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ELECTRICAL CHARACTERISTICS OF THE MEMBRANE OF AN IDENTIFIED INSECT MOTOR NEURONE

BY G. F. GWILLIAM* AND M. BURROWS
Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ

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SUMMARY

1. The electrical properties of the membrane of an identified locust motor neurone, the fast extensor tibiae in the metathoracic ganglion, have been investigated to determine: the distribution of excitable and inexcitable membrane; the impulse initiation zone; and the conduction velocity of the spike in the ganglion and in the axon.
2. The waveform of extracellularly recorded spikes indicates that the transition from inactive to active membrane occurs along the region of the neurite which bears many arborizations within the neuropile.
3. Measurements of the delay between orthodromically or antidromically evoked spikes, recorded at the soma and other points along the neurite, place the impulse initiating zone close to the transition between active and inactive membrane.
4. Within the ganglion, the spike is conducted at different velocities over different parts of the neurite. The average velocity within the ganglion is, however, only about a seventh of that in the axon (0·54 $m.s^{-1}$ against 4·1 $m.s^{-1}$).

INTRODUCTION

Insect motor neurones have now been sufficiently well characterized morphologically and physiologically (e.g. locust, Hoyle & Burrows, 1973; Burrows & Hoyle, 1973; Tyrer & Altman, 1974; Wilson, 1979: cockroach, Fourtner & Pearson, 1977: dragonfly, Simmons, 1977*a*: cicada, Simmons, 1977*b*: moth, Truman & Reiss, 1976) that it is possible to describe the general characteristics as follows. The cell body, which is unipolar and devoid of synapses, lies in a multi-layered cortex intermingled with the cell bodies of local and interganglionic interneurones. The single process or neurite from the cell body enters the neuropile, broadens, and gives rise to a plethora of side branches before emerging from a ganglion or the brain as an axon. Synaptic interactions with other neurones occur at the neurite and at finer branches. The cell body typically does not generate an actively propagated action potential or spike. Action potentials are thought to be initiated at some distance from the cell body and then reflected into it by what is presumed to be passive propagation. The spike initiating zone is generally assumed to be near the emergence of the axon from a

* Permanent address: Biology Department, Reed College, Portland, Ore 97202, U.S.A.

brain or ganglion (Hoyle, 1970), an idea supported by the absence of over-shooting spikes in the neuropilar processes of motor neurones (Pearson & Fourtner, 1975). In Crustacea this supposition has been substantiated for a motor neurone which innervates muscles of the eyecup of a crab (Sandeman, 1969) and has been incorporated into a generalized model for crustacean motor neurones (Evoy, 1977) that differs little from that proposed for insect ones (Hoyle, 1970). Briefly, in insects it is assumed that the spike-initiating zone is at the boundary between active axonal membrane and the passive membrane thought to characterize the so-called 'integrating zone' (expanded portion of neurite-bearing side-branches (Sandeman, 1969; Hoyle, 1970)) and the soma. Certain experimental manipulations can alter the properties of the membrane of the soma. For example, when the axon is cut or in the presence of colchicine (Pitman, Tweedle & Cohen, 1972*b*; Pitman, 1975; Goodman & Heitler, 1979), the soma becomes capable of supporting a predominantly sodium-dependent overshooting action potential. In the presence of intracellular citrate ions or external tetraethylammonium ions, the soma of a cockroach motor neurone is capable of supporting overshooting action potentials, which are, however, calcium-dependent (Pitman, 1979).

In order to provide some indication of where the transition from active to inactive membrane occurs and to derive from this some information about the spike initiating zone, we have examined a motor neurone in the metathoracic ganglion of the locust. This motor neurone, the fast extensor of the tibia of a hind leg, is an identified individual and, together with its symmetrically arranged counterpart that innervates the other hind leg, is the largest in the ganglion. It produces a characteristic rapid twitch of the extensor tibiae muscle with each spike. Extracellular recording within the neuropile suggests that the transition from inactive to active membrane occurs along the region of the neurite that bears side-branches. Spikes may also arise from this region.

MATERIALS AND METHODS

Adult male and female locusts *Schistocerca americana gregaria* (Dirsh) were obtained from our own crowded laboratory culture. The meso- and metathoracic ganglia were exposed by a ventral dissection, leaving as much of their tracheation intact as was compatible with the recording procedure. The ganglia were stabilized upon a wax-covered platform. The thorax was perfused with a constant flow of saline (Usherwood & Grundfest, 1965) modified by the addition of 90 mM sucrose and 5 mM magnesium chloride.

The electrodes, used for recording both intra- and extracellularly within the ganglion, were glass micropipettes filled with 2 M potassium acetate or 10% cobalt chloride and with d.c. resistances of 20–40 MΩ. Before recording, the sheath of the ganglion was treated with a 1% (w/v) solution of protease (Sigma, type VI) for 2–4 min. A pair of 50 μm stainless-steel wires insulated but for their tips were used to record the activity of the extensor tibiae muscle in a hind leg and to stimulate the axon terminals of the fast extensor motor neurone to elicit antidromic spikes. All observations were made at 18–21 °C. The results are based upon 43 locusts in which simultaneous recordings were made from the soma of the fast extensor tibiae motor

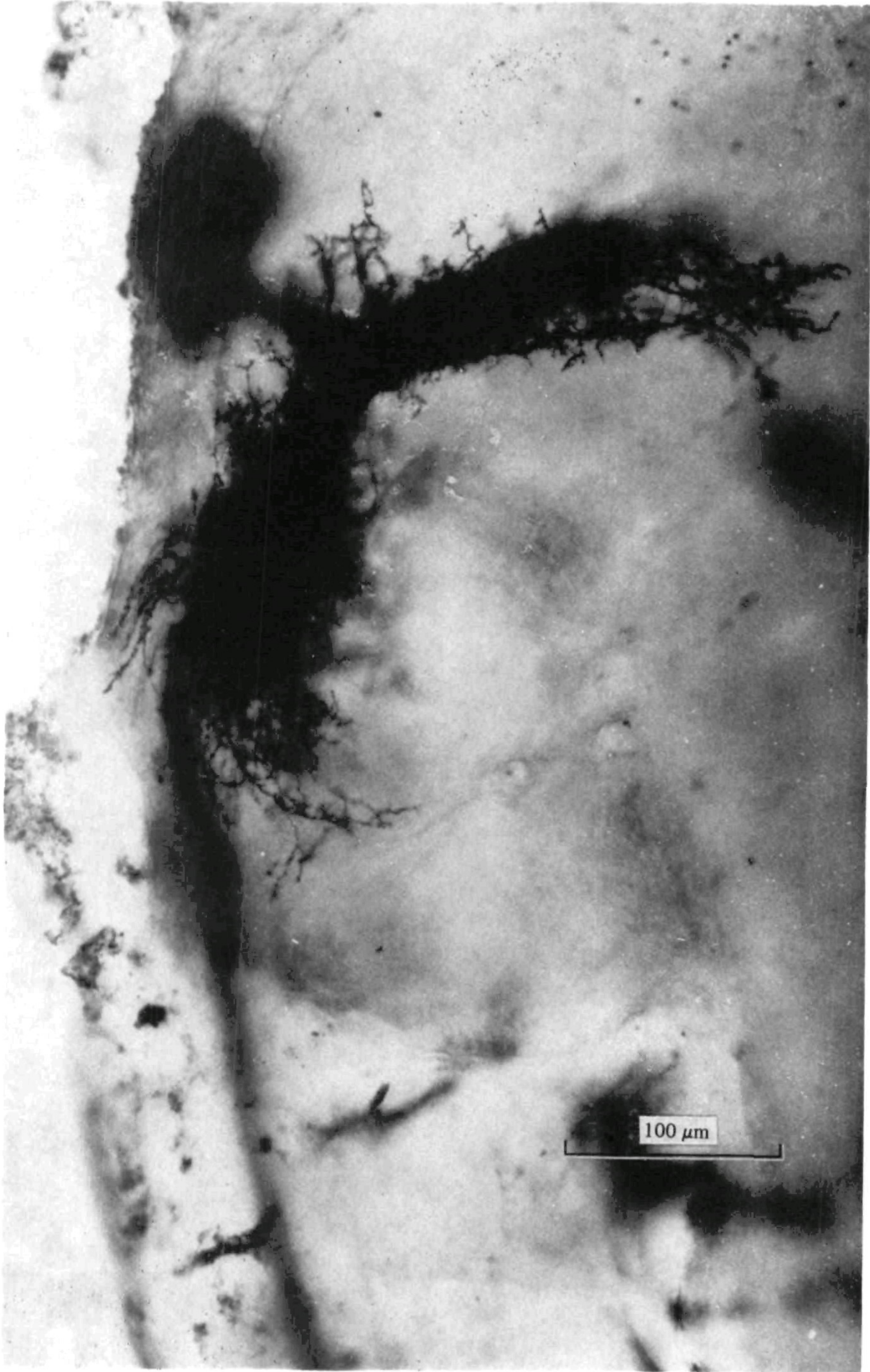

Fig. 1. The morphology of the fast extensor tibiae motor neurone as seen from the dorsal surface of an intact metathoracic ganglion. The neurone was physiologically characterized, injected with cobalt chloride and the stain subsequently intensified with silver. The cell body is on the ventral surface of the ganglion and the axon enters nerve 5 at the bottom left. Anterior is at the top.

neurone (FETi) and from one or more intra- or extracellular sites of this neurone within the neuropile.

Three methods were used to determine the position of the electrode within the neuropile.

(1) By far the best was to mark the intra- or extracellular position by ejecting cobalt from the tip of the electrode (Pitman, Tweedle & Cohen, 1972*a*). The soma of the motor neurone was also injected with cobalt at the same time but from a different electrode. After silver intensification of the cobalt (Bacon & Altman, 1977) the neuropile recording site was visible as an intense spot against the more lightly stained remainder of the motor neurone in a fixed, cleared whole ganglion.

(2) The position of the electrode was recorded with reference to a 100 μm grid (Burrows & Hoyle, 1973) laid over the surface of the ganglion.

(3) An ocular micrometer was used to measure the linear distance between the electrode in the soma and the second electrode in the neuropile. The position of the electrode in the neuropile could then be superimposed upon a drawing of the motor neurone from a ganglion of similar size. The recording sites have been numbered, with the soma given the designation 1 and the axon 15 (see Figs. 4–6).

The length of the neurite from the cell body to its emergence from the ganglion in nerve 5 was determined in the following way. Camera lucida drawings of the motor neurone, which was stained with cobalt, were made from dorsal and lateral aspects (Fig. 2) in order to obtain x, y and z coordinates. Reference points on these coordinates were defined so that $x > 0$ was anterior, $y > 0$ was toward the right-hand side and $z > 0$ was toward the dorsal surface of the ganglion, the origin being at the inflexion of the neurite (Figs. 1, 2). The distance (d) between any two points along this length of the neurite is given by

$$d = \sqrt{[(x_1 - x_2)^2 + (y_1 - y_2)^2 + (z_1 - z_2)^2]}.$$

Using this method, the actual length of the neurite is 1·3 times that derived from a two-dimensional projection viewed dorsally.

RESULTS

Identification of FETi

Of all the motor neurones within the metathoracic ganglion, the fast extensor tibiae (FETi) has the largest soma and, when it spikes, produces a most distinctive movement of a hind leg. The FETi is the only fast motor neurone to the extensor tibiae muscle, and the position of its cell body is quite separate from the only other excitatory motor neurone to this muscle, the slow extensor tibiae (Burrows & Hoyle, 1973). The soma of FETi is sometimes visible on the ventro-lateral margin of the ganglion and can therefore then be penetrated under visual control. Extra- or intracellular recording from other parts of FETi can be made by careful but blind probing within the neuropile. Spikes of FETi at any of the recording sites can be identified in the following ways.

(1) Depolarizing the soma or a neuropilar process with an intracellular electrode evokes an orthodromic spike, which is always accompanied by a large potential in the extensor tibiae muscle and a rapid extension of the tibia. As a consequence of this

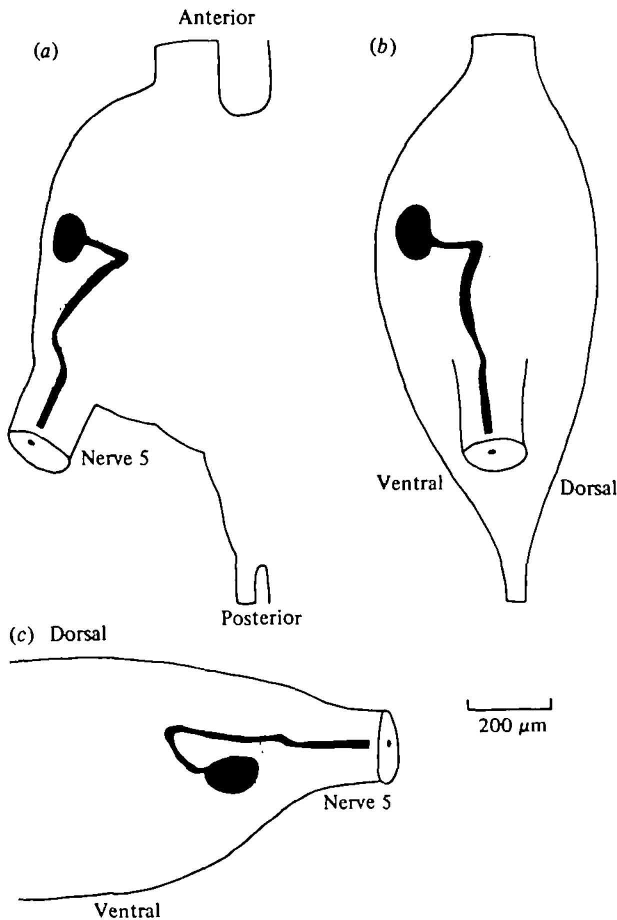

Fig. 2. The undulating path of the main neurite of the fast extensor tibiae motor neurone within the metathoracic ganglion. The drawings were made with a camera lucida from a whole ganglion in which the motor neurone had been stained by the intracellular injection of cobalt. (*a*) Dorsal view. (*b*) side view. (*c*) Anterior view. The dotted lines indicate the extent of the arborizations.

spike, other neurones, most notably flexor tibiae motor neurones (Hoyle & Burrows, 1973), may often spike. They can be distinguished from FETi spikes by their later and more variable occurrence.

(2) Stimulation of the axon terminals within the extensor tibiae muscle to evoke a rapid extension of the tibia is always accompanied by an antidromic spike recorded in the soma or elsewhere in the FETi neurone. Recordings from neuropilar processes of FETi were always made simultaneously with one from the soma. This provided an additional safeguard for the identification procedure by allowing correlation of the spikes from the two sites.

(3) The FETi motor neurone could be stained by the injection of cobalt from the recording microelectrode in the soma or in the neuropile to reveal its unique morphology (Fig. 1).

The morphology of FETi

The soma lies within the cortex of cell bodies on the ventro-lateral aspect of the ganglion and measures 95–130 μm in its longest dimension when stained with cobalt and silver intensified (Fig. 1). A single process emerges from the soma and runs dorsally and medially for 90–100 μm before abruptly turning more posteriorly (Fig. 1). This main process or neurite continues to course dorsally and laterally through the neuropile and emerges from the ganglion as an axon in nerve 5. An impression of the three-dimensional course of the neurite through the neuropile can be gained from the drawings in Fig. 2. The length of the main neurite from the soma to the start of the axon in nerve 5 in this adult locust is 650 μm. As it leaves the soma, the main neurite has a diameter of about 10 μm and this increases to as much as 15 μm throughout most of the neuropile before narrowing to 5–7 μm as it leaves the neuropile. The axon in nerve 5 has a diameter of 15 μm. These measurements were made after silver intensification of the cobalt stain and are therefore necessarily dependent upon this and the other histological procedures used, namely fixation, dehydration, clearing and mounting.

There are two main regions of branches which emerge from the main neurite. The first arises at the abrupt turn of the main neurite with many parallel branches, some of which are as much as 5 μm in diameter, extending 320 μm to the mid-line of the ganglion (Fig. 1). The second arises from the posteriorly running portion of the main neurite (Fig. 1). The branches are shorter, typically no more than 50–100 μm and emerge in all directions, but the longest are the medial ones. The main neurite is devoid of branches as it emerges from the neuropile to enter nerve 5.

Form of the FETi spike

An antidromic spike of FETi initiated in the muscle and recorded in the axon as it emerges from the ganglion (Site 14 in Fig. 4) has a duration at half height of 1·02 ms ± S.D. 0·04 ($n = 5$), an amplitude of 66·6 mV ± S.D. 8·4 ($n = 5$) so that it overshoots the 50–60 mV resting potential (Fig. 3*a*). There is a hyperpolarization of 7 mV which persists for over 15 ms after the spike. In the soma, by contrast, the duration of the antidromic spike at half height is 3·01 ms ± S.D. 0·45 ($n = 7$) and the amplitude is only 25·1 mV ± S.D. 5·8 ($n = 7$) (Fig. 3*a*). The after-hyperpolarization is also prolonged and reduced in amplitude. There is a delay of 0·55 ms between the initial inflexion of the spike in the axon (site 14) and that in the soma (Fig. 3*a*). The peak of the spike in the soma follows that in the axon with a delay of 1·7 ms (Fig. 3*a*). An orthodromic spike initiated by depolarization of the soma (Fig. 3*b*), or by synaptic inputs, undergoes similar changes in waveform and delay when compared at the axon and the soma. In the soma, the orthodromic spike, elicited by depolarization of the soma, is of smaller amplitude, and briefer duration but with an enhanced after-potential as compared with the antidromic spike (Fig. 3*b*, and Hoyle & Burrows, 1973; Goodman & Heitler, 1979). Within the neuropile the shape of either the antidromic or orthodromic spike depends upon the recording site. As the electrode is moved progressively from sites 2–6 the spike shows a progressive increase in its rate of rise, a decrease in duration and an increase in amplitude. At site 8, a 70 mV spike can be recorded which overshoots the resting potential (Fig. 3*c*).

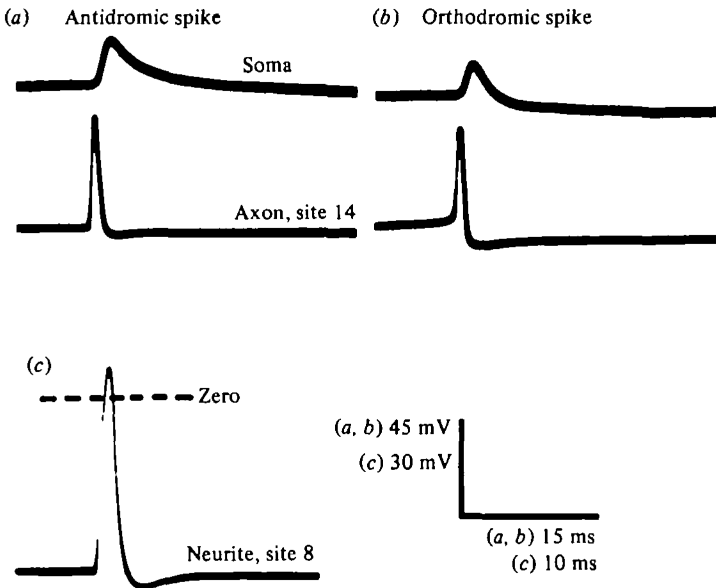

Fig. 3. The action potential of FETi as recorded by intracellular electrodes in the soma and in the axon. (*a*, *b*) A comparison of the antidromic spike (*a*) with the orthodromic spike (*b*) recorded from the same positions in one locust: soma, upper trace, axon (site 14, see Fig. 4) lower trace. The spike overshoots the rather low resting potential of this particular neurone. (*c*) A 70 mV overshooting action potential recorded from site 8 in the neurite.

It is clear, therefore, that the membrane at, and close to, the cell body differs from that of the axon in being unable to support a propagated action potential. In order to determine where 'active' membrane (able to actively propagate a regenerative spike) gives way to 'inactive' membrane (unable to carry a regenerative spike) the following series of extracellular recordings were made.

The extracellular field potential of the spike

An antidromic spike was elicited by stimulation of the axon terminals of FETi in the extensor tibiae muscle and recorded in the soma by an intracellular electrode. At the same time a second microelectrode was moved through the neuropile to record the extracellular spike of FETi at different points along the length of the neurite (Fig. 4). Recordings were made with the electrode just above the neurite. This was verified by dye marking or by making a small vertical advance of the electrode whereupon it entered the neurite of FETi. Using the results from four recording sites in one locust as an example, the extracellular spike is seen to have a predominantly monophasic, positive waveform at the neurite close to its emergence from the cell body (recording site 2, Fig. 4*a*). Where the axon is about to enter nerve 5 (recording site 12), the extracellular waveform is biphasic (Fig. 4*a*) or triphasic (Fig. 4*b*). At recording site 4, just after the abrupt posterior turn of the neurite, the waveform is still monophasic, but 160 μm away and still within the region of dense branches the waveform is now biphasic or triphasic (Fig. 4*a*). The usual interpretation of such waveforms (e.g. Woodbury, 1963) suggests that the monophasic potentials are

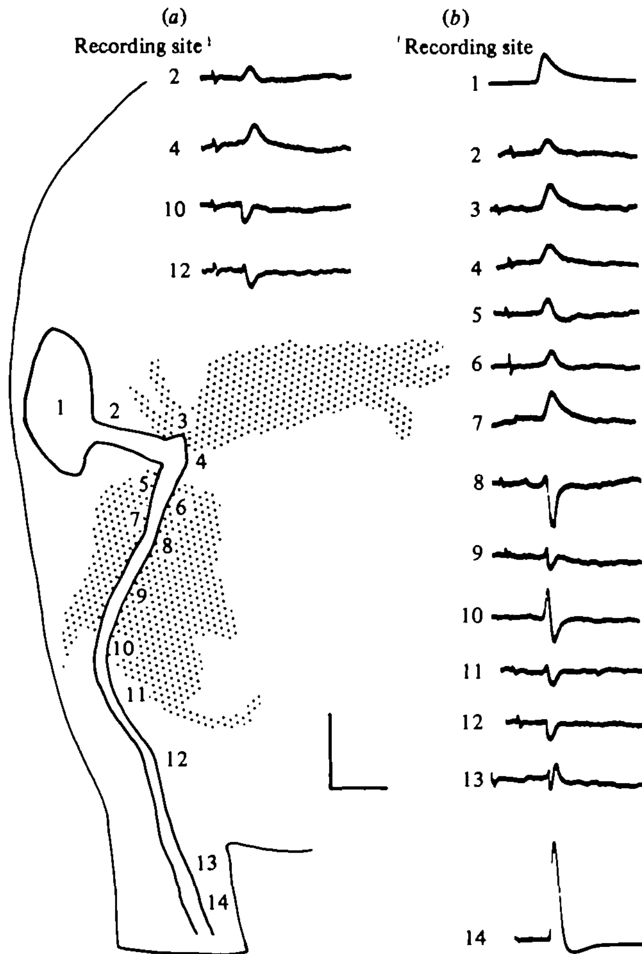

Fig. 4. The extracellular field potential arising from an antidromic spike in FETi along the course of the neurite. The diagram on the left indicates the sites from which recordings were made: the stippled area indicates the extent of the arborizations. (*a*) Extracellular recordings from four sites along the neurite of one locust. The recordings are aligned vertically with the stimulus artifact as the reference point. (*b*) Recordings from 14 different sites taken from many locusts. The records are arbitrarily aligned at the peaks of the spikes. Recordings from sites 1 and 14 are intracellular and the rest are extracellular. Note the change in waveform which occurs between sites 7 and 8. An upward deflexion is positive in the extracellular records. Calibration, voltage, extracellular 5 mV, intracellular 50 mV; horizontal 4 ms and 50 μm.

associated with inactive membrane and the bi- or triphasic potentials with active membrane.

In any one locust it was not possible to record from more than four different sites before inflicting damage that would necessarily invalidate further observations. To pin-point more accurately the site at which the monophasic waveform changed to a bi- or triphasic one, results from many locusts were pooled. Representative recordings at 14 different recording sites from the soma to the axon indicate that the transition between the monophasic and biphasic or triphasic waveform occurs in the region of recording sites 7 and 8 (Fig. 4*b*). Here the neurite approaches its most dorsal position

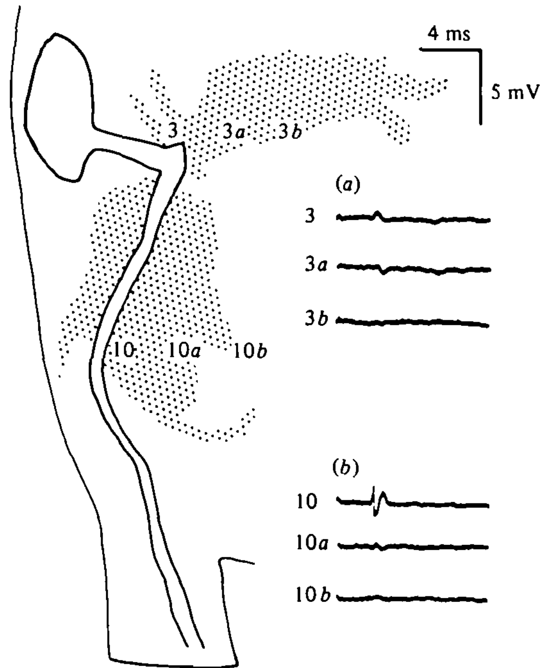

Fig. 5. The extracellular field potential arising from the antidromic spike in FETi and recorded medial to the main neurite. (a) The extracellular electrode is moved medially from site 3 (inactive membrane) but remains within the arborizations. (b) In the same locust, the electrode is now moved medially from site 10 (active membrane), to site 10b outside the arborizations. In both tracks of the electrode there is a similar decrease in the amplitude of the potential with increase in distance from the neurite.

within the ganglion and still bears a profusion of branches. This was confirmed by dye marking; sites from which bi- or triphasic potentials are recorded are within the region of dense branches. There are some limitations to the accuracy with which the recording sites can be marked in this region, because the neurite runs obliquely and dorsally. The electrode approaches the ganglion vertically from the ventral surface so that a small shift along either of the horizontal axes could lead to a much larger error in estimating the position along the neurite. Nevertheless sites 5 and 9 are clearly different in each locust but sites 6, 7 and 8 may not be.

All of the extracellular recordings in Fig. 4 were obtained before the neurite was penetrated for intracellular recordings. This was to circumvent possible changes in the extracellular waveform due to damage inflicted by the microelectrode (cf. Eccles, 1964). For example, in one locust extracellular recording initially indicated that the underlying membrane was 'active'. Subsequent penetration led to a repetitive sequence of spikes. When the electrode was withdrawn, the extracellular potential indicated that the membrane was now 'inactive'. It took 20 min for the extracellular potential to assume the characteristics of an underlying active response.

Whenever the electrode was moved laterally or medially away from the main neurite and into the region of branches, it was noticed that only a small, or often no, extracellular potential could be recorded. This suggests that the main neurite and not the many side branches is the source of the large waveform. As a more rigorous test of the distribution of the extracellular spike potential, an electrode sampled in a

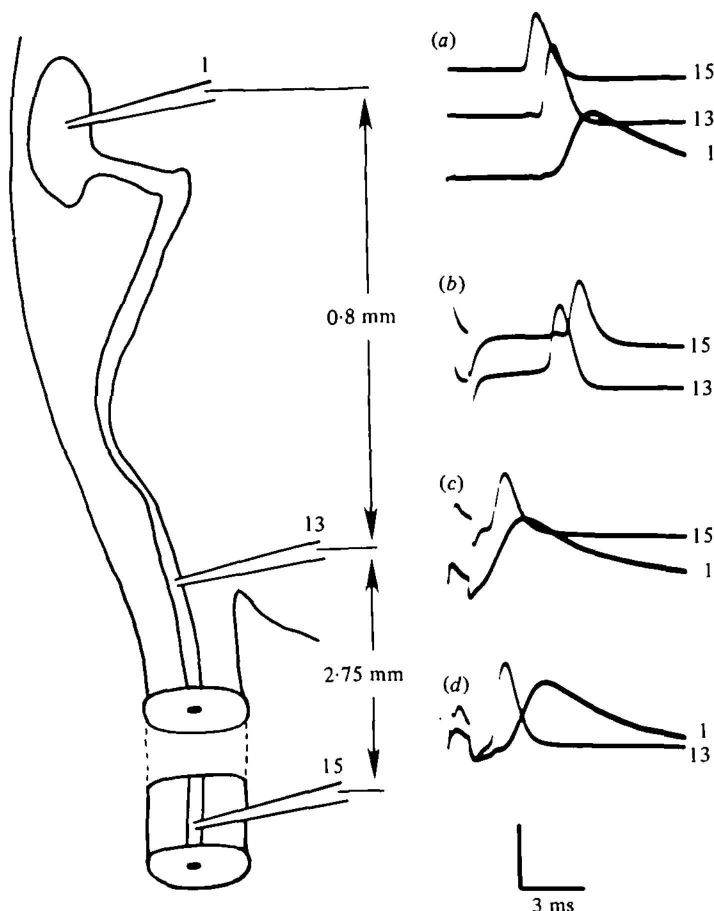

Fig. 6. Conduction velocity of the spike in the axon and in the neurite of FETi. The diagram shows the positions of the three intracellular electrodes and the length of the neurite or axon between them. (*a*) An antidromic spike evoked by stimulation at the muscle is recorded sequentially by the three electrodes. (*b*) A brief pulse of current is applied through the electrode in the soma. It triggers the sweep of the oscilloscope and evokes an orthodromic spike at sites 13 and 15. (*c*) The pulse of current is applied through the electrode at site 13, and then (*d*) at site 15 to evoke an orthodromic spike at the other two sites. Calibration: voltage, soma 30 mV, neurite (13) or axon (15) 75 mV.

random order points in a track medial from recording site 3 and from site 10 (Fig. 5*a*, *b*). The recording sites 3a and 3b were within the medially directed side branches, whereas those at 10a and 10b were outside the branches of FETi. In both tracks of the electrode there is a progressive diminution of the potential toward the mid line (Fig. 5*a*, *b*).

Conduction velocity of the spike

In order to determine the conduction velocities of the spike along the active and inactive regions of the neurone within the ganglion, and to compare these velocities with that in the axon, the following two experiments were performed. Measurements were restricted to the antidromic spike evoked by stimulation at the muscle.

First, three intracellular electrodes were placed in the motor neurone (Fig. 6). One was in the soma, the second 800 μm away at the edge of the ganglion near the emergence of nerve 5, and the third 2750 μm further away in the axon in nerve 5. With this arrangement of the electrodes the overall conduction velocity of the spike within the ganglion could be compared with that in the axon (Fig. 6*a*). The electrodes were shown to be in the same neurone by passing current through each in sequence and recording the evoked spike at the other two (Fig. 6*b–d*). Within the ganglion the antidromic spike is conducted with a velocity of 0·54 $m.s^{-1}$ (range 0·5–0·8 $m.s^{-1}$, $n = 4$) and over the initial part of the axon at 4·1 $m.s^{-1}$ (range 3·4–4·2 $m.s^{-1}$, $n = 4$). The antidromic spike is conducted with the same velocity between the axon recording site and the muscle as it is in the initial part of the axon.

The intent of the second experiment was to determine the conduction velocity of the spike between points within the neuropile. One electrode was inserted into the soma and a second was moved progressively along the neurite from the edge of the ganglion toward the soma to record the extra- or intracellular spike. The axon decreases in diameter as it enters the ganglion from nerve 5, and the conduction velocity decreases to about 0·8 $m.s^{-1}$ between sites 14 and 11. From site 6 to the soma, a region of inactive membrane and for the greater part devoid of branches, the velocity increases to about 0·9 $m.s^{-1}$.

Spike initiating zone

The region from which orthodromic spikes are normally initiated can be estimated by comparing the delay of an orthodromic spike with that of an antidromic spike recorded at the soma and various points in the neurite. The rationale behind the experiment is this. If the spike initiating zone is distal to both the soma and the neurite electrode, then the time taken for an antidromic and an orthodromic spike to travel between these two recording sites will be the same. If, however, the spike initiating zone lies between the soma and the neurite electrode, then the delay, as measured at these two sites, will be *less* for an orthodromic than that for an antidromic spike. This is because at any particular time the orthodromic spike will be travelling in opposite directions away from the spike initiating zone (toward the soma and toward the electrode in the distal neurite). In contrast the antidromic spike initiated at the muscle travels in one direction, first passing the neurite electrode before invading the soma. Two electrodes were therefore inserted into the soma, while a third was moved along the neurite. Antidromic spikes were elicited by stimulation at the muscle and orthodromic ones by depolarizing the soma with one of the electrodes. Between the soma and an electrode placed where the axon enters nerve 5 (site 14), the antidromic spike had a latency of 1·56 ms whereas the orthodromic spike had the shorter latency of 1·26 ms. On the argument presented, this would, not unexpectedly, place the spike initiating zone within the ganglion somewhere between these two points. The neurite electrode was then moved toward the soma (site 11) and the measurements repeated. The antidromic spike had a latency of 1·25 ms while that of the orthodromic spike was 0·91 ms. This would place the spike initiating zone between site 11 and the soma and therefore within the region of neurite that bears the profusion of side branches. The zone can be delimited still further by recalling that the membrane between the soma and the region between sites 6 and 8 is inactive membrane (Fig. 4).

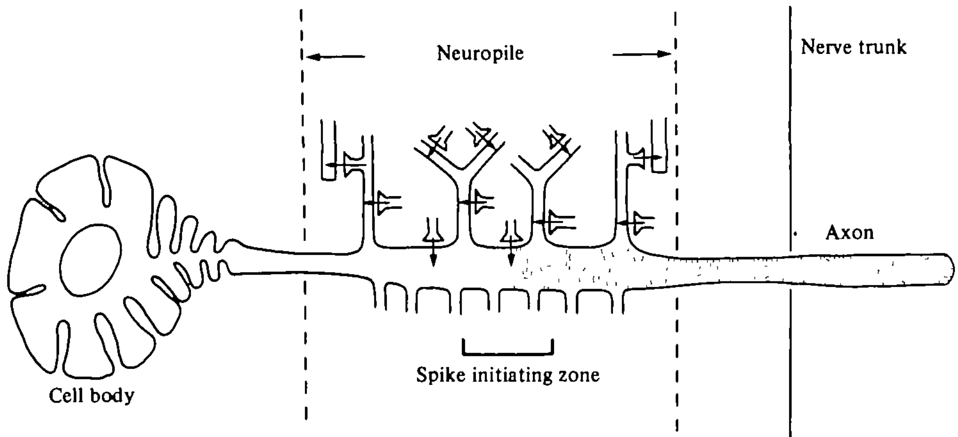

Fig. 7. A diagram of an insect motor neurone that incorporates the results of this paper. The soma is invaginated by glial processes that form the trophospongium at the point where the neurite emerges. In the neuropile the expanded main neurite bears a profusion of branches. Possible synaptic sites are indicated upon the main neurite, upon primary and secondary branches. Some branches are presynaptic to other neurones. The transition from 'active' (stippled) to 'inactive' membrane occurs along the region of neurite that bears side branches. The main neurite may narrow before leaving the ganglion as the axon.

Moreover overshooting action potentials have been recorded from site 8 (Fig. 3*c*). Combining these data suggests that the spike initiating zone lies between sites 8 and 11, perhaps close to the region of transition from active to inactive membrane.

DISCUSSION

This paper provides evidence concerning the electrical characteristics of the membrane of an insect motor neurone that have heretofore simply been assumptions incorporated into a generalized model (Hoyle, 1970). Its findings can be incorporated into a drawing of a simplified insect motor neurone (Fig. 7). In presenting such a stylized drawing we are acutely aware of the dangers of generalizing from one example. Indeed our results indicate that it may not be possible to generalize about the characteristics of arthropod motor neurones to the extent that had been thought possible (Evoy, 1977).

In the motor neurone we have studied, the FETi, the part of the neurite bearing a considerable number of side branches is capable of supporting a propagated action potential (Figs. 3, 4, 6). This result is contrary to the usual assumption that the region of neurite bearing branches is electrically inexcitable (Hoyle, 1970; Evoy, 1977). The conclusion presented here is based upon the waveform of the extracellularly recorded spike at different points along the path of the neurite within the neuropile. The interpretation of these waveforms is in accord with that presented for neurones in other animals (e.g. Edwards & Ottoson, 1958; Sandeman, 1969; Llinas, Bloedel & Roberts, 1969) and is consistent with the description presented by Woodbury (1963). The interpretation is based upon the extracellular rather than intracellular waveforms because of the problems encountered whenever multiple penetrations of a small neurite were attempted. The usual result of repeated intracellular penetrations was

damage that reduced the resting potential, and altered the shape of the spike and made its amplitude variable. Hence it was difficult to determine whether the action potential was overshooting. Even the interpretation of the extracellular records can be complicated by the possibility of damage caused by the electrode. For example, on occasion the somata of vertebrate motor neurones have been considered to be inexcitable on the basis of extracellular recordings (Freygang, 1958; Freygang & Frank, 1959), when in fact the reverse is true, and the more likely interpretation is that the membrane was damaged (Eccles, 1964). The possibility of damage influencing the shape of the extracellular waveform was minimized here by always obtaining extracellular records *before* the neurone was penetrated. The neurone was then penetrated, the electrode withdrawn and the same extracellular potential recorded. Any damage of the motor neurone immediately manifested itself as a series of spikes and a consequent series of rapid movements of a hind tibia. The intracellular recording from the soma that always accompanied the extracellular recordings from the neurite apparently inflicted no damage upon the neurone. The motor neurone would function normally for 2–4 h with an electrode in its soma. Another problem lies in determining accurately the position of the electrode within the ganglion. However, the most reliable method, that of cobalt marking, clearly showed that recordings interpreted as arising from active membrane were made within the region of the neurite from which numerous side-branches arise. The consistency with which the shape of the waveform could be correlated with particular positions of the electrode in different locusts is a strong argument for the conclusions drawn (Figs. 4, 7).

Spike initiating zone

The most straightforward way to determine the spike initiating zone would be to find the place from which a spike can be initiated with the smallest externally applied current. Although this approach has proved successful for a motor neurone innervating eye muscles in a crab (Sandeman, 1969), the method is not practicable here because of the undulating course described by the neurite within the neuropile. Therefore it is difficult to position an extracellular electrode at points equidistant from the neurite along its length. Under these circumstances an apparently low threshold would not necessarily be indicative of the spike initiating zone.

The method which was finally adopted, of comparing the delay between either an orthodromic or an antidromic spike recorded at the soma and different points on the neurite, could only indicate the likely *region* at which the orthodromic spikes arose. To locate the spike initiating zone more accurately would have required recordings from many more sites along the neurite of one locust with the attendant problems described before. Nevertheless, the results obtained are consistent with those observations which relate the electrical characteristics of the membrane to the extracellularly recorded waveform (Fig. 4). They are also consistent with the demonstration of an overshooting action potential in the region of the FETi neurite which bears many branches (Fig. 3*c*). The unexpected finding that electrically excitable membrane and the spike initiating zone are within what had previously been supposed to be the 'passive' integrating region of the neurone, must inevitably raise functional questions about this arrangement that are outside the scope of this present paper. Amongst th

many kinds of information that is now needed is the identification of the input and output synapses of this motor neurone, relating their distribution to the particular characteristics of the membrane that have been revealed here.

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