

MULTIPLE SITES OF SPIKE INITIATION IN A BIFURCATING LOCUST NEURONE

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SUMMARY

Recordings were made from the metathoracic dorsal unpaired median neurone to the extensor tibiae muscle (DUMETi) in the locust. This is a bifurcating neurone with axons exiting both sides of the ganglion, whose soma can support a full action potential. Four different spike types were recorded in the soma, each of which we associate with a different region of the neurone. These were (1) a soma (S) spike of 70–90 mV, (2) a neurite (N) spike of 20–40 mV, occurring between the axon hillock and axon branch point, (3) and (4) axon (A) spikes of 8–15 mV, occurring distal to the branch point on the left and right axons. Each of these regions must therefore have its own spike initiation site.

At spike frequencies greater than about 10 Hz at room temperature or 1–5 Hz at 32 °C (the preferred environmental temperature of the locust) the S-spike may fail, revealing A-spikes, or more rarely N-spikes. A-spikes usually consist of two more-or-less separate components, A_l and A_r , which can be correlated with action potentials in the left and right axon branches by recording spikes extracellularly in the peripheral nerves on each side. Occasionally single component A-spikes occur when an action potential is initiated in only one axon, and fails to propagate across the branch point to the contralateral axon. Thus, action potentials may occur independently in the branches of this bifurcating neurone. After unilateral axotomy only S-spikes and N-spikes are recorded, indicating that action potentials no longer fail to propagate across the branch point. Anatomical asymmetries in the axon branches of DUMETi have been correlated with physiological asymmetries recorded in the soma of the same neurone.

INTRODUCTION

On the dorsal surface of the thoracic and abdominal ganglia of the locust and cockroach (and probably other insects) there occur groups of neural somata clustered about the midline. These neurones are unpaired; they do not occur as bilaterally symmetrical homologues. Rather each soma gives rise to a single short neurite which bifurcates into (usually) symmetrical processes that approach the lateral margin at each side of the ganglion, and in some cases exit through peripheral nerves (Plotnikova,

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1969; Crossman *et al.* 1971). The cells were thus termed dorsal unpaired median (DUM) neurones (Hoyle *et al.* 1974). The somata of these neurones are capable of carrying large, overshooting action potentials 70–110 mV in amplitude (Crossman *et al.* 1971). This is in contrast to the numerous somata located on the ventral surface of the ganglion, in which only attenuated action potentials (usually 2–5 mV) are recorded, having been electrotonically propagated from more or less distant regions of active membrane (Crossman *et al.* 1971; Hoyle & Burrows, 1973).

The largest DUM neurone in the locust metathoracic ganglion has axons emerging bilaterally in nerve five (nomenclature of Pringle, 1939). These axons terminate in the extensor tibiae (ETi) muscles, which provides the power for the locust jump (Hoyle *et al.* 1974). Only one DUM neurone innervates the ETi muscles, and therefore this identified neurone is referred to as the dorsal unpaired median neurone innervating the extensor tibiae (DUMETi). DUMETi has not been shown to form conventional neuromuscular junctions with the muscle fibres. Both the terminals and the pre-terminal axons are filled with large (60–170 nm) dense core vesicles (Hoyle *et al.* 1974). No direct mechanical or membrane potential response has been detected in the muscle in response to stimulating DUMETi. This led to the suggestion that DUMETi has an innervating neurosecretory role, rather than normal motor function (Hoyle *et al.* 1974). Although stimulation of DUMETi produces no mechanical effect of itself, it can modulate an intrinsic myogenic rhythm in the ETi muscle (Hoyle & O'Shea, 1974; Hoyle, 1974; Evans & O'Shea, 1978), and it can modulate the electrical and mechanical effect produced by the slow excitatory motoneurone innervating this muscle (O'Shea & Evans, 1977). The modulatory effects of DUMETi can be mimicked by the application of low concentrations of octopamine to the isolated muscle (Hoyle, 1974; Evans & O'Shea, 1977, 1978), and the soma of DUMETi has been shown to contain octopamine (O'Shea & Evans, 1977).

An antidromic spike in the axon of a DUM neurone initiated by electrical stimulation of the peripheral terminals does not always initiate an overshooting action potential in the soma. Crossman, Kerkut & Walker (1972) found that during maintained high-frequency stimulation of the peripheral axon of an unidentified DUM neurone in the cockroach the first few stimuli initiated full size action potentials in the soma. A point of inflexion then appeared in the rising phase of the soma (S) spike, and this was followed by a progressive dissociation between the S-spike and a residual, smaller depolarizing wave, until finally the soma spike failed completely. The residual wave was identified as the axon (A) spike propagating electrotonically into the soma but failing to initiate a soma spike, a situation analogous to that described for various molluscan neurones (see for example, Tauc, 1962*a, b*).

We examine the various kinds of spikes recorded in the soma of an identified DUM neurone in the locust, DUMETi, and attempt to relate them to neural geometry. Four different spike types were recorded, and these we associate with four different regions of the neurone. Anatomical asymmetries occurring naturally in development, and as a result of surgery, are correlated with changes in the spike types recorded in the soma.

MATERIALS AND METHODS

Adult male and female locusts (*Schistocerca nitens*) were obtained from crowded laboratory colonies maintained at 32 °C and 50% humidity, with a 16/8 h light/dark cycle. The locust was restrained by embedding its legs in Plasticene, and the nervous system exposed by dissecting from the dorsal aspect. A longitudinal cut was made through the dorsal cuticle and dorsal longitudinal flight muscles, and the pleuron on each side, with its attached dorso-ventral flight muscles, was folded outwards. The gut was removed. Cutting the meso- and metathoracic pleuro-sternal apophyses freed the thoracic central nervous system so that a waxed platform could be placed under the metathoracic ganglion. The ganglion was stabilized on this platform with small pins placed through the tracheal sacks and fatty tissue surrounding the ganglion. The following physiological saline was used: NaCl, 140 mM; KCl, 10 mM; CaCl₂, 5 mM; TES buffer, 5 mM (pH adjusted to 7.2 with a few drops of NaOH). A heating coil could alter the temperature of the saline just before it entered the thoracic cavity. Usually the metathoracic ganglion was partially isolated from the rest of the nervous system by cutting the connectives and some of the peripheral nerves. This reduced the probability of spontaneous activity of the thoracic muscles, and hence dislodgement of microelectrodes through movement. Under these conditions the ganglion remained healthy for several hours, as judged by the occurrence of normal resting potentials, spike amplitudes, spike activity, and synaptic activity in DUMETi, and other neurones.

Recordings were made from the soma of DUMETi (diameter about 50 µm) using glass microelectrodes filled with 3 M potassium acetate, with resistances in the range 20–50 MΩ. Current could be passed into the cell via a bridge circuit built into the high input impedance pre-amplifier. The position of the soma of DUMETi was extremely variable, and finding it involved making more-or-less random penetrations over about two-thirds of the dorsal surface of the ganglion. Once penetrated, DUMETi could be distinguished from other DUM cells by correlating spikes recorded intracellularly with spikes recorded extracellularly from the peripheral axons of DUMETi. These axons occur in nerve 5b₁d (by analogy with the mesothoracic nervous system described by Campbell, 1961) in the metathoracic femora. This nerve runs across the ventral surface of the extensor tibiae muscle, and was exposed by removing the flexor tibiae muscle. The nerve was recorded and stimulated using paired stainless steel hook electrodes. Nerve 5b₁d contains the axons of four neurones; DUMETi, the fast extensor tibiae, the slow extensor tibiae and the common inhibitor. The extracellular spikes of DUMETi and the common inhibitor neurone were of similar amplitude and often occurred in close succession, but DUMETi spikes could be identified because at room temperature and low spike frequencies they were always temporally paired in the nerves 5b₁d of the two sides, which was not the case with the other neurones. Furthermore, DUMETi is the only neurone of these four to have its soma on the dorsal surface of the ganglion.

Intracellular staining of DUMETi was achieved by penetrating the soma with an electrode containing 0.2 M cobalt chloride. Cobalt ions were injected with depolarizing current pulses. The cobalt ions were precipitated as a sulphide salt by the addition of 5% ammonium sulphide in saline for several minutes. The ganglia were then

washed in saline, fixed in Carnoy's solution for 7 min, dehydrated (5 min in 95% ethanol, 2 washes 10 min each in 100% ethanol) and cleared in methyl benzoate. The ganglia were initially viewed and photographed as whole-mount preparations. They were then paraffin-embedded and cut in 15 μ m serial sections. The cobalt profiles were silver-intensified using a modification of Timm's sulphide silver method as described by Tyrer & Bell (1974) and further modified by Goodman (1977).

RESULTS

Recordings from DUMETi, at room temperature show that in the dissected locust activity ranges from a complete absence of spontaneous spikes up to tonic frequencies of about 0.5 Hz. Tonic spikes are clearly synaptically driven (Fig. 1*a*) and there is no evidence of autoactivity. Spikes may be elicited in response to a wide range of environmental stimuli, including tactile, auditory and visual sensory inputs (e.g. Fig. 1*b*). However, all specific sensory modalities appear to habituate very rapidly, and even such shock tactics as high intensity electrical stimulation of major sensory

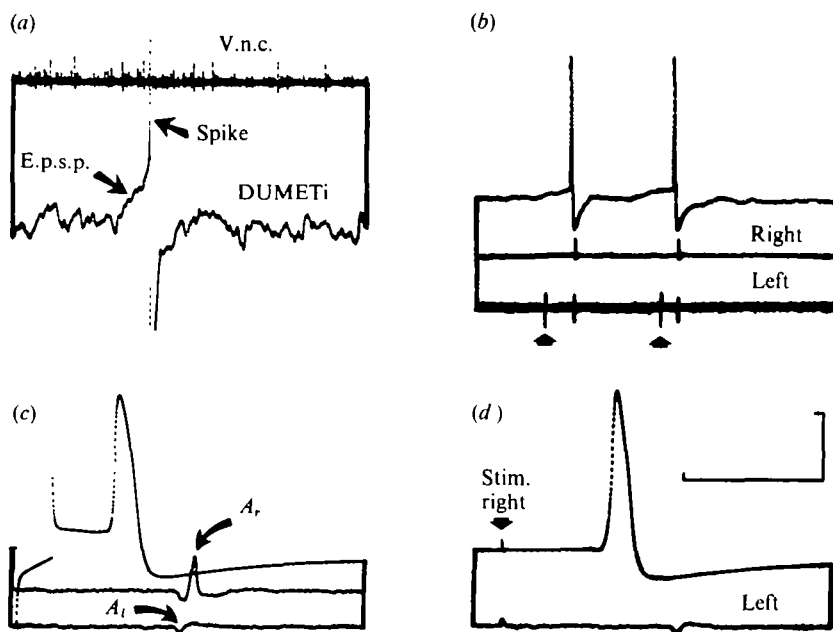


Fig. 1. Modes of activation of DUMETi. (a) Spontaneous EPSPs give rise to a spike in DUMETi (lower trace, clipped by the oscilloscope screen), and do not correlate with activity recorded extracellularly from one of the connectives between the pro- and mesothoracic ganglia (upper trace). (b) A light off/on stimulus causes two soma spikes in DUMETi (upper clipped by top of oscilloscope screen), which can be correlated with axon spikes recorded in the right peripheral nerve 5b₁d (middle) and the left, where two spikes from the common inhibitor are also recorded (arrows, lower). (c) A short duration pulse of depolarizing current is injected into the soma, and initiates an S-spike (upper). Axon spikes are recorded extracellularly in the right (middle) and left (lower) peripheral axons. (d) The right peripheral axon is stimulated, and an S-spike recorded in the soma (upper), and an axon spike in the left peripheral axon (lower). Calibration: vertical (a) 4 mV, (b) 20 mV, (c, d) 40 mV; horizontal (a) 800 ms, (b) 400 ms, (c, d) 20 ms.

nerve trunks in the leg or the ventral nerve cord fail to elicit spikes in DUMETi with any regularity. The only way experimentally to produce spikes in DUMETi both repeatedly and predictably is directly to stimulate the neurone itself. A spike can be elicited by injection of small amounts of current (1–3 nA) across the somatic membrane. This stimulation regime will, under most circumstances, cause spikes in both branches of the neurone, and these can be recorded extracellularly in the periphery (Fig. 1*c*). Usually current injected into the soma initiates a spike in the neuropil which propagates back and secondarily initiates a soma spike (originally described by Hoyle *et al.* 1974). A soma spike can also be initiated by electrical stimulation of the peripheral axon on one side. This causes an antidromic spike to propagate centripetally to the branch point within the ganglion, then centrifugally down the contralateral branch and simultaneously somatopetally up the neurite to the soma. Successful propagation of the spike across the branch point can be checked extracellularly from the contralateral peripheral axon (Fig. 1*d*). It is this stimulation regime which was originally used to demonstrate the A-spike/S-spike separation (Crossman *et al.* 1972), and which we used here to examine the various spike components.

S-spikes, A-spikes, and N-spikes

Soma spikes in DUMETi will usually occur after each stimulus delivered to one of the peripheral axons at frequencies up to 5 Hz at room temperature. However, stimulation at higher frequency can cause loss of the S-spike. In one stimulus train at 10 Hz (Fig. 2) the first 15 stimuli initiated soma spikes, but with succeeding stimuli there was an increasing probability of soma spike failure. Stimuli 16–21 initiated alternately S-spikes and A-spikes, then the A-spikes predominate over the S-spikes, until stimuli 65–100 elicit only A-spikes. There is an unmistakable difference between S-spike and A-spike; the S-spike mean amplitude is 89 mV, whereas the A-spike mean amplitude is only 12 mV. The amplitude of the S-spike is very constant throughout the stimulus train (s.d. = 2% of mean), but the amplitude of the residual A-spike is much more varied (s.d. = 28% of mean). Examination of the A-spike reveals it to be composed of discrete components. When a spike in the contralateral peripheral branch of DUMETi indicates that transmission has successfully crossed the ganglion, the A-spike in the soma can usually be seen to be the summated waveform of two components, more or less separated by an inflexion on the rising wavefront (Fig. 3*a, b*). Much of the variability in A-spike amplitude results from the variations in temporal summation of these two components. However, when there is no spike recorded in the contralateral periphery, and therefore transmission had failed at some point in the neurone, the A-spike recorded in the soma consists of only one component (Fig. 3*c*). The A-spike amplitudes in which contralateral transmission failed form a group within the overall A-spike group with reduced mean and variation (10 mV \pm 10%, Fig. 2). There is no overlap in amplitude between the single-component A-spikes where contralateral transmission fails, and the double-component A-spikes where contralateral transmission succeeds: the single-component spikes are all smaller than the double-component spikes.

We conclude that the two components present in the A-spike when transmission across the ganglion succeeds are the attenuated waveforms of two spatially separate

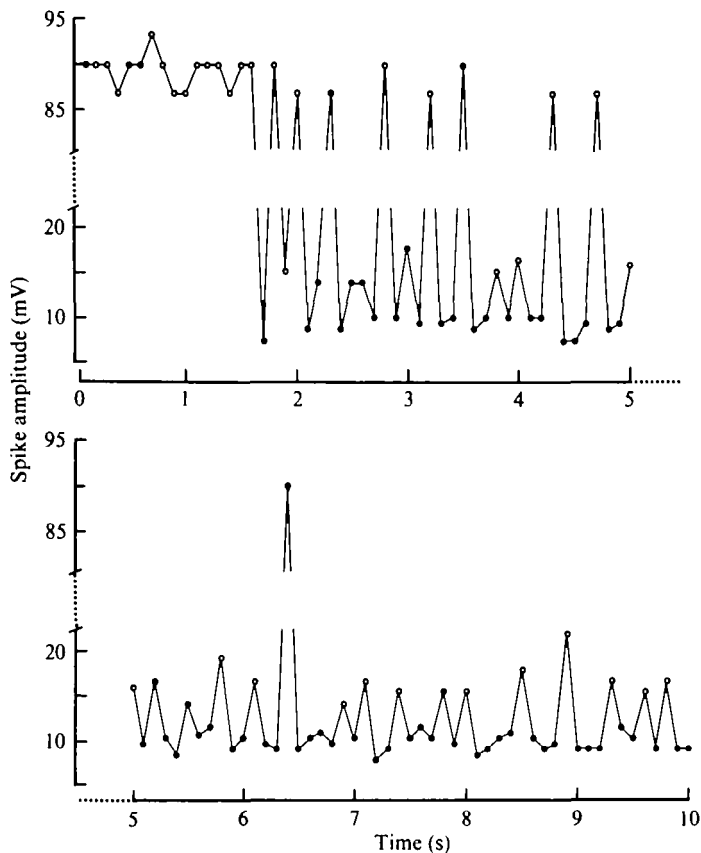


Fig. 2. A graph of the amplitude of spikes recorded in the soma of DUMETi upon stimulating one axon branch in the periphery at 10 Hz, against time. The closed circles are single-component axon spikes in which no spike was recorded extracellularly in the contralateral periphery. The open circles are double-component axon spikes and soma spikes in which a spike was recorded in the contralateral periphery.



Fig. 3. Spike components in DUMETi. The right peripheral axon is stimulated and recordings made from the left peripheral axon (lower) and the soma (upper). (a) Two stimuli sequences are superimposed. The first initiates a soma (S) spike and a left peripheral axon spike, the second initiates a double-component axon spike (A) and a left peripheral axon spike with a slightly longer latency. (b) A single stimulus initiates a double-component A-spike in which the inflexion separating the two components can be clearly seen (arrow), and a spike in the left peripheral axon. (c) A single stimulus initiates a single-component axon spike, and fails to initiate a spike in the left peripheral axon.

active membrane potentials in DUMETi, one from each side of the branch point. Thus, stimulation of one of the peripheral axons of DUMETi causes an antidromic action potential to propagate centripetally. At some point within the ganglion distal to the branch point the action potential fails, and further propagation is then electrotonic. The depolarizing wave spreads past the branch point, both up the neurite towards a region of spike initiation near the soma, and contralaterally towards a region of spike initiation in the opposite branch. If the attenuation in the neurite is sufficient then the depolarizing wave will fail to cross the somatic threshold, and no soma spike will occur. If the attenuation to the contralateral branch is sufficient no spike will be initiated there, and transmission across the ganglion will fail. In this case all that will be recorded in the soma is a single-component A-spike from the original stimulated axon. If, however, the depolarizing wave propagates to the contralateral side with sufficient amplitude, it will elicit a spike there. This then propagates centrifugally as an action potential which can be recorded in the contralateral peripheral axon, and centripetally as a passive electrotonic potential. It does not initiate a second action potential in the original stimulated axon, presumably because this is still refractory, but it can be recorded in the soma shortly following the original depolarizing wave. This is the second component of the two-component A-spike which occurs when propagation across the ganglion is successful. Assuming a symmetrical neurone, the latency of the two components of the A-spike recorded in the soma is a direct measure of the time between failure of the spike in one axon branch and initiation of the spike in the other axon branch. This time is variable within a single preparation, as can be seen in Fig. 3(a), where two stimuli sequences are superimposed. In the first sweep an S-spike occurs: propagation from the stimulated axon to the soma is sufficient to elicit a soma spike, and propagation to the contralateral side initiates a spike there so that an action potential can be recorded extracellularly in the peripheral axon with a certain latency from the stimulus. In the second sweep the S-spike fails, but the A-spike is composed of two components and once again a spike can be recorded in the contralateral peripheral axon. However, this spike has a longer latency than the previous spike. Examples will be introduced below in which the variation between the two components of the A-spike recorded in the soma itself is seen. The variation in the amplitude of the single-component A-spike recorded in the soma (Fig. 2) confirms that electrotonic transmission varies. We do not know whether this variation is due to changes in the exact location of the A-spike failure within the ganglion, or changes in the passive properties of the membrane due to synaptic inputs.

Occasionally spike heights intermediate between the full S-spike and the two-component A-spike are observed, particularly in the early stages of a stimulus sequence when the S-spike first starts to fail (Fig. 4a). Such intermediate spikes were not recorded over the complete amplitude range between S- and A-spikes. The largest intermediate spikes are about 40 mV, and smaller intermediate spikes occur over the range from this maximum down to the size of two-component A-spikes (about 12 mV). These intermediate spikes usually lack the afterhyperpolarization characteristic of the S-spike. We interpret these intermediate spikes as being neurite spikes (N-spikes), i.e. spikes occurring in the link segment between the branch point and the soma. Presumably, the largest N-spikes fail in the neurite close to the soma, while the smaller spikes fail at progressively more distant points on the neurite, until failure occurs distal to the branch point and a two-component A-spike is recorded in the

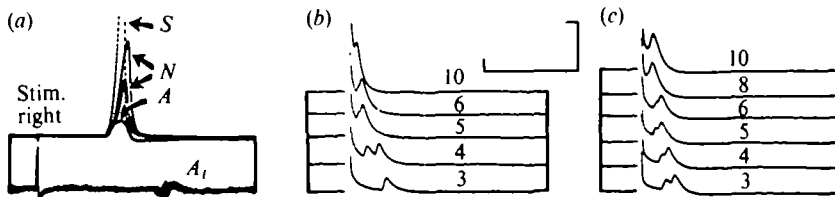


Fig. 4. (a) The right axon of DUMETi is stimulated at 10 Hz and recordings made from the soma (upper) and left peripheral axon (lower). Four non-consecutive stimuli are superimposed. One stimulus produced an S-spike, two produced N-spikes of varying amplitude, and one produced a double-component A-spike. A peripheral spike was recorded in the left axon with each stimulus. (b, c) Depolarizing pulses of 0.1 ms duration and varying amplitudes (noted in nA in the figure) are superimposed on a constant 2 nA hyperpolarizing current injected into the soma. They produce single- and double-component A-spikes with varying degrees of summation between the components. Calibration: vertical, 20 mV; horizontal (a) 20 ms, (b, c) 40 ms.

soma. It must be emphasized that separate N-spikes are a relatively rare event; in many cases the S-A spike transition is made with no intervening N-spikes (although N-spikes, like A-spikes, may presumably occur simultaneously with S-spikes, but be masked by the greater amplitude of the latter).

We conclude from these experiments that each DUMETi can carry four different spike types each associated with distinct regions of the neurones: one in each of the axon branches, one in the neurite, and one in the soma. Each of these spiking regions is separated from the others by a region of membrane in which action potentials fail (about the branch point and in the proximal portion of the neurite), and therefore each spiking region can have its own spike initiation site (SIS) (see Fig. 5). (Spike initiation site does not, of course, necessarily imply that spikes are initiated in response to synaptic input.)

The S-spike in DUM cells can be abolished by injecting hyperpolarizing current into the soma (Crossman *et al.* 1972). In one experiment hyperpolarizing current of 2 nA was sufficient to abolish the S-spike in DUMETi. Short duration (0.1 ms) depolarizing current pulses of increasing intensities (3–10 nA) were then superimposed on the hyperpolarizing 2 nA d.c. (Fig. 4*b, c*). At low intensities a single A-spike is initiated. As the intensity is increased a second A-spike occurs, which is initially well separated from the first, but with further increase in intensity it merges with the first spike to achieve a two-component A-spike which is approximately twice the amplitude of the single-component A-spike first initiated.

Peripheral stimulation of one of the DUMETi axons at 10 Hz causes a hyperpolarization of the soma of some 10 mV (Fig. 6*a*). This is not merely a summation of the afterhyperpolarization of the S-spikes, since it is maintained even after S-spike failure, and the membrane potential only recovers over the course of some 5 s after cessation of stimulation. Although this hyperpolarization may contribute to the S-spike failure, it is not the sole cause, since stimulation at 5 Hz produces a similar hyperpolarization, but does not cause failure of the S-spike (Fig. 6*b*). Stimulation at both 5 and 10 Hz causes an increase in the afterhyperpolarization of the S-spike. Neither the cause of the increased afterhyperpolarization, nor the cause of the overall membrane hyperpolarization is known (but see section 'The effect of unilateral axotomy' below).

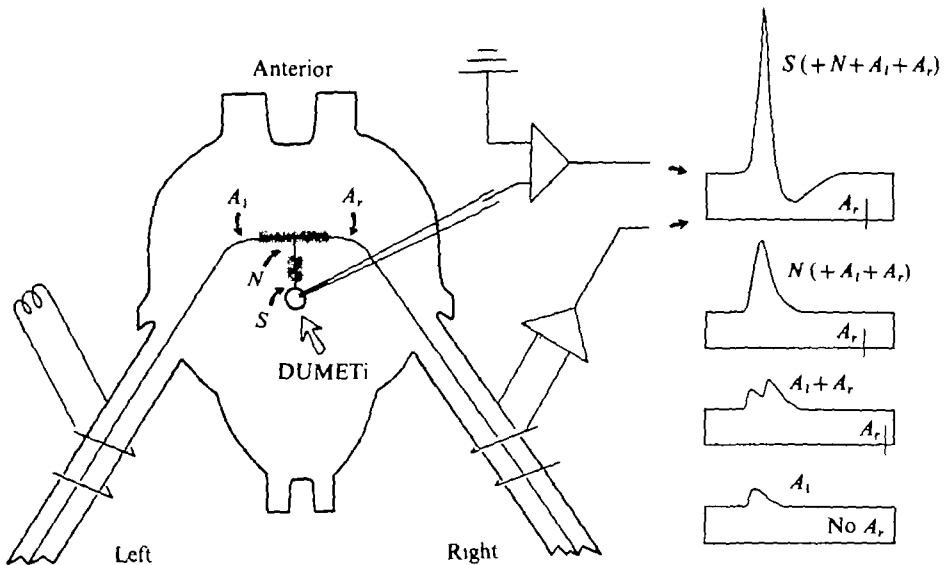


Fig. 5. A diagram to show the experimental situation and results described so far, and our interpretation of these results. On the left is the metathoracic ganglion, showing the soma of DUMETi and the major axon branches. The left axon is stimulated, and recordings made intracellularly from the soma, and extracellularly from the right axon. Extracellular electrodes were in fact placed on the extensor nerve sb_1d , rather than as diagrammed on the whole nerve five. On the right typical results are diagrammed, showing the four spike components we identify: the S-spike, N-spike, double-component A-spike, and single-component A-spike. Within the ganglion the stippled areas show the regions of DUMETi where spikes may fail, and the proposed regions of spike initiation are arrowed.

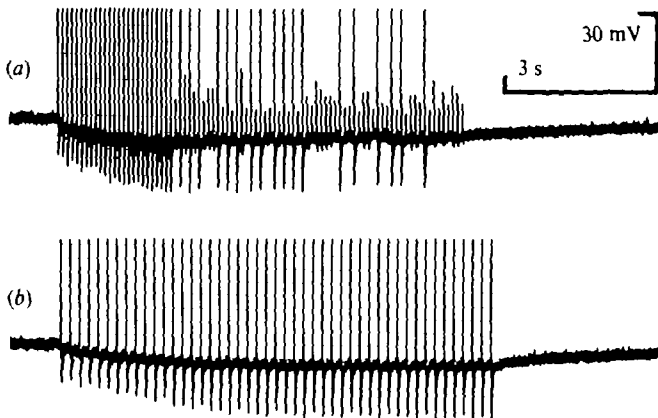


Fig. 6. Spikes are recorded in the soma of DUMETi while one peripheral axon branch is stimulated at (a) 10 Hz and (b) 5 Hz. The S-spikes are clipped by the recording device. The membrane hyperpolarizes by about 10 mV in both situations.

Failure of S-spikes with increasing temperature

The experiments reported so far in this paper, and all previous experiments on DUM cells described in the literature, have been carried out at room temperature (19–22 °C). However, the preferred environmental temperature of the locust is

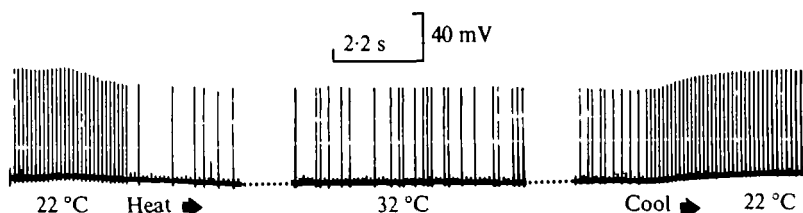


Fig. 7. One of the peripheral axons of DUMETi is stimulated at 1 Hz and spikes are recorded in the soma. At room temperature S-spikes follow the stimulus one for one, but as the temperature is increased to 32 °C the S-spike starts to fail, leaving one- and two-component A-spikes. On cooling the preparation back to room temperature the S-spike failures cease. Changes in membrane potential and spike height are a good monitor for the time course of the change in temperature (Heitler *et al.* 1977).

greater than 30 °C (Strelnikov, 1935, 1936), and the locusts used in our experiments were reared in, and thus acclimated to, an insectary maintained at 32 °C. Increasing the temperature from 22 to 32 °C has been shown to cause a number of reversible effects in locust neurones (Heitler, Goodman & Rowell, 1977) including a decrease in spike threshold, a decrease in spike amplitude and duration, a membrane hyperpolarization, and a transient decrease in membrane resistance. In view of these effects, the spike properties of DUMETi have also been examined over this temperature range.

As stated above, the S-spike in DUMETi follows stimulation of the peripheral axon at 1 Hz in a one-to-one manner at room temperature. If the preparation temperature is increased during such a stimulus regime, there is a decrease in S-spike amplitude. In one preparation (Fig. 7) the S-spike amplitude at 22 °C was 90 mV, and it decreased to 77 mV at 27 °C. Further increase in temperature to 32 °C caused frequent S-spike failure, revealing double-component A-spikes of 6 mV amplitude, and single-component A-spikes of 3.5 mV amplitude. During the initial heating phase there was a high frequency of S-spike failures, which then declined somewhat to a steady state in which about 55% of the stimuli resulted in S-spike failure. This slight decrease in the failure rate is probably due to recovery from the transient decrease in membrane resistance which accompanies initial heating (Heitler *et al.* 1977). Usually temperature changes occurred in less than 30 s, steady state was achieved within 2 min, and many preparations were maintained at steady state for up to 15 min. In the steady-state conditions of Fig. 7, 87% of the A-spikes were single-component spikes in which contralateral transmission failed. Thus at the preferred environmental temperature of the locust in this preparation even low frequency stimulation at 1 Hz failed to initiate S-spikes more than half the time, and when the S-spike failed the A-spike of the stimulated axon branch rarely succeeded in initiating a spike in the contralateral axon branch.

In some preparations DUM neurones show spontaneous spikes at room temperature (which could be recorded extracellularly in the periphery before penetration of the soma). At a tonic spike frequency of about 0.5 Hz a normal S-spike is recorded (Fig. 8a). On increasing the temperature of the preparation the spike frequency usually increases, and the S-spike is preceded by a two-component pre-potential, the A-spike (Fig. 8b). Occasionally, the S-spike fails altogether. At maintained temperature (30 °C) the spontaneous spike frequency of one preparation increased to about 3 Hz, and the S-spike failed completely. The two components of the A-spikes had varying latency and degrees of summation, and about 20% of the A-spikes had only a single

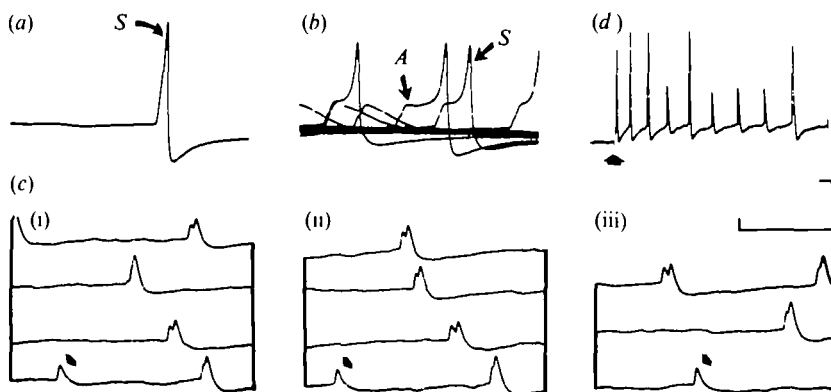


Fig. 8. The effect of temperature on spontaneous spikes in DUM neurone. (a) At 22 °C a normal S-spike is recorded. (b) At 27 °C the S-spikes and A-spikes start to dissociate, and occasional S-spike failures occur. (c) (i-iii) At 30 °C in this preparation no S-spikes occur, and only double- and single- (arrowed) component A-spikes are recorded. (d) If the cell is prevented from spiking at this temperature by injecting hyperpolarizing current, and then the current is switched off (arrow), a few S-spikes occur initially as the cell starts to spike again. The data in this figure are from a DUM neurone not positively identified as DUMETi owing to failure of the extra-cellular electrodes. Calibration: vertical (a, b, d) 20 mV, (c) 10 mV; horizontal (a, c) 80 ms, (b) 40 ms, (d) 800 ms.

component (Fig. 8c). The neurone could be prevented from spiking by injecting hyperpolarizing current, and upon release from this hyperpolarization the first few spikes had the full size S component. However, as spiking continued the S-spike failed, leaving just the one- and two-component A-spikes (Fig. 8d).

The above result is typical of preparations in which DUMETi shows a relatively high frequency of spontaneous (synaptically driven) spikes. However, in some preparations DUMETi shows no spontaneous activity, and even quite violent mechanosensory stimulation only elicits one or two spikes, even at high temperatures. In these circumstances, peripheral recordings always reveal paired bilateral axon spikes when a response is elicited; no independent action potentials occur unilaterally. Failure in transganglionic transmission can be demonstrated in these preparations by stimulating the axon on one side and recording on the other, but the failure occurs at a higher stimulation frequency than in preparations which show a greater level of spontaneous activity. Thus, in one preparation where DUMETi showed no spontaneous activity, spikes followed the contralateral stimulus at up to 10 Hz at 33 °C, failure only occurring when the stimulus frequency was increased to 14 Hz. However, in other preparations where DUMETi showed no spontaneous activity, failure occurred at this temperature at 1–2 Hz. We thus conclude that the probability of successful transganglionic transmission of the DUMETi axon spike is in general inversely proportional to spike frequency and temperature, but that there is considerable variation from preparation to preparation.

Two effects of increasing temperature are a membrane hyperpolarization and a decrease in spike amplitude. We have already discussed the possible effects of membrane hyperpolarization on spikes in DUMETi. Spike amplitude can be experimentally reduced at room temperature by bathing the nervous system in a sodium-free saline. This eventually abolishes all spike activity but before this a sequence of

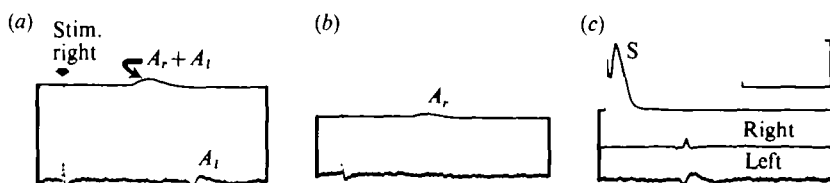


Fig. 9. The effect of saline containing no sodium (Goodman & Heitler, 1977) on spikes in DUMETi. (a, b) The right axon is stimulated, and recordings made from the left axon (lower) and the soma (upper). After about 10 min the S-spike fails and a double-component A-spike of reduced amplitude is recorded in the soma (a). A few minutes later only single-component A-spikes are recorded, and contralateral transmission fails (b). (c) Injection of depolarizing current into the soma at this point produces an S-spike of reduced amplitude (upper), and spikes in the right (middle) and left (lower) peripheral axons. Calibration: vertical (a, b) 20 mV, (c) 40 mV; horizontal 20 ms.

events similar to that found on heating occurs (Fig. 9). With maintained stimulation at 1 Hz of one of the peripheral axons the first effect of zero-sodium is loss of the S-spike, revealing a low amplitude double-component A-spike. A little later contralateral transmission fails, and the A-spike has only a single component. At this stage a reduced-amplitude S-spike can still be elicited by injecting depolarizing current into the soma, and this initially causes axon spikes in both branches of the neurone. Later the S-spike only initiates an action potential in one axon branch. Later still, spikes can no longer be elicited by intrasomatic depolarization or peripheral axon stimulation. On returning to a saline containing normal concentrations of sodium the reverse sequence of events is observed. Obviously the reduction in spike amplitude induced by low sodium does not precisely mimic that produced by increasing temperature particularly in terms of spike duration, but nonetheless the parallel sequence of events observed under these two conditions suggests that the decrease in spike amplitude produced by an increase in temperature may be one of the factors contributing to both S-spike failure and contralateral transmission failure in DUMETi at higher temperatures.

Axonal asymmetry in DUMETi

In most preparations the two components of the A-spike are approximately equal in amplitude. However, in one preparation there was a striking asymmetry in the A-spike components which enabled us to determine which component belonged to the axon spike of each side. This preparation was spontaneously active at about 0.6 Hz at room temperature, and on heating to 35 °C the frequency increased to 4–6 Hz. At this temperature and frequency the S-spike failed more than 60% of the time. Stimulating one of the peripheral axons caused either a double- or a single-component A-spike, and in the double-component spikes, the components were of different amplitudes and well separated. Stimulating the right side axon produced a two-component A-spike in which the first component was 8.8 mV in amplitude and the second component 3.7 mV (Fig. 10a). Stimulating the left axon produced a two-component spike where the first component was 4.1 mV, and the second component varied between 7.4 mV (Fig. 10b) and 5.9 mV (Fig. 10c). In this way we were able to distinguish between the two components and show that the larger component (A_r) was associated with a spike in the right side axon, and the smaller component

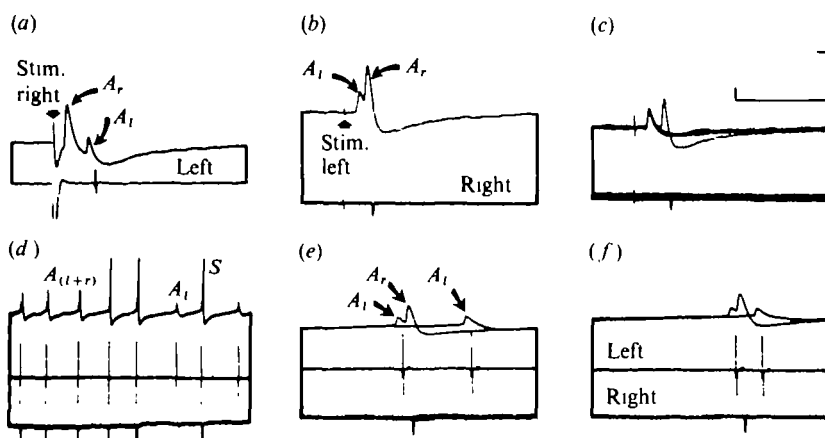


Fig. 10. Asymmetric A-spikes recorded from DUMETi in one preparation, with the temperature maintained at 30–32 °C. (a) Stimulating the right peripheral axon causes a double-component A-spike in the soma (upper), in which the first component, A_r , is larger than the second, A_l . A spike is recorded in the left peripheral axon (lower). (b) Stimulating the left peripheral axon reverses the order of the components in the soma. (c) Five consecutive stimuli of the left axon are superimposed, only one of which initiates a double-component A-spike and a spike in the right peripheral axon. (d) Spontaneous spikes in DUMETi recorded in the soma (upper) and the left (middle) and right (lower) peripheral axons. (e, f). The same on an expanded time scale. Two traces are superimposed, and no S-spikes occur. A_l always precedes A_r in the double-component spikes, and no single A_r spikes occur. Calibration: vertical (a–c) 10 mV, (d–f) 20 mV; horizontal (a–c, e–f) 80 ms, (d) 800 ms.

(A_l) with a spike in the left side. Occasionally contralateral transmission failed on stimulating the left axon, and then the large A-spike component, A_r , was missing (Fig. 10c). In two sequences stimulating the left side, the latency between A_l and A_r , recorded in the soma increased from 7.0 ms (Fig. 10b) to 12.9 ms (Fig. 10c), and the total conduction time from stimulating the axon in the periphery on the left side to recording the axon spike in the periphery on the right side increased from 25.5 ms to 31.6 ms – an increase of 6.1 ms which is almost entirely accounted for by the 5.9 ms increase in the A_l – A_r latency. In the sequence with the longer latency (Fig. 10c) A_r is 5.8 mV in amplitude, compared to the shorter latency sequence (Fig. 10b) where A_r is at least 7.3 mV. A_l amplitude is unchanged at 4.1 mV, as is its latency from the stimulus. This suggests that some change has occurred between the branch point and the site of A_r -spike initiation; either some synaptic input in this region has decreased the space constant and increased the time constant, or the site of A_r -spike initiation itself has moved slightly distally. In all cases (including other preparations) the A-spike component of a given side is slightly larger when initiated by direct stimulation of that side, rather than by the electrotonically conducted A-spike component resulting from stimulation of the contralateral side. This suggests that for both sides the site of spike failure is slightly closer to the soma than the site of spike initiation, or that the passive propagation of the first A-spike across the branch point alters the membrane properties of this region in such a way as to increase attenuation of the somatopetal passive propagation of the second A-spike.

In this preparation there was an asymmetry not only in the amplitudes of the A-spike components, but also in the pattern of spontaneous activity. Over the course

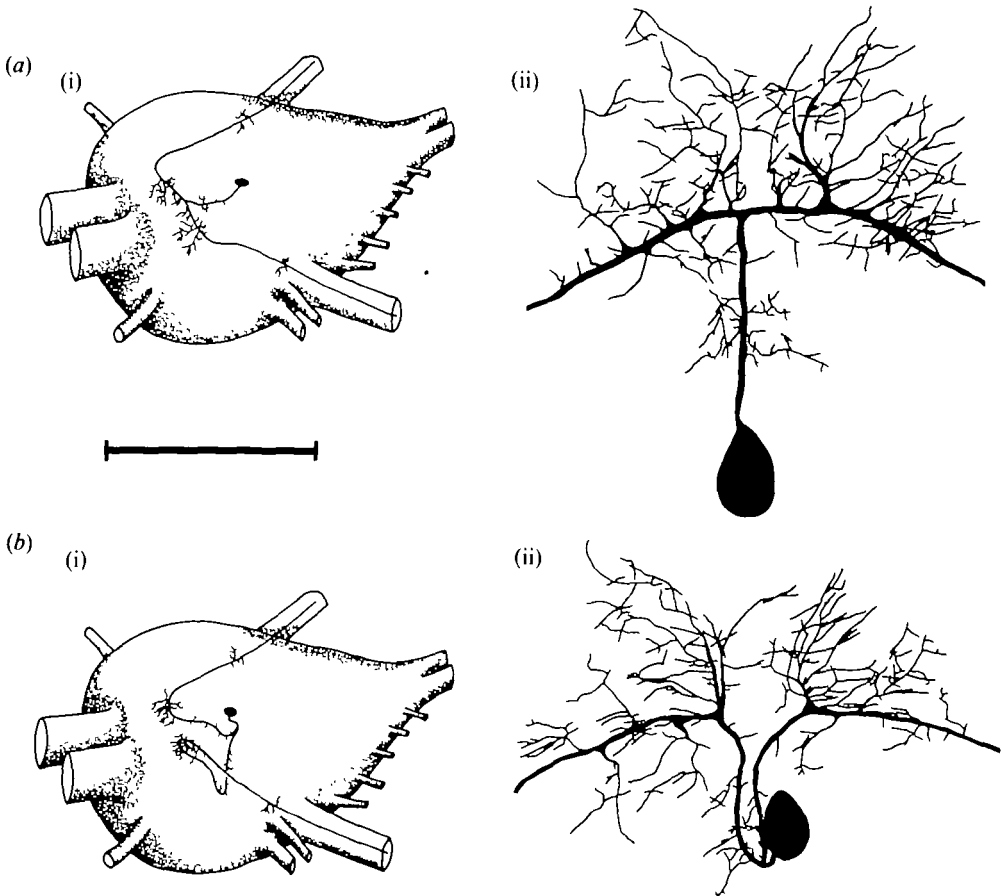


Fig. 11. The anatomy of DUMETi as revealed by cobalt sulphide staining, drawn semi-diagrammatically from whole-mounts of the metathoracic ganglion (i), and reconstructed from horizontal sections of the ganglion intensified by precipitation of silver in a modification of Timm's method (ii). (a) A normal DUMETi in which left and right axon spikes were of approximately equal amplitude. (b) A DUMETi in which the right axon spike was much bigger than the left axon spike (see text). Note the large ventral loop in the left axon branch. Calibration: (i) 0.6 mm (approx.), (ii) 200 μ m.

of a minute at 30 °C 297 spikes were recorded in the left peripheral axon, of which 66 were not paired with spikes in the right axon. No spikes were recorded in the right axon which were not paired with spikes in the left axon. In other words, 22% of the spikes recorded intracellularly were single-component A_i -spikes, but no single-component A_r -spikes were recorded (Fig. 10d). Furthermore, in the double-component A-spikes A_i always preceded A_r (Fig. 10e, f). It would thus appear that in this preparation all the spontaneous spikes were being initiated on the left side of the ganglion, and that in 22% of the cases electrotonic propagation to the right side was insufficient to elicit a spike there. Unilateral A spikes continued to occur (at reduced frequencies) at temperatures as low as 24–26 °C, in contrast to most preparations where axon spikes at these temperatures were always paired.

Upon completion of the physiological experiments on this preparation, DUMETi

was penetrated with an electrode filled with cobalt chloride. The cobalt was injected with positive current pulses and then precipitated as the sulphide salt. We were thus able to examine the anatomy of this DUMETi, which showed a definite physiological asymmetry, and compare it with the anatomy of a normal DUMETi in which the two axon spikes were approximately equal in amplitude (Fig. 11*a*). In a normal, physiologically symmetrical DUMETi, a neurite arises from the soma on the dorsal surface of the ganglion and travels ventrally and somewhat anteriorly for about 50 μm . It then travels anteriorly in the horizontal plane for a further 120 μm , moves dorsally and anteriorly about 30 μm , and bifurcates into the lateral axon processes at a T-junction. A few small dendritic processes arise from the neurite as it approaches the branch point, but no major arborizations are found in this region. From the T-junction the axon branches travel laterally and then posteriorly, staying approximately in the same horizontal plane, to exit from the ganglion in nerve five. Major dendritic arborizations project anteriorly from the lateral branches on either side of the T-junction, and the axon diameter is increased in this region relative to the neurite or the more distal axon. Some minor processes arise from the axon branches just before they exit the ganglion but the axon diameter does not increase in this region. These fine branches ramify amongst the large axons of motoneurones leaving the ganglion through nerves four and five. In the DUMETi that showed the physiological asymmetry (Fig. 10*b*) the bifurcation occurs much closer to the soma, at a point where the neurite of a normal DUMETi bends anteriorly. The right axon branch then continues anteriorly and horizontally before bending laterally at approximately the position of the T-junction in the normal DUMETi. The left axon branch, however, plunges abruptly ventrally for about 100 μm , before turning dorsally and anteriorly and moving into the plane of the T-junction of a normal DUMETi. It then bends laterally to parallel the path of the lateral axon branch of a normal DUMETi. The major dendritic arborizations occur in the normal positions on the lateral branches. Thus in the DUMETi that showed the physiological asymmetry the ventral loop of the left axon branch adds approximately 150 μm of neuropil process between the soma and the major dendritic arborization of the left axon branch. If the spike initiation site is distal to the dendritic arborizations on the lateral axon branches, then the increased distance of the left spike initiation site from the soma, relative to the right, might explain the reduced amplitude of A_i relative to A_r , as recorded in the soma.

The effects of unilateral axotomy on DUMETi

The somata of insect motoneurones do not usually carry overshooting action potentials. However, a few days after sectioning the peripheral axon of cockroach motoneurones, overshooting action potentials can be recorded in the soma (Pitman, Tweedle & Cohen, 1972). This also occurs in the locust metathoracic fast extensor tibiae motoneurone and fast flexor motoneurones and is thought to be due in part to an increase in the number of sodium channels present in the somatic membrane (Goodman & Heitler, 1977, also in preparation). Unilateral axotomy of DUMETi is a common natural occurrence since the locust is capable of autotomizing one of its back legs, if, for instance, it is restrained by a hind tibia. Thus, many locusts with only one hind leg occur in nature, and yet presumably DUMETi must still be

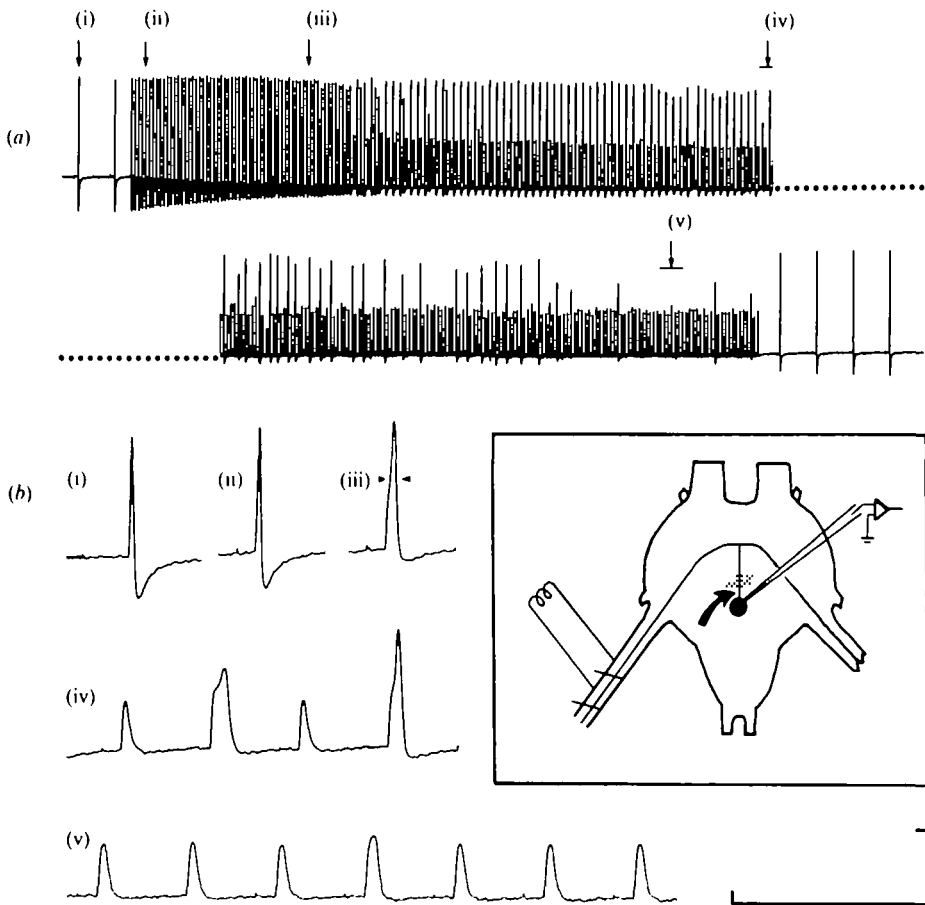


Fig. 12. The effect of unilateral axotomy on DUMETi. (a) The remaining peripheral axon is stimulated initially at 1 Hz, then for a burst of about 3 s at 10 Hz, and then again at 1 Hz. (b) Selected spikes and spike sequences (i-v) are shown on an expanded time scale. Note the increasing duration of the S-spike with progressive stimuli (e.g. horizontal arrows in (iii)), and the decrease in the afterhyperpolarization, which recovers on returning to stimulation at only 1 Hz. Only large amplitude, single-component, residual spikes are observed when the S-spike fails. The inset shows the experimental situation, with the stippled area the proposed region of N-spike failure, and the arrow the site of S-spike initiation. Calibration: vertical, 70 mV; horizontal (a) 4 s, (b) 160 ms.

capable of normal function, since it still innervates the remaining hind leg. Thus, it is interesting to find out what effects, if any, unilateral axotomy has on DUMETi.

Recording from DUMETi 5 days after inducing autotomy by pinching (and twisting, if need be) one of the hind legs show that the soma carries overshooting action potentials which are indistinguishable in size and shape to those recorded in DUMETi when both hind legs are intact (Fig. 12; Goodman & Heitler, 1977). Repetitive stimulation of the remaining axon at 10 Hz causes failure of the soma spike at room temperature. However, the residual depolarizing wave recorded after S-spike failure is very different from the one- and two-component A-spikes recorded under similar circumstances when both legs are intact. The residual spikes are very much larger

than the normal A-spikes, with mean amplitude of 34 mV (s.d. $\pm 8\%$), compared to an average A-spike when DUMETi is intact of about 12 mV. The residual spikes consist of only one component (Fig. 12*b*). We were unable to fractionate this large residual spike even after several minutes of stimulation at 10 Hz, although there was an overall slight reduction in its amplitude with continued stimulation. Occasionally spikes are initiated intermediate in amplitude between the full S-spike and the residual spike. However, these intermediate spikes clearly consist of a pre-potential of about 34 mV, the residual spike, with a second added depolarization (Fig. 12*b*). These intermediate spikes have an afterhyperpolarization, which is not normally found for N-spikes in the intact neurone (Fig. 4*a*), and so we interpret them as partial soma spikes. The large residual spikes we interpret as being N-spikes. Thus, axotomy has converted the neural membrane about the branch point and neurite from a region in which spikes fail, into membrane which carries a full action potential. Stimulation of the remaining axon of a unilaterally axotomized DUMETi causes an action potential to propagate without interruption past the branch point and up the neurite to some position close to the cell body, perhaps the axon hillock. If this potential then fails to initiate an S-spike, an N-spike of approximately 34 mV amplitude is recorded in the soma, comparable to the largest N-spikes recorded when DUMETi is intact. Nerve five, containing the axon of DUMETi, was cut about 15 mm distant from the ganglion. Recordings were made 4–5 days after axotomy, at which time the proximal section of the cut axon was still functional. Thus the absence of spike failure at the branch point is not due to an unloading effect resulting from degeneration of the cut axon back to the branch point.

Repetitive stimulation reveals some differences between the S-spike of an axotomized and an intact DUMETi which are not obvious with single stimuli. The first difference is the occurrence of partial soma spikes. In an intact DUMETi we did not record spikes intermediate in size between the 40 mV of the largest N-spikes and the 70–90 mV S-spikes, whereas in the axotomized DUMETi a number of such spikes occurred (Fig. 12). Secondly, in the intact DUMETi the afterhyperpolarization increases on repetitive stimulation, despite an overall membrane hyperpolarization (Fig. 5), whereas in the axotomized DUMETi there is a similar membrane hyperpolarization, but the afterhyperpolarization of the S-spike decreases. This reduction is associated with an increase in the duration of the S-spike, measured at the depolarization above the N-spike pre-potential (Fig. 12*b* (iii)). This perhaps indicates a reduction in the delayed potassium current initiated by succeeding spikes. Upon cessation of stimulation there is a gradual recovery of the afterhyperpolarization.

DISCUSSION

Independent action potentials

DUMETi is essentially a T-shaped neurone where the horizontal bar represents the two axon branches which eventually exit to the periphery, and the vertical bar represents the neurite leading to the soma at its base. In this paper we show that each of four topographic regions of the neurone (the soma, the neurite, and the two axon branches) can have a separate action potential, and hence there must exist four functional sites of spike initiation. The existence of the separate SISs is due to the failure

of the membrane separating the SISs to support action potentials. We do not know whether this failure occurs under all circumstances. Thus at low spike frequencies and at room temperature, when an S-spike is recorded in the soma, the axon branches spike in unison. This may be because the membrane connecting the axon branches carries a full action potential, or alternatively it may be because the S-spike is sufficiently large to ensure synchrony of spiking in the two axons. However, at higher spike frequencies and/or higher temperatures spike failure definitely does occur somewhere in the axon branches distal to the branch point, and in the neurite. Similar frequency-dependent spike failure has been demonstrated in a number of other systems (e.g. Grossman, Spira & Parnas, 1973). We consider the possible mechanisms for spike failure in these regions fall into two broad categories. One is that the ionophore composition is such that the membrane is not capable of supporting a full action potential under conditions in which spikes fail. The other is that changes in axon diameter about the branch point and at the axon hillock constitute regions of low safety factor beyond which the spikes may not propagate. We cannot clearly distinguish between these possibilities with our data, but evidence suggests that the latter mechanism is less likely. The considerable variation in the amplitude of the N-spike suggests that it may fail at varying locations in the neurite, and yet the diameter of the neurite remains approximately constant from the branch point to the axon hillock. Furthermore, axotomy is known to cause a change in ionophore composition such as to convert membrane which is normally non-spiking into membrane capable of carrying spikes (Goodman & Heitler, 1977), and unilateral axotomy of DUMETi abolishes A-spikes, leaving only S-spikes and large N-spikes. This is consistent with the idea that axotomy has converted membrane about the branch point and in the neurite into spiking membrane. We cannot discount the possibility that axotomy has altered the geometry of the neurone in this region, but any such alteration would have to affect the unoperated side, as well as the operated side. We conclude that in the normal animal, the most likely explanation for the failure of spikes between the various SISs is that the membrane in these regions is unable to support full action potentials (at least at high temperatures and/or high frequencies) by reason of its ionophore composition.

Our chief aim in this work has been to relate some of the neurophysiological characteristics of DUMETi to its geometry. However, the finding that under experimental conditions action potentials may occur in one axon branch of the neurone but not in the other raises the question of whether synaptically driven spikes can be differentially produced in the two axon branches in a controlled manner, or whether the independent action potentials are merely the result of a probabalistic process in which SIS depolarizations very close to threshold succeed or fail in spike production as a result of random fluctuations. In the course of these experiments we attempted to initiate synaptically driven spikes specifically in only the axon branch of one side by mechanical and electrical stimulation of sense organs and nerves on that side of the locust. However, we were unable to find any stable correlation between the stimulus and spikes in either branch of DUMETi; it appears that the synaptic inputs which drive DUMETi are several levels of interneurones removed from primary sensory inputs. Only one preparation exhibited any spontaneous output bias. This was the asymmetric neurone, in which single axon spikes occurred only in the left axon.

However, the asymmetric geometry of this neurone means that we are unable to discount the possibility of an altered relationship between the SISs and the sites of excitatory synaptic input. In other words, the asymmetric output of this neurone may not be a result of a controlled and modifiable bias, but rather simply an inherent bias due to anatomical or physiological asymmetry.

Thus although DUMETi can under experimental conditions produce action potentials independently in its two axons, we have been unable to obtain evidence that it does this in a way which is physiologically or behaviourally useful. One reason for this is our ignorance of the spatial distribution of synaptic inputs. There are five areas of dendritic arborization: one on the distal region of the neurite, one on either side of the branch point, and one on each axon branch just before it exits the ganglion. The dendritic arborizations are presumably either regions of excitatory synaptic input, inhibitory synaptic input, or synaptic output. Any inhibitory synaptic input occurring between the two axon SISs may serve a gating function by increasing the electrical separation of the SISs, and thus increasing the probability of unilateral spikes. If independent excitatory synaptic inputs occur close to the axon SISs, this would obviously allow controlled independent spike initiation in the two axon branches. Unfortunately, we cannot make any functional distinction between areas of dendritic arborizations; this would require intracellular recordings from the neuropil processes, which, owing to their small size, would be very difficult.

We have only observed independent spike initiation in spontaneously active DUMETi's at temperatures of 30–35 °C (except in the special case of the asymmetric DUMETi). At room temperature the frequency of spontaneous spikes is much lower, the S-spike rarely fails, and all observed A-spikes were bilaterally paired. Although the preferred temperature of the locust is 32 °C, the locust is competent at room temperature, which may suggest that independent initiation of spikes in the two axon branches of DUMETi does not play an important role in the function of the neurone. However, all our observations are made from heavily dissected and restrained preparations in which the metathoracic ganglion was isolated from most of the rest of the nervous system. It is quite possible that in the intact free-walking locust DUMETi spike frequencies are much higher, and then S-spike failure and independent action potentials may occur even at room temperature. The fact that at the preferred environmental temperature of the locust S-spike failure and independent action potentials occur even in the dissected preparation may support this suggestion.

Multiple sites of spike initiation have now been demonstrated in a variety of invertebrate motor- and interneurons. Many of these studies have been concerned with dendritic spikes which propagate electrotonically to a single-spike initiation site in the axon (Sandeman, 1969*a, b*; Zucker, 1972). The multisegmental tactile interneurons of crayfish provide an example in which dendritic spikes initiate axon spikes at more than one site in a single dendritic arborization (Calabrese & Kennedy, 1974). In the locust the lobular giant movement detector generates spikes in response to both visual and auditory stimuli and has two modality-specific sites of axon spike initiation, one located at each end of the axon (O'Shea, 1975). In these cases the various sites of spike initiation all give rise to spikes in the same axon. Independent spikes do not occur in different regions of the neurone's axon, just independent sites of spike initiation. However, the large neurones of the cardiac ganglion in the lobster have

branching axons in which independent spikes do occur. The ganglion has only five large motoneurons, but physiological evidence indicates that these give rise to at least twelve independent efferent axons (Friesen, 1975). The large cardiac neurones appear to have a normal motor function; they cause cardiac muscle contractions (Maynard, 1955; Anderson & Cooke, 1971). The independent action potentials may thus be advantageous in terms of neural economy, one neurone acting as two or three separate neurones. The function of DUM neurones in insects is not certain, but the branching geometry and independent axon spikes (if they occur in a controlled manner) may have similar economic advantages.

Unilateral axotomy

Loss of one of the metathoracic legs of the locust, whether this occurs naturally through autotomy, or experimentally through surgery, leads to asymmetry in DUMETi: one of its axon branches is now axotomized. Unlike bilaterally paired neurones, DUMETi must remain competent under these conditions since it still innervates the remaining intact leg. The major effect observed in DUMETi after unilateral axotomy is loss of two of the four sites of spike initiation. The spike recorded in the soma now only occurs in one of two forms, a soma spike or a large neurite spike. We interpret this to mean that the region of membrane about the branch point in which spikes may fail in the intact animal has now been converted into a fully active region of membrane in which spikes never fail, thus providing spike continuity between the two axon spike initiation sites and the neurite spike initiation site (Goodman & Heitler, 1977). The only remaining region where spikes can fail is in the neurite. The constancy of N-spike amplitude (relative to when DUMETi is intact) suggests that the area of the neurite in which spikes may fail has been reduced in the axotomized preparation to one localized region, thus ensuring a relatively constant attenuation factor between this region and the soma, and hence the constancy of N-spike amplitude. The large size of the N-spike suggests that failure occurs close to the soma, possibly at the axon hillock where the neurite expands into the soma. Failure of spikes at this point may be caused by a low safety factor due to the sudden change in neurone diameter, rather than the existence of non-spiking membrane.

Axonal asymmetry

DUMETi is an identified neurone which, like other identified neurones, shows a high degree of constancy in the general shape of its axons and major dendritic arborizations. However, given that phenotypic variability in identified neurones must be the substrate on which natural selection acts in the evolution of such neurones, we might expect to find occasional variations in the major branching patterns and physiology if we examine the same neurone in enough individuals from the sexually reproducing population. Recent studies on the locust and other invertebrates have demonstrated just such variability in identified neurones. Major variability in the location of neural somata has been observed in the pond snail *Lymnaea* (Benjamin, 1976), but the axonal branching and synaptic physiology of the neurones was normal. We too find variability in the position of the soma of DUMETi which does not appear

to affect the physiology of the neurone. The soma location, evidently, is not very important to these aspects of neural function. Variabilities have also been observed in the axonal morphology of various identified neurones (Burrows, 1973; Goodman, 1974), and in at least one case, the ocellar interneurons of the locust, studies using isogenic locusts have shown that anatomical variability in identified neurones can have a genetic basis (Goodman, 1977, 1978). The question raised by such findings is whether an anatomical abnormality in a particular neurone leads to a physiological abnormality in that neurone, or whether some regulatory mechanism compensates for the anatomical abnormality and allows the neurone to have a normal physiology.

To our knowledge, the finding in one particular animal of a physiological asymmetry of DUMETi correlated with an anatomical asymmetry is the first test of this question. In this asymmetrical cell, no hypothetical regulatory mechanism in development or early experience had compensated for the extra 150 μm of neurite interposed between the two axonal SISs. Rather, the resultant physiology of the neurone reflected the additional membrane in terms of the amplitudes of the axon spikes recorded in the soma, and perhaps in the asymmetric output of the neurone. Furthermore, our data suggest, but are insufficient to prove, that the extra membrane has increased the independence of the two axon SISs, in that unilateral axon spikes occur at a lower temperature than normal. We may speculate that if such anatomical variability has a genetic component, then in the course of evolution variation in the amount of non-spiking membrane between the axon SISs may bias the output of DUMETi towards its two possible extremes: two axons acting in unison or two axons behaving as two independent neurones.

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