UNPAIRED MEDIAN NEURONES IN A LEPIDOPTERAN LARVA (*ANTHERAEA PERNYI*)

I. ANATOMY AND PHYSIOLOGY

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Summary

The anatomy and physiology of two unpaired median neurones (MC1 and MC2) with bilaterally symmetrical axons in abdominal ganglia 3, 4, 5 and 6 of *Antheraea pernyi* larvae were studied. Intracellular dye filling of MC1 and MC2 revealed that they were distinguishable from all other neurones in the ganglia and that they both had axons projecting out of the ganglia in right and left nerves 1. The two cells were identical in their central anatomy and physiology, but could be distinguished from one another by their peripheral branching patterns. The significance of these patterns was investigated by detailed study of the neural and muscular anatomy of the proleg-bearing segments 3, 4, 5 and 6. The peripheral axons of MC1 and MC2 were exclusively associated with nerve trunks that could be traced to blocks of muscle.

Intracellular recordings of the two median cells characteristically showed overshooting soma action potentials that were followed by a long afterhyperpolarization lasting many seconds. Simultaneous recordings from median cells in the same ganglion revealed that MC1 and MC2 shared an excitatory synaptic drive that largely determined their patterns of firing. Recordings from median cells in different ganglia showed that the common synaptic drive was also shared by median cells in different segments. Selective lesions of the ventral nerve cord indicated that the synaptic drive to MC1 and MC2 originated in the suboesophageal ganglion. These cells were similar in anatomy and physiology to the median cells in several other insects.

Introduction

Bilaterally symmetrical nerve cells with bifurcating axons have been described in a number of insect species, mostly after backfilling dye into nerves that innervate muscle blocks (locusts and cockroaches, see Hoyle, 1978; crickets, Davis & Alanis, 1979; Hemiptera, Davis, 1977; Coleoptera, Christensen & Carlson,

1981, 1982; Lepidoptera, Taylor & Truman, 1974; Casaday & Camhi, 1976; Rheuben & Kammer, 1980; Kondoh & Obara, 1982). The association of these cells with nerve trunks that innervate muscles (Hoyle, Dagan, Moberly & Colquhoun, 1974; Davis, 1977; Davis & Alanis, 1979; Kondoh & Obara, 1982; and the present study) suggests that this class of neurones may be involved in the control of the musculature in these phylogenetically diverse groups of insects. In the locust and cockroach many bilaterally symmetrical neurones have been shown to have overshooting action potentials in their somata (Crossman, Kerkut, Pitman & Walker, 1971). In contrast, most insect neurones appear to lack soma spikes (Orthoptera, Hoyle & Burrows, 1973; Dictyoptera, Fourtner & Pearson, 1977; Odonata, Simmons, 1977; Lepidoptera, Rind, 1983).

The present study investigates the anatomy and physiology of two unpaired median neurones (MC1 and MC2) in the abdominal segments of *A. pernyi*, and includes a detailed description of the muscular anatomy of the segment as no previous accounts in the literature were adequate. Results indicated that the two median cells investigated in the present study were uniquely identifiable neurones. Several physiological and anatomical similarities between MC1 and MC2 and the dorsal unpaired median (DUM) neurones of locusts and cockroaches were noted.

Materials and methods

Rearing methods

Caterpillars of the Chinese oak silkmoth *Antheraea pernyi* were raised from hatching to their third instar in batches of 50–100 in constant light conditions at 18–28°C, depending on the rate of growth required. The animals were fed on beech, hawthorn, oak or sweet chestnut leaves during the summer, and on evergreen oak leaves during the winter. Nutritional deficiencies of evergreen oak were overcome by spraying freshly cut leaves with the water-soluble components of the diet of David & Gardiner (1966). To reduce the spread of infectious diseases, the third-instar animals were transferred to individual polystyrene containers in which a small piece of moistened 'oasis' was placed. Animals were fed daily until spinning started at the end of the fifth instar. Five days after spinning, pupae were removed from their cocoons to monitor their development, and were kept at 10–28°C, depending on when adults were required. Following eclosion, moths were kept in the dark and observed until they had mated. Eggs were surface-sterilized in 5% sodium hypochlorite solution in dilute detergent, rinsed in distilled water then allowed to develop at 18–28°C until hatching. Using these methods a continuous culture of *Antheraea pernyi* was maintained through-out the year.

The preparation

The dorsal surface of fifth-instar *Antheraea pernyi* larvae was smeared with petroleum jelly, sharpened tungsten hooks were inserted into the head capsule and anal skin flap and the animal was chilled at 0°C until just immobilized

(normally 8–12 min). It was then suspended in a dissection chamber, a dorsal incision was made from the tail to the head capsule and the resulting flaps of skin were fixed onto swivelling pins at the side of the chamber. The gut and silk glands were ligatured and removed, care being taken not to disrupt the tracheal supply to the ganglion under study. Saline modified from Weevers (1966) (in $mmol\,l^{-1}$: NaCl, 20; KCl, 30; $MgCl_2$, 20; $CaCl_2$, 3; sucrose, 160; NaH_2PO_4, 10·9; Na_2HPO_4, 4·1; pH 6·6) was then superfused through the body cavity at a rate of $1–2\,ml\,min^{-1}$. Abdominal ganglion 3, 4, 5 or 6 was then lifted onto a vinyl-covered stainless-steel platform and pinned out with small insect pins approximately 2 mm long. Up to 20 such pins were inserted into connective tissue and large tracheae to immobilize the ganglion for recording. The connective tissue sheath covering the ganglion was focally softened by applying a 1 % protease solution (Sigma Type VI) in a flat-bottomed pipette for 8–12 min. Microelectrodes pulled on a Brown & Flaming pipette puller (Sutter Instrument Co.), filled with $3\,mol\,l^{-1}$ KCl or $2\,mol\,l^{-1}$ potassium acetate solution, with resistances of $20–50\,M\Omega$ were used to make intracellular recordings. Penetration of median cells was aided by the use of a piezoelectric prodder (Weevers, 1980) driven by square wave pulses from a stimulator. Signals from the microelectrode were passed through a home-built electrometer with internal bridge balance circuitry, displayed on an oscilloscope (Tektronix 5111A) and simultaneously recorded on a Racal Store 4 FM tape recorder. Intracellular recordings of muscle activity were made in a similar way except that electrodes were of lower resistance ($1–10\,M\Omega$) and were mounted on a flexible silver wire (Woodbury & Brady, 1956).

Extracellular recordings of neural activity were made *en passant* from nerve trunks with a chloride-coated silver wire hook electrode which could be retracted into a paraffin-filled glass sleeve. Recordings were made between the hook and an indifferent electrode placed in the body cavity *via* an Isleworth A103 pre-amplifier. Nerve trunks were stimulated by a fine pair of chloride-coated silver wires insulated except at their tips. Recordings were played into a Digitimer DS900 transient recorder and plotted on a Gould x,y plotter.

Dye filling of neurones

For intracellular dye filling, microelectrodes were filled with $100\,mmol\,l^{-1}$ hexammine cobaltic chloride, 3 % Lucifer Yellow (Stewart, 1981) or 4 % horseradish peroxidase (Boehringer Mannheim, grade 1) in $0·5\,mol\,l^{-1}$ potassium acetate solution. Dye was injected into physiologically identified cells by passing 1–10 nA constant-current pulses, 500 ms long at 1 Hz for 5–30 min. Cobalt-filled cells were then treated with dilute ammonium sulphide solution (two drops in 10 ml of phosphate buffer, pH 7·0) for up to 5 min, rinsed in fresh phosphate buffer and fixed in 5 % formaldehyde in buffer for 30 min. After washing overnight in running tap water, ganglia were rinsed twice in distilled water and then intensified using the method of Bacon & Altman (1977). Ganglia were then dehydrated in ethanol, cleared in methyl benzoate and xylene, and mounted in neutral Canada balsam.

Cells filled with horseradish peroxidase were processed according to the method of Roberts & Clarke (1982). Lucifer Yellow fills were fixed in 5 % formaldehyde in 100 mmol l^{-1} phosphate buffer pH 7·0, dehydrated in ethanol and mounted in Fluoromount (Edwin Gurr Ltd).

Vital staining of caterpillars

The general neuroanatomy of fifth-instar caterpillars was revealed by *intra vitam* injection of 1–2 ml of 0·17 % Methylene Blue in saline, reduced with Rongalite (see Pantin, 1946, for exact details). After 15 min the animal was dissected in the normal way from the dorsal midline, either under phosphate buffer (pH 7·0) or under saturated ammonium molybdate solution. Six preparations were studied in detail in this way.

Results

Of the many neurones with cell bodies in the midline of the abdominal ganglia of larval *Antheraea pernyi*, only two were recorded that had axons projecting bilaterally and symmetrically in both nerves 1 of the ganglion. These neurones were named median cells 1 and 2 (MC1 and MC2), and were found to be uniquely identifiable. The anatomy of the segment in fifth-instar larvae is described first to provide a frame of reference for the description of the morphology of the two median cells.

The peripheral nervous system of abdominal segment 5

Methylene Blue staining of nerve trunks in the abdomen revealed fine branches that were only seen with great difficulty in living material. Fig. 1 shows data collected from six preparations using this technique and subsequently confirmed in many *in vivo* preparations. The diagrams show the structure of nerves at four stages of dissection starting after the removal of the gut, silk glands, gonads and fat body. The four stages progress from the innermost layer of longitudinal muscles outwards to the oblique muscles directly below the cuticle. The nomenclature of nerve branches was based on the system of Pipa & Cook (1959) as it was felt that the system of Beckel (1958) was too inflexible to allow for the variability of branching seen in different preparations.

Fig. 1. Four stages in the dissection of the nervous system of the fifth abdominal segment of fifth-instar larval *Antheraea pernyi* are shown, starting with the innermost layer and progressing outwards towards the cuticle. For the sake of clarity the gut, silk glands, fat body and most of the tracheae have been omitted. The nomenclature is based on the system of Pipa & Cook (1959). Each diagram shows half of an abdominal body segment, with the ventral midline at the top and the dorsal midline at the bottom, the segment being flattened for the purposes of dissection. AC, anterior connective; PC, posterior connective; vs, ventral scolopale organ; ls, lateral scolopale organ; mro, muscle receptor organ; sp, spiracle; da, dorsal aorta.

Fig. 1

The named nerve trunks were all consistently identifiable in different animals; finer branching was extremely variable but that represented in Fig. 1 is typical. The pattern of branching was basically similar to that described in the earlier studies of Libby (1959) and Beckel (1958) (in *Hyalophora cecropia*) with the following exceptions. Beckel's nerve A11 which was shown to run from N1b3 near the spiracle and anastamose with N2d in the next anterior segment was not seen in *Antheraea pernyi* (see Fig. 1C). Libby (1959) also failed to record this branch. Second, the long branch of N2b (see Fig. 1C) was shown by Beckel as being distally attached to a branch of N1c; this connection was never seen. Four anastamoses were seen; N1 joined mN both near the ganglion and near the spiracle. The small branch mNa (see Fig. 1A) which anastamosed with N2c of the next anterior ganglion was that shown by Taghert & Truman (1982), in *Manduca sexta*, to contain the axons of three identified bursicon-containing cells. The fourth anastamosis was again with mN where N1b3 joined it just ventral to the spiracle.

The targets of each of the named branches are summarized in Table 1. Nerves N1b1, N1b2, N1c1, N1c2, N1f1, N1f2, N1f3 and N2b were never seen to have any connections with muscle fibres and appeared to innervate either the cuticle or identified sense organs. Nerve trunks N1a1, N1a2, N1a3, N1b3, N1d, N1e, N1g, N1h and N1j could only be traced to bundles of muscle fibres; no branches could be traced to run to the cuticle or to identified sense organs.

The motor units of abdominal segment 5

Motor units were characterized in the following way. By placing a fine stimulating electrode on a muscle fibre it was possible to stimulate a single motoneurone and all of its collaterals, and by using a movable intracellular recording electrode the extent of the motor unit could be defined. An extracellular recording electrode was placed on the proximal root of the nerve and used to check that only a single antidromic spike was elicited by stimulation.

The results obtained using these methods are shown in Fig. 2 (for the sake of simplicity the gut, silk glands, fat body, nervous system and tracheae have been omitted). The nomenclature used is that of Lyonet (1762) and Forbes (1914), except for muscles π and ψ, which were not identified by these authors. The shading of the muscles reflects the source of their innervation, the 19 stippled motor units were supplied by motoneurones with somata in the next anterior segmental ganglion *via* N1. Four motor units (crosshatched) were supplied by motoneurones with cell bodies in the same segment's ganglion and axons in N1. Ten muscles (striped) were supplied by motoneurones with axons in N2. The spiracle opener (spo) is a non-muscular ligament (Beckel, 1958) and the alary muscle, heart and spiracle closer were innervated by the median nerve (mN).

Of all the muscles tested, only one (γ) was shown to be innervated by more than one axon. All fibres in this muscle showed two sizes of EJP in spontaneous activity and in response to graded stimulation of N2. All other motor units had only one size of spontaneous or evoked EJP. No evidence for inhibitory input was seen in any muscles in the abdomen of *Antheraea pernyi* larvae.

Table 1. *Targets of peripheral nerve trunks and their innervation by axons of MC1 and MC2*

Name of nerve	Traced to somatic muscles?	MC1 or MC2	Peripheral target(s) traced
mN	?	−	Heart, spiracle, neurohaemal organ
mNa	?	−	Anastamoses with N2c of anterior segment
N1	yes	both	Dorsal and ventral muscles and sense organs
N1a1	yes	both	Muscles b,c,d,e
N1a2	yes	both	Muscles d,e,f,g
N1a3	yes	both	Muscles f,g,i
N1b1	no	−	Ventral and lateral cuticle
N1b2	no	−	Ventral scolopale organ
N1b3	yes	MC1	Muscles θ,q and l, anastamosis with mN
N1c1	no	−	Anterior lateral cuticle
N1c2	no	−	Dorsolateral scolopale organ
N1d	yes	MC2	Muscles E,H,F
N1e1	yes	MC2	Muscles C,B,L
N1e2	yes	MC2	Muscle of muscle receptor organ
N1f1	no	−	Nucleus of muscle receptor organ
N1f2	no	−	Posterior dorsal cuticle
N1f3	no	−	Anterior dorsal cuticle
N1g	yes	MC2	Muscle QR
N1h	yes	MC2	Muscles QR,A,I,D,G
N1j	yes	MC2	Muscles A,D,G
N2	yes	−	Muscles of proleg, ventral cuticle
N2a	yes	−	Muscles x,p,t,π
N2b	no	−	Ventral and proleg cuticle
N2c	?	−	Anastomosis with median nerve
N2d	yes	−	Muscles r,z,ξ,δ,γ,ψ

Innervation by the median cells indicates that an axon branch was physiologically traced into the nerve trunk on one or more occasions.

The central morphology of the median cells

Two neurones, MC1 and MC2, were recorded that had axons in both right and left nerves 1 of abdominal ganglia and were distinguishable from all other neurones by this criterion and by their characteristic intracellular physiology. It was of interest, therefore, to examine their central morphology to determine whether these two cells were uniquely identifiable, to see how they differed from one another and to compare their anatomy with that of DUM (dorsal unpaired median) cells in other insects. Intracellular dye fills of the two median cells were obtained on many occasions; typical examples are shown in Fig. 3. The most striking feature of the cell was the symmetry of its axons, dendrite distribution and branching at least to the level of secondary branching. The posteriorly sited soma gave rise to a single neurite that ran forward without branching quite deep within the neuropile. At a level just behind the roots of nerve 1 the neurite rose sharply,

Fig. 2

and bifurcated into two axons which ran over the dorsal surface of the neuropile and then out of right and left nerves 1 of the ganglion. Using the method of McKenzie & Vogt (1976) the vertical distribution of dendrites was mapped for four regions of the neuropile (see Fig. 4) from the fine focus control on a microscope. This indicated that dendritic branching was largely restricted to the dorsal, dorsomedial and dorsolateral regions of the neuropile.

Intracellular dye filling indicated that there were no significant differences between the central morphology of MC1 and MC2; in fact, they could only be distinguished by their peripheral branching pattern. This is shown by the dye fills drawn in Fig. 3. Apart from characteristic differences in peripheral axon branching, the only difference between MC1 and MC2 was that MC1 tended to have the more dorsal cell body. However, this was not a reliable distinguishing characteristic. In 78 % of preparations where measurements were recorded (32 out of 41) MC1 had its cell body within 50 μm of the dorsal ganglion surface (measured with the fine advance of the micromanipulator). MC2 had a dorsally located soma in only 38 % of preparations (10 out of 36). There was no significant tendency for one cell body to be more posteriorly located than the other.

Peripheral branching of the median cells

Peripheral axons of the median cells were traced with three methods. First, antidromic impulses were evoked by stimulation of peripheral nerve trunks and were recorded in the cell soma; this indicated the presence of an axon branch at the point of stimulation. The paucity of synaptic input from nerve 1 made this method reliable and quick. Orthodromic tracing used the presence of extracellular potentials recorded in peripheral nerve trunks and time-locked to intracellularly recorded soma spikes. This method was slow, it often required signal averaging and could damage peripheral nerves. The third method was to inject dye into the soma of the median cell and let it diffuse into the axons. This method was rather capricious; however, it did give unequivocal confirmation of the basic branching patterns.

Fig. 2. Four stages in the dissection of the fifth abdominal segment of fifth-instar larval *Antheraea pernyi* are shown to reveal the structure of the somatic musculature and its source of innervation. The figures start with the innermost layer of fast longitudinal muscles and progress outwards towards the cuticle. For the sake of clarity the gut, silk glands, fat body, tracheae and nervous system have been omitted. The 19 stippled muscles were innervated by motoneurones with somata in the next anterior ganglion and axons in nerve 1. The four crosshatched muscles were innervated by motoneurones with somata in the ganglion of segment 5 and axons in nerve 1. The ten striped motor units were supplied by motoneurones with axons in nerve 2. The nomenclature is that of Lyonet (1762) and Forbes (1914). gm, strap-like muscle connected to the side of the gut. This muscle had a very different appearance from the other somatic muscles and did not appear to be innervated by any nerve branches arising from the abdominal nerve cord. spc, spiracle closer muscle; mro, muscle receptor organ; spo, spiracle opener ligament (non-muscular); am, alary muscles; da, dorsal aorta.
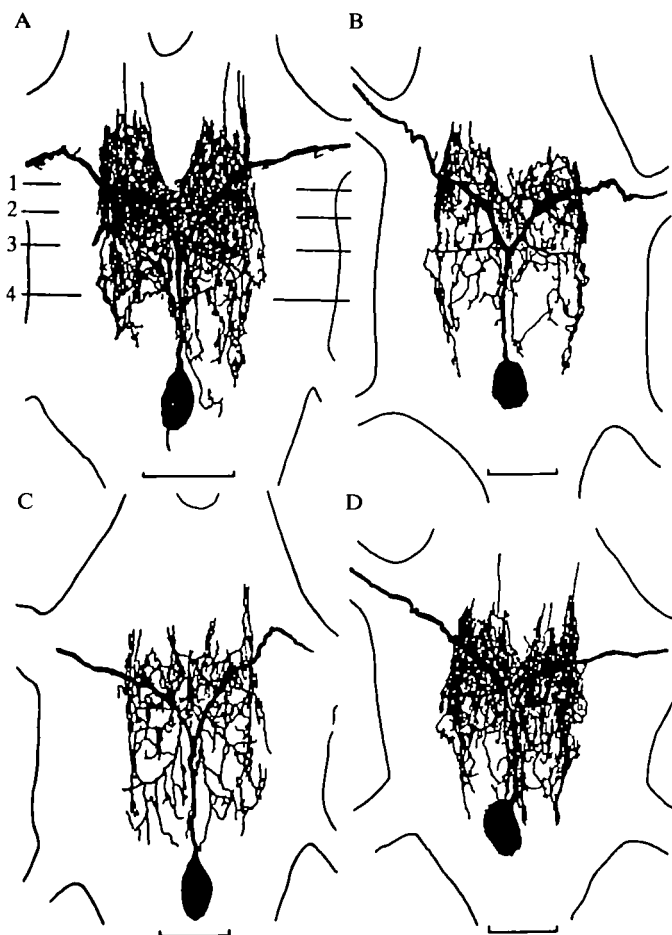
Fig. 3. A comparison of the central anatomy of MC1 and MC2 in different ganglia. Four *camera lucida* drawings of dorsal projections of dye-filled cells are shown. The neurones are so similar as to be indistinguishable from one another in their central anatomy; they could only be reliably identified by their peripheral projections. Scale bar, $100\,\mu$m. (A) MC1, ganglion 5; intensified cobalt fill. The four numbered lines down the side of this figure show the 'plane of section' in Fig. 4. (B) MC1, ganglion 4; intensified cobalt fill. (C) MC2, ganglion 6; horseradish peroxidase fill. (D) MC2, ganglion 4; intensified cobalt fill.

MC1 was distinguished by the presence of an axon branch in N1b3 whereas the other median cell (MC2) always had an axon branch in N1 beyond the spiracle. No neurone with branches in both of these nerves was ever seen. The branch of MC1 in N1b3 could usually be traced no further than the anastamosis with the median nerve in *Antheraea pernyi*. In a few preparations, axons of MC1 could be traced a short distance down the nerve rami running from N1b3 to muscles 1,q,θ and the spiracle closer muscle, but in no case was extensive branching found in any of these muscles. Both MC1 and MC2 sometimes had axons in the branch of N1a which

innervated the ventral longitudinal and oblique muscles. In 53 % of preparations where detailed tracing was carried out (9 out of 17) MC1 had axon branches traced into N1a; 79 % of MC2 cells had axons in N1a (11 out of 14 cells). The extent of this branching was highly variable but could never be traced beyond the major nerve trunks innervating blocks of muscle.

The distribution of axons was very variable. In summary, occasionally branches of either MC1 or MC2 were found in every branch of N1 that innervated blocks of muscle but they were never found in any nerve trunks that were not seen to innervate muscle blocks (see Table 1). A significant detail of this tracing was that the latency of antidromic impulses often increased dramatically within a short distance along a nerve trunk innervating a block of muscle, presumably as the axon narrowed near its termination. Conduction velocity varied from $1\,\mathrm{m\,s^{-1}}$ in major nerve trunks (e.g. N1) to $0.02\,\mathrm{m\,s^{-1}}$ in the ventral muscle block. The extent of median cell branching was very limited in comparison with that of motoneurones; within muscle blocks, branches were only detected in nerves supplying large
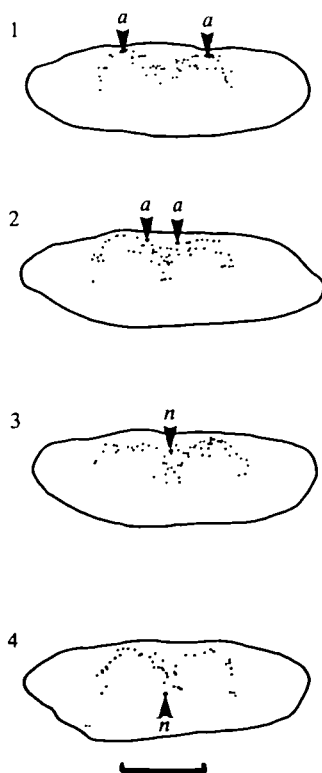

Fig. 4. The distribution of branches of MC1 within the fifth abdominal ganglion. Using the method of McKenzie & Vogt (1976), the depths of dendrites at four levels in the ganglion (see Fig. 3A) were mapped. Comparison with transverse sections of other ganglia confirmed that the dendrites of the median cell 1 were restricted to the outer regions of the dorsal neuropile. The position of the central neurite (*n*) and the axons (*a*) are marked. Scale bar, $100\,\mu\mathrm{m}$.
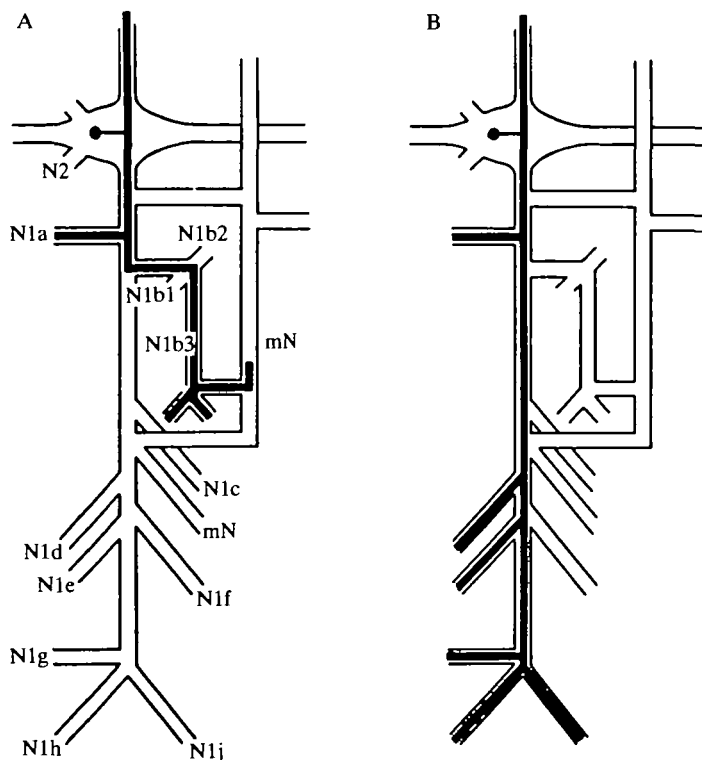
Fig. 5. Peripheral branching of MC1 and MC2. (A,B) Schematic diagrams of axon branching patterns typical of MC1 and MC2, respectively, traced physiologically in ganglion 5 of the same preparation. The main nerve trunks are labelled as in Fig. 1.

bundles of fibres, never in the fine branches that ramified over single muscle fibres. Typical patterns of branching of MC1 and MC2 from a single preparation are shown schematically in Fig. 5.

The neurophysiology of MC1 and MC2

The apparent resting potential of the two median cells compared with the baseline potential when the microelectrode was outside the ganglion was $-19 \cdot 2 \pm 4 \cdot 2$ mV ($N = 10$). It is likely, however, that this does not represent the true difference in potential between the inside of the cell and the surrounding extracellular fluid. Whilst advancing the microelectrode through a ganglion, a complex series of potentials was often recorded before a median cell was penetrated, even when the cell was clearly visible as the most dorsal neural soma. Typically, a negative potential of up to -70 mV (probably as a perineurial glial cell was penetrated) was the first event recorded; this was typically followed by an apparent positive potential averaging $+33 \cdot 1 \pm 4 \cdot 0$ mV ($N = 10$), which was presumed to represent a transperineurial potential, as has been reported in the cockroach (Treherne & Schofield, 1981). Thus a better estimate of the real resting
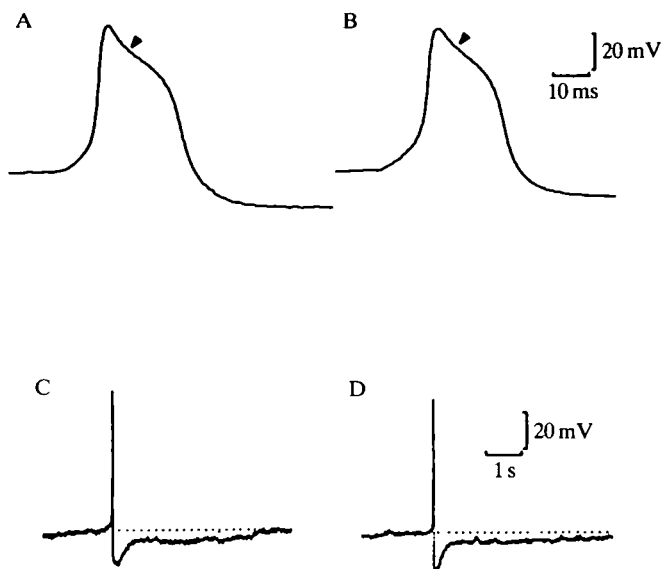
Fig. 6. (A,B) Soma action potentials of MC1 and MC2, respectively, had a characteristic shape with a pronounced inflection on the falling phase (arrowheads). Impulses were of very slow time course; at half peak amplitude they lasted from 10 to 25 ms. (C,D) MC1 and MC2 impulses are shown on a slower time scale. Immediately following each soma action potential was an afterhyperpolarization up to 20 mV below resting potential and up to 400 ms long at half peak amplitude. This was followed in most median cells by a long afterhyperpolarization which profoundly depressed the excitability of the neurone for up to 20 s.

potential of the median cells is given by the difference between the apparent resting potential and the transperineurial potential, i.e. $-52\cdot3 \pm 8\cdot2$ mV ($N = 10$).

Median cell impulses

One of the distinguishing characteristics of MC1 and MC2 was the presence of long-duration overshooting action potentials recorded in the neural soma. Impulse duration averaged $21\cdot9 \pm 3\cdot8$ ms ($N = 14$) for MC1 and $20\cdot4 \pm 7\cdot2$ ms ($N = 12$) for MC2 measured at half peak amplitude, and had a distinctive profile with a pronounced inflexion on the falling phase (see Fig. 6). The peak amplitude varied between preparations but usually lay between 70 and 90 mV above resting potential (average peak amplitude for MC1 $83\cdot6 \pm 13\cdot8$ mV, $N = 14$; for MC2 $74\cdot8 \pm 11\cdot5$ mV, $N = 12$).

Action potentials in the soma of the median cells were followed by a large afterhyperpolarization 10–20 mV below resting potential (MC1, $-15\cdot0 \pm 5\cdot2$ mV, $N = 14$; MC2, $-14\cdot3 \pm 4\cdot6$ mV, $N = 12$). The durations of the initial hyperpolarizations were (average for MC1) 350 ± 89 ms ($N = 14$) and (for MC2) 289 ± 85 ms ($N = 12$), measured at half peak amplitude. Following the initial hyperpolarization was an even longer duration afterpotential (LAH) (see Fig. 6C,D) that lasted up to 20 s after each impulse and was up to 8 mV in peak amplitude below resting
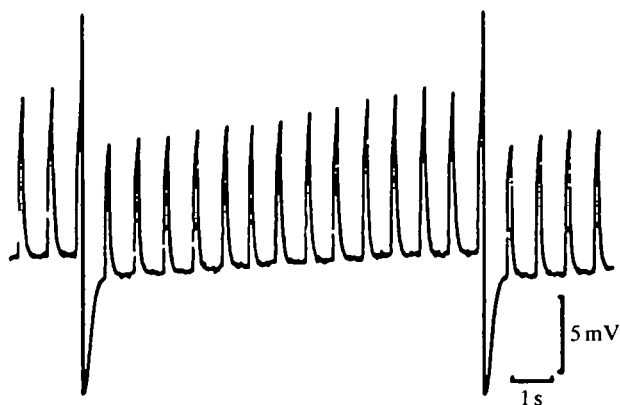
Fig. 7. 0·3 nA depolarizing constant current pulses injected into the soma of MC1 evoked impulses when the cell was at its normal resting potential. However, during the long afterhyperpolarization evoked by a previous spike, this size of current pulse no longer produced impulses, indicating that the threshold for spike initiation was increased by the long afterhyperpolarization.

potential [average for MC1 $-4·8 \pm 1·7$ mV ($N = 14$), for MC2 $-4·1 \pm 1·7$ mV ($N = 12$)]. This potential was visible in about 80 % of median cells recorded and had a strong depressive effect on the spontaneous firing rate. In some median cells there was a measurable increase in cell conductance during the course of LAH. It was apparent that the resting potential of the median cells was usually poised very close to the threshold for spike initiation, since injection of even small amounts of depolarizing current evoked impulses. However, during the course of LAH, the current threshold for production of impulses increased (see Fig. 7). This indicates that the effect of even small hyperpolarizations of the median cell membrane on the spontaneous firing rate of these neurones could be quite profound. The appearance of the long afterhyperpolarization depended on the presence of a preceding soma spike; in Fig. 8, antidromic action potentials elicited soma spikes that were followed by an LAH; this was unaffected by a short hyperpolarizing current pulse injected through the recording electrode into the median cell. However, when the hyperpolarizing current pulse was just sufficiently strong to block the initiation of a soma spike, no long afterhyperpolarization was evoked.

Synaptic activity in MC1 and MC2

Spontaneous synaptic activity was seen in all recordings from median cells where the ventral nerve cord was intact. These spontaneous synaptic potentials were all depolarizing and varied in size from 1 to 5 mV in different preparations (average size $2·9 \pm 1·0$ mV for 21 preparations). Their frequency also varied considerably, from 5–20 distinguishable events per second were seen in immobile preparations; however, this increased during spontaneous muscular activity.

Paired recordings from MC1 and MC2 in the same ganglion revealed that the two cells showed a similar temporal pattern of firing, although the spikes did not
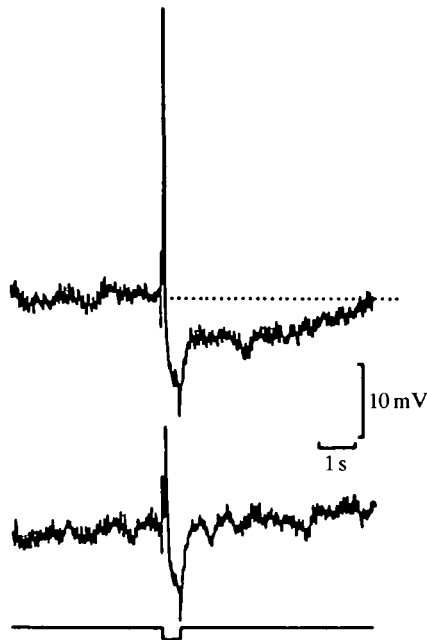
Fig. 8. The two traces show antidromic impulses initiated at the same time as a 500 ms hyperpolarizing current pulse was injected into the median cell soma. The same amplitude of current was used in each case; in the top trace it was not sufficient to block the soma spike which was followed by a long afterhyperpolarization (LAH) lasting over 5 s. In the lower trace the same hyperpolarizing current pulse was just sufficient to block the initiation of a full soma spike (because the LAH from a previous soma spike had not quite fully decayed). In this case no LAH was seen, strongly suggesting that the soma spike causes the long afterhyperpolarization.

correlate one-for-one and the basal rate of firing often differed between the two neurones (see Fig. 9A). At higher gain it was clear that MC1 and MC2 shared much common input; in fact, synaptic events correlated exactly one-for-one in the two cells in all recordings (Fig. 9B). No evidence was found for direct connections between the two median cells in the same ganglion; impulses initiated by intracellular current pulses in one cell evoked no time-locked synaptic potentials in the other cell even when the putative postsynaptic cell was systematically depolarized and hyperpolarized. In view of the slowness of the peripheral effects of these neurones (Brookes, 1988), similarly slow modulatory influences were looked for; in spite of this no evidence for any sort of interaction was seen on membrane potential, input impedance or firing rate.

Paired recordings of median cells in different ganglia also showed identical common synaptic input, with events in one cell being followed one-for-one after a short delay by events in the more posterior cell (Fig. 10B). The delay between synaptic events recorded in different ganglia indicated that the presynaptic
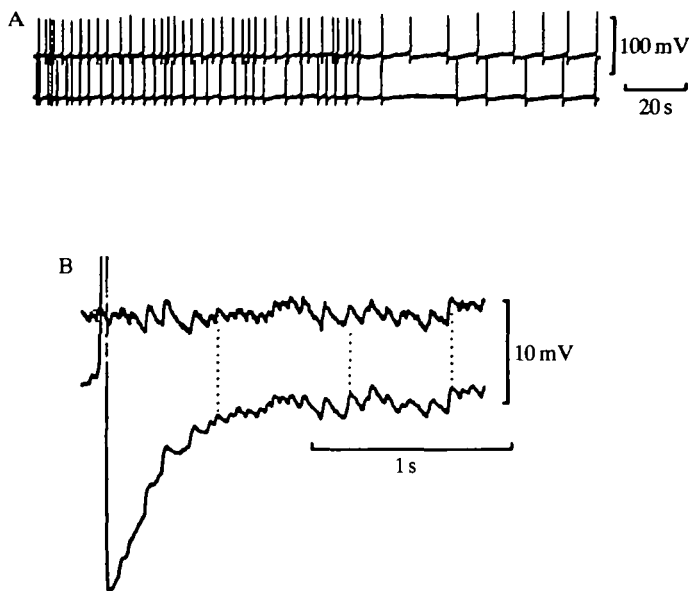
Fig. 9. (A) Simultaneous recordings of MC1 and MC2 in the same ganglion indicated that, although the two cells had different basal firing rates and impulses did not correlate one-for-one, nevertheless the temporal patterning of firing in the two neurones was similar. (B) Paired recordings of MC1 and MC2 at higher gain revealed that the two cells had very similar synaptic activity. In fact, synaptic events correlated one-for-one in the two neurones; dotted lines show corresponding events.

neurones conducted impulses from the anterior to the posterior of the animal at a conduction velocity that varied from 0·2 to 1·0 m s^{-1} in different preparations.

The source of the tonic synaptic input was investigated by selective lesions of the nervous system in a preparation where MC1 was simultaneously recorded in ganglia 4 and 6 (Fig. 10A,B). The two cells received identical synaptic input when the two connectives were intact. However, severing the left connective between the ganglia led to a disruption of the one-to-one correspondence of synaptic events (Fig. 10C), not all EPSPs in the anterior cell were followed by an EPSP in the more posterior neurone. Cutting the left connective anterior to ganglion 4 led to a resumption of one-to-one correspondence (Fig. 10D). Cutting the right connective between the ganglia led to a virtual silencing of spontaneous synaptic activity in the median cell in ganglion 6 (Fig. 10E). In fact, cutting both connectives at any point posterior to the suboesophageal ganglion along the ventral nerve cord always abolished spontaneous synaptic activity in all median cells posterior to the lesion. Lesion of the circumoesophageal connectives did not significantly affect the appearance of spontaneous EPSPs. These results taken together indicate that the excitatory drive for the median cells arose predominantly in the suboesophageal ganglion, that the axons conveying it descended the length of the nerve cord, that it was not completely synchronized in the two sides and that it did not cross from
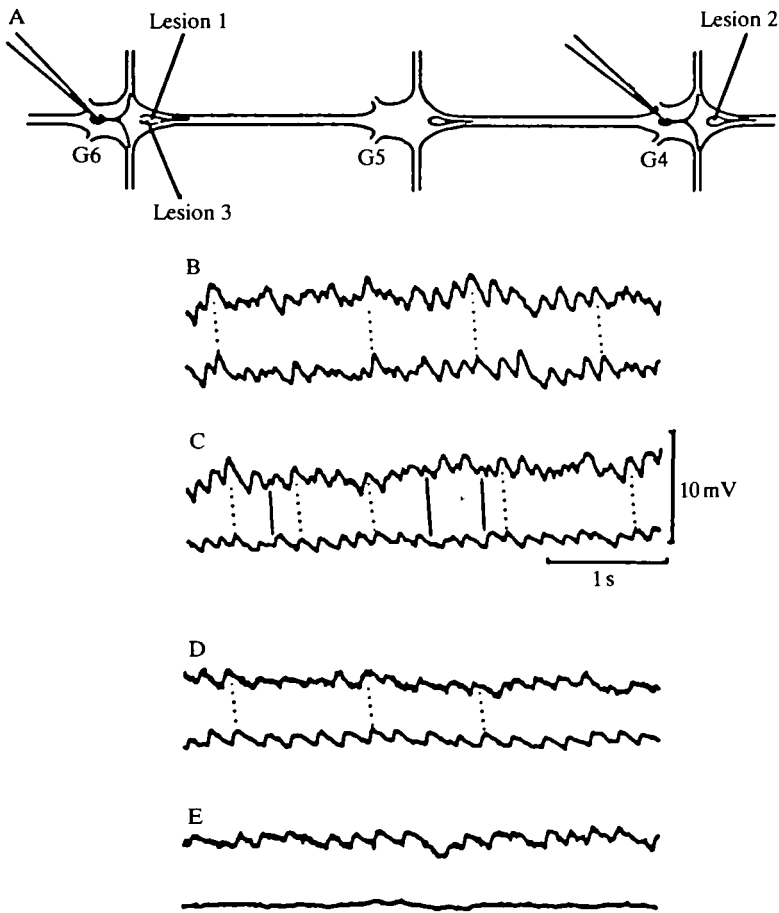
Fig. 10. (A) A diagram showing the sites of the lesions that produced the activity displayed in B–E. (B) Control. Both connectives were intact along the length of the animal. Synaptic events correlate one-for-one in the two median cells in ganglia 4 and 6. Dotted lines indicate corresponding synaptic events in the two cells. (C) Cutting the left connective between ganglia 4 and 6 disrupted one-for-one correspondence of synaptic events. Each EPSP in ganglion 6 was preceded by an event in ganglion 4. However, some events in ganglion 4 were no longer followed by an EPSP in the median cell in ganglion 6. The solid lines indicate some synaptic events which appeared in the more anterior ganglion that were not followed by a corresponding event in the more posterior ganglion. (D) Severing the left connective anterior to ganglion 4 caused a resumption of one-for-one correspondence of potentials, since both cells now only received input from the right connective. (E) Cutting the right connective anterior to ganglion 6 virtually silenced the spontaneous activity recorded in the more posterior median cell.

one side to the other within the abdominal ganglia. Electrical stimulation of the cut ends of connectives evoked synaptic activity similar to the spontaneous activity seen in preparations with an intact nerve cord. Interestingly, stimulation of the

peripheral nerves 1 and 2 also evoked EPSPs in most preparations, indicating that there were inputs other than those descending from the suboesophageal ganglion.

Discussion

The anatomy of abdominal segments

The muscles of several families of lepidopteran larvae have been described elsewhere (Cossidae, Lyonet, 1762; Notodontidae, Lubbock, 1859; Lasiocampidae, Noctuidae and Sphingidae, Forbes, 1914; Saturniidae, Beckel, 1958; Libby, 1959; *Galleria mellonella*, Randall, 1968; *Manduca sexta*, Rheuben & Kammer, 1980). However, the description given here is based on both anatomical and physiological evidence and thus describes for the first time the muscular anatomy in terms of motor units. The most striking feature of the segmental anatomy was the extreme simplicity of the innervation and musculature. The association of MC1 and MC2 axons with nerve trunks that innervated bundles of muscle fibres rather than with nerve trunks running to identified sense organs or to the cuticle was very striking.

In none of the Methylene Blue or *in vivo* preparations were peripheral nerve cell bodies seen on segmental nerves 1 and 2, although such neurones have been described in the stick insect and in the blowfly larva (Finlayson & Osborne, 1968), and in the median nerve of three families of lepidopteran larvae (Hinks, 1975; Griffiths & Finlayson, 1982). In the noctuid, arctiid and sphingid caterpillars examined by these authors, peripheral neural somata were confined to the distal end of the median nerve near the alary muscles, and were never seen on branches supplying somatic muscles. Since axons of the median cells were never traced to the distal median nerve it is unlikely that they synapse onto or interact directly with any peripheral neurosecretory cells.

The morphology of median cells 1 and 2

The consistent central anatomy of the median cells revealed by intracellular dye filling in the present study indicates that these cells are uniquely identifiable. They probably correspond to the bilaterally symmetrical neurones described by Taylor & Truman (1974) in abdominal ganglion 4 of larval *Manduca sexta*. MC1 and MC2 have also been recorded and dye-filled in abdominal segments 3, 4, 5, and 6 in *A. pernyi.*

MC1 and MC2 share a number of features with DUM cells that have been studied extensively in locusts and cockroaches. First, they share the characteristic bilateral symmetry of their branching (Crossman *et al.* 1971). Second, they have action potentials of relatively long duration that invade their cell bodies. Third, they have axons that can be traced into nerves supplying blocks of muscle, a feature of many of the unpaired neurones stained by backfilling from peripheral nerve trunks in cockroach and locust ganglia (Hoyle, 1978), in a cricket (Davis & Alanis, 1979), in a hemipteran (Davis, 1977), in fireflies (Christensen & Carlson, 1981, 1982) and in silkmoth larvae and adults (Taylor & Truman, 1974; Casaday &

Camhi, 1976; Rheuben & Kammer, 1980; Kondoh & Obara, 1982). Lastly, MC1 and MC2 have been shown to have modulatory effects on a number of somatic muscles (Brookes, 1988) similar to those described for DUMETi in the locust (Evans & O'Shea, 1977; O'Shea & Evans, 1979). However, there are also a number of differences between MC1 and MC2 in *Antheraea pernyi* larvae and DUM cells in locusts and cockroaches. Both cells in this study had extensive peripheral branching. Several DUM cells with exclusively central branching have been described (Crossman *et al.* 1971), but no such cells were recorded in *A. pernyi* larvae. The long afterhyperpolarization seen after impulses in MC1 and MC2 has not been reported in DUM cells in other insects. The one-for-one correspondence of synaptic events in MC1 and MC2 was not reported for DUM cells in the locust metathoracic ganglion, although common synaptic inputs were seen (Hoyle & Dagan, 1978).

The significance of soma action potentials

MC1 and MC2 both had overshooting soma action potentials which distinguished them from the majority of insect neurones. It appears that the biophysical basis of the difference between spiking and non-spiking neural somata in insects is due to the presence of a stronger repolarizing outward potassium current in non-spiking somata which prevents the initiation of overshooting action potentials (Goodman & Heitler, 1979; Miyazaki, 1980). Goodman & Heitler (1979) speculated that DUMETi had a reduced outward potassium current as a mechanism for prolonging the axon spike, thus potentiating transmitter release. Such a mechanism might be expected to cause increased soma excitability. They support their reasoning with the observation that the soma spike of DUMETi is unlikely to play an important physiological role as it often failed when recordings were made at 32°C, the temperature at which the locusts were raised.

Although the suggestions of Goodman & Heitler (1979) are attractive for explaining the presence of a soma spike in DUMETi, it has been shown that the soma spike of MC1 and MC2 correlates with the appearance of a long afterhyperpolarizing potential (LAH) that strongly limits their excitability. The effect of this potential was to maintain a low basal rate of discharge which could be overridden by increased descending synaptic drive during periods of excitability. As such, it would appear that the soma spikes of MC1 and MC2 and the associated LAHs probably play a significant role in determining the firing of the cells. Another possible role for the soma spikes in these cells could be to coordinate impulse initiation in the two axons. During prolonged periods of recording, soma spikes always evoked impulses in axons that could be detected peripherally. Consideration of the symmetry of the cells suggests that there may be two spike initiation sites, as has been reported for DUMETi in the locust (Heitler & Goodman, 1978); in spite of this, axon spikes were extremely rarely seen during intracellular recording. Thus, bilateral coordination of firing in the axons may be another role for the soma spike. This is of obvious adaptive significance

considering the widespread peripheral effects of the median cells (see Brookes, 1988).

The suboesophageal ganglion has classically been associated with the initiation and maintenance of movement in insects (see, for example, Roeder, 1963; Kien, 1983). In the light of the involvement of MC1 and MC2 with the modulatory control of somatic muscles (see Brookes, 1988) it is perhaps not surprising that much of their synaptic input should arise in this ganglion. The sharing of synaptic input between median cells in different ganglia is likely to have the effect of ensuring that the entire somatic musculature supplied by these cells receives a similar pattern of modulatory input.

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