monosynaptic latency of 2-4 ms measured for the central chemical connection between the N cell and the L motoneurone (Nicholls and Purves, 1970). This makes it seem unlikely that it is an electrical synapse. Alternatively, assuming such a synaptic delay of 4.0 ms and a conduction distance of 5.0 mm, a not unreasonable N_L cell action potential conduction velocity of 0.3 $msec^{-1}$ is obtained. It should be borne in mind that the N cell will not have a uniform conduction velocity in the peripheral nerve. It is most likely that a reduction in conduction velocity will occur on propagation of the action

potential into the fine side branches and fine terminal coils of the N_L cell hence the synaptic delay is likely to be far less than that calculated in the present study.

The present discussion is based on the assumption that the SRN epsp is mediated by a perpheral synapse at the site of the coiled N_L terminals. An alternative possibility is that a central synaptic connection from the N_L cell to the SRN is present in the ganglionic neuropile. Synaptic potentials generated within the central SRN terminal branches following activity within the N_L cell would then be passively transmitted to the SRN soma in accordance with the large space constant of the SRN axon. Figure 42 shows the psp recorded from the SRN cell body following intracellular stimulation of the N_L cell soma before and after section of the N_L cell axon branch in the MA nerve root. After section of this N_L cell axon branch, the epsp previously recorded from the SRN soma was abolished. Such a result is incompatible with the hypothesis of a centrally located synapse. The result of cutting the AA nerve root however must also be considered (fig. 43). This proceedure cuts the SRN axon in the AA nerve as well as the N_L cell axon branch to the proximal fan of the SRN whilst leaving the N_L cell axon branch in the MA nerve root to the distal SRN fan intact. Surprisingly, this proceedure also resulted in the abolition of the epsp from the SRN soma. As it is known that the N_L cell distributes coiled terminals to both the proximal and distal SRN dendrites (Blackshaw et al. 1982), it is likely that both sites of contact are involved and are necesary in the generation of the SRN epsp.

Of the remaining ipsilateral mechanosensory neurones, only the medially situated N cell (N_M) failed to evoke an SRN epsp. Intracellular stimulation of both the medially and laterally placed P cells and all three ipsilateral T cells elicited epsp's in the peripheral SRN cell body. The examination of electron micrographs of the SRN dendrites and associated N_L cell terminals has revealed the presence of numerous other unidentified axon profiles closely apposed to the surface of the SRN fan (Blackshaw et al. 1982). Although it awaits to be directly demonstrated with the use of morphological techniques that the unidentified processes are indeed P or T cell terminals, the electrophysiological experiments have indicated a number of candidate neurones which may project to the peripheral SRN dendrites. Two exceptions however are the medial pressure cell (P_M) and the dorsal touch cell, (T_D). Both of these neurones have receptive fields in the dorsal half of the leech body wall and both have a single axon branch in the dorsal branch of the posterior nerve root (Baylor and Nicholls, 1968). It appears unlikely therefore that either of these neurones may contact the peripheral SRN dendrites in the AA nerve root directly. An alternative explanation for the synaptic connectivity observed between these cells may lie in either a direct central connection within the ganglion, or a poly-synaptic central connection from the P_M or T_D cell to a neurone that projects to the peripheral SRN fan. That a poly-synaptic pathway exists between the P_M and the SRN was suggested in the experiment in figure 45b. Summation of two epsp's in the SRN soma is shown in response to the first two of three action potentials elicited in the cell body of the P_M cell. The failure of the third epsp following the last P_M action potential

suggests that more than a single synapse is interposed in the pathway.

7.1 The function of the peripheral efferent innervation of the SRN

The role played by the T, P and N cells, in the efferent control of the SRN must be considered with respect to their principle function as mechano-receptors. Tactile stimulation, pinching or proding of the leech body wall evokes rapid shortening of the animal, a proceedure which involves drastic changes in its overall length. This activity is mediated by activity within pressure, nociceptive and touch mechanosensory neurones and which is relayed either monosynaptically within the segment to the large longitudinal motoneurones, or distributed poly-synaptically and intersegmentally via the S cell fast conducting system (Nicholls and Purves, 1970 ; Bagnoli et al. 1975 ; Magni and Pellegrino, 1978). Activity within the mechanosensory neurones may, via a peripheral "axon reflex", evoke depolarising synaptic activity in close proximity to the receptor region of the SRN. This synaptically evoked SRN depolarisation would then strongly modulate the hyperpolarising SRN response produced during the subsequent tension increase during the longitudinal muscle contraction. Hence mechanosensory efferent innervation of the SRN will exert a positive bias upon SRN afferent activity. Large hyperpolarisations of SRN E_m which may result from excessive shortening of the animal will be offset. The input/output

relationship of the SRN and its post-synaptic elements will therefore presumably be maintained within the more sensitive region of the SRN's response curve.

8.0 Afferent modulation of the motor output

In the present study a close morphological association was observed between the central branches of the SRN and the dendritic arbourisation of a number of excitatory motoneurones to the longitudinal muscle. For example direct projections from both the L cell and the ventral inhibitory neurone (cell 2) to the SRN were observed (figures 49 and 54). It is somewhat surprising therefore that imposed changes in SRN membrane potential produced by the injection of current into the cell body produced no consistent change in L cell somatic action potential frequency. During these experiments sufficient current was injected into the SRN to elicit voltage deflections in the SRN cell body of between 10 and 20 mV. Taking into account the length constant of the SRN axon and the distance between the peripheral cell body and central terminals, potential changes of between 3.7 and 7.4 mV were calculated to occur at the central synaptic zone. Such a pre-synaptic depolarisation was considered adequate to elicit a potent post-synaptic response. Sand et al. (1975) elegantly demonstrated that injection of small amounts of current into the hair cells of the superficial lateral line organs of Necturus sufficient to change the hair cell membrane potential by between 1 and 9 mV could effectively modulate impulse

activity in the post-synaptic afferent fibres. Similarly, in a study of the crustacean T fibre to promotorneurone synapse by Blight and Llinas (1980), a pre-synaptic voltage change of 5.8 mV was found sufficient to produce an e-fold increase in the amplitude of the peak post-synaptic potential.

In response to small pre-synaptic depolarisation of the T fibre, Blight and Llinas (1980) were also able to record large epsp's with superimposed spikes from the motoneurone cell body in the thoracic ganglion of the crab. The responses consisted of large epsp's (5 mV - 20 mV) the amplitude of which rose linearly as the pre-synaptic depolarisation increased. On occasion the epsp amplitude was sufficiently large to completely shunt the antidromic motoneurone spike such that only the underlying posy-synaptic potential was recorded from the cell body. In the present experiments the absence of post-synaptic potentials from the intracellular L cell recordings in response to activity within the SRN points to the possibility that the intracellular somatic recording point was a considerable distance, and therefore electrotonically remote from the presumed site of synaptic contact between the two cells. Because leech motoneurone cell bodies do not have overshooting action potentials, the site of motoneurone action potential initiation is thought to be located near the point where the axon leaves the ganglion and is hence remote from the cell body. This point is also close to the region of the L cell, shown in figure 49, where numerous points of contact are found between the SRN and the L motoneurone. The further possibility exists therefore that modulation of L motoneurone activity in response to SRN input may occur at the point of SRN and

L cell contact and remain undetected by recording at a distance in the L cell body. It may be more appropriate therefore to monitor activity extracellularly from the L cell axon within the anterior nerve root as well as from its central cell body. Such a proceedure may well reveal any modulatory activity of the SRN input upon the motor outflow from the segmental ganglion.

Activity in another motoneurone within the segmental ganglion, cell 3, the excitatory motoneurone to the dorsal longitudinal muscle was monitored extracellulary in the dorsal posterior nerve (DP) in response to imposed changes in SRN membrane potential (fig.56). However no obvious or consistent alteration in the discharge frequency of this cell was observed following either depolarisation or hyperpolarisation of the SRN E_m. To record activity from this dorsal excitatory motoneurone was however not a critical test of the modulatory influence of the SRN. It had not been shown for example whether or not the incoming SRN axon did indeed contact this particular motoneurone in the manner shown previously for the L cell and the ventral inhibitory motoneurone.

The problem of detection of functional connections between pairs of neurones has been investigated by a number of techniques. Of these methods, the method of coherence analysis is considered to be one of the most sensitive measures of association between pairs of signals (Ellaway et al. in press). This method (Brillinger et al. 1976) was applied to the SRN-motoneurone system of the leech in order to negate the inherent problems associated with recording

synaptic potentials and their consequent effects at a distance. In the present case, extracellular spiking activity was monitored directly from the MA nerve root before and during random imposed activity in the SRN. In this study the pathway of central connections from the SRN to motoneurones could be considered to be a black-box. Only the degree of coherence between input and output and the dependence of the coherence of the outputs upon the input was considered. From the results of this work in the present study a rather complex picture has emerged. It appears that no direct association exists between activity within the SRN and any of the individual efferent units investigated, thus confirming the earlier intra and extracellular investigation which also failed to detect any such direct functional contact. It is clear however that the coherent activity of all three of the units studied was highly dependent upon the SRN input ; moreover it appears that the coupling effect of the SRN input upon the three motoneurone spike trains is driven via one of these units itself. It is unfortunate that these three efferent units were not identified in this experiment, however the findings from the coherence data raise one or two interesting implications. Firstly, we may not assume that the segmental reflex loop proposed by Kristan and Stent (1976) to explain the opposition of longitudinal muscle stretch, is as simple as initially described. Secondly, SRN afferent actvity appears to have a widespread effect in co-ordinating the activity of a number of efferent units within the segment. Such co-ordinating activity between individual motoneurones will form the basis for reflex integration within the animal.

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