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Inhibitory motor neurones supply body wall muscles in the locust abdomen

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Summary

Inhibitory motor neurones in the abdominal ganglia of the locust *Locusta migratoria* were identified by combining extra- and intracellular electrophysiology, labelling of motor neurones by peripheral nerve backfills, and immunocytochemistry directed against the inhibitory transmitter γ-aminobutyric acid. The fifth and sixth abdominal ganglia were studied in particular detail, although general findings were verified in all other abdominal segments.

In each abdominal ganglion half, there are two inhibitory motor neurones, CIa and CIb, which supply dorsal (CIa) and ventral (CIb) longitudinal muscles. Their cell bodies are located in the next anterior ganglion to where the axons leave the ventral nerve cord *via* nerve 1. Both inhibitors have contralateral somata in the posterior ventral soma cortex, looping primary neurites and bilateral dorsal arborisations. There are homonomous (segmentally homologous) motor neurones in the fused abdominal neuromeres, the thoracic ganglia, and at least the third subesophageal neuromere.

These body wall inhibitors are distinctly different from the limb muscle inhibitors, $CI_{1\text{-}3}$, described previously. This is signified, for example, by the fact that both types of inhibitory motor neurones coexist in the prothoracic segment and innervate leg and body wall muscles, respectively.

Key words: locust, *Locusta migratoria*, common inhibitory motor neurone, abdominal muscle, segmental homology (homonomy), GABA.

Introduction

Innervation of limb muscles by inhibitory motor neurones is a general feature of arthropods, required due to their particular muscle structure and innervation. Common inhibitory (CI) motor neurones, which supply most, or all, muscles of an appendage, subserve the adjustment of muscle performance according to behavioural requirements – postural control, walking, etc. – in all arthropods studied so far (for a review, see Wiens, 1989) (see below). While structure (Watson et al., 1985) and function (Hale and Burrows, 1985) of limb muscle inhibitors are well understood, the same is not true for inhibitory control of the remaining muscles of the body. In the case of the insect abdomen, it is indeed not clear whether or not the body wall muscles are supplied by inhibitory motor neurones, although inhibitory innervation has been demonstrated electrophysiologically in locust (Yang and Burrows, 1983) and bushcricket (Consoulas and Theophilidis, 1992; Consoulas et al., 1993).

On the one hand, inhibitory motor neurones might well be reduced or absent in body segments without appendages. Elimination of appendages and their musculature in the course of evolution also abolished the need for the neuronal control of these structures. Accordingly, the complement of neurones which constitute the segmental ganglia in the abdomen is considerably reduced when compared to the thoracic, limb-bearing segments. Even whole neuroblasts, and thus the neuronal progeny generated by them, are missing from the abdominal ganglion primordia in insects (e.g. Shepherd and Bate, 1990). Indeed, the neuroblast which gives rise to two of the three limb muscle inhibitors in the thorax (Wolf and Lang, 1994) is among those missing in the abdomen. Accordingly, inhibitory motoneurones, where present in the abdomen, may not be homologues of the inhibitors innervating the limb muscles.

On the other hand, the peculiarities of arthropod muscle that require inhibitory control, namely sparse and polyneural innervation with (partly) overlapping motor units (for reviews, see Rathmayer, 1990, 1998), are the same in limb and body wall muscles. Inhibitory motor neurones speed up the mechanical response of a muscle to changes in excitatory motor neuron discharge through the selective pre- and postsynaptic inhibition of ‘slow’ motor neuron activity, and through its effects on the muscle fibres (Rathmayer and Erxleben, 1983; Rathmayer, 1998). Common inhibitors therefore facilitate rapid alternating limb movements, such as executed during fast walking (Ballantyne and Rathmayer, 1981; Wolf, 1990, 1992). And although the adjustment of muscle contraction properties according to changing behavioural requirements is perhaps less demanding in body wall as opposed to walking leg muscles, inhibitory innervation of body wall muscles would still appear necessary where

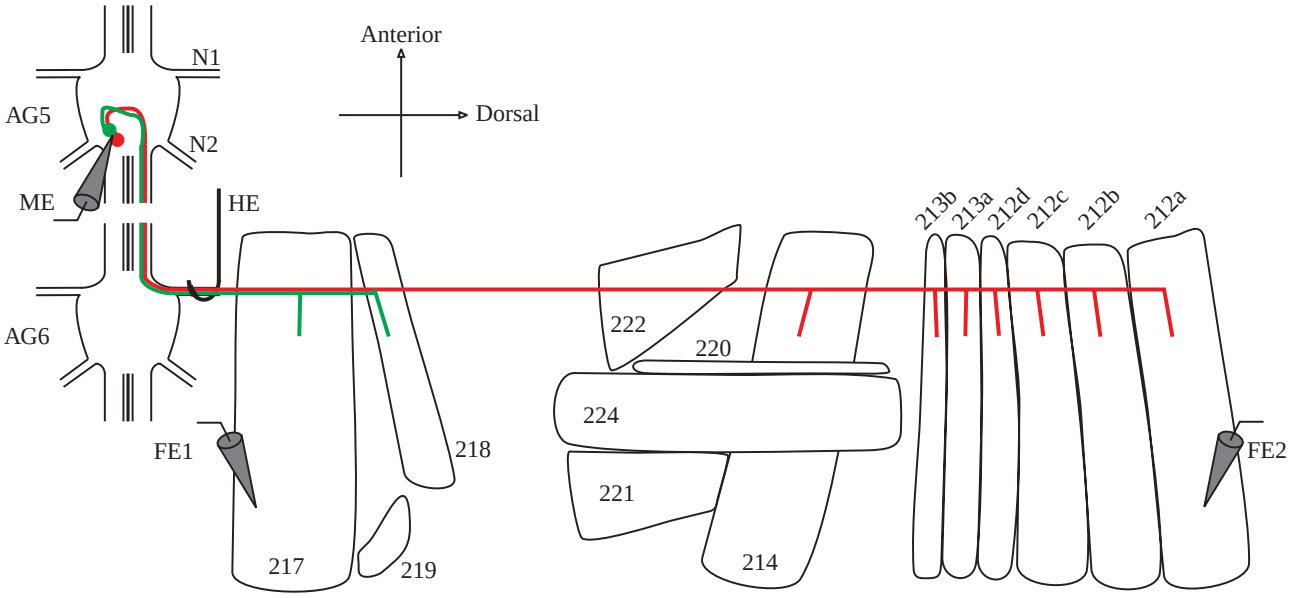

Fig. 1. Schematic diagram of the right set of body wall muscles in the locust abdominal segment 6, their inhibitory motor neuron supply, and the experimental arrangement. The body wall is shown flattened out, dorsal to the right and ventral to the left (nerve cord marks ventral midline), anterior to the top. Muscles are numbered according to Snodgrass (1935); 212, 213 and 214, dorsal, 217 and 218 ventral intersegmental muscles. Innervation by CIa is shown in red, by CIb, in green. Placement of intracellular recording electrodes is indicated in ventral and dorsal intersegmental muscles (FE1 and FE2, flexible electrodes) and in motor neuron soma (ME, microelectrode), allowing identification of inhibitory muscle innervation and staining of neurones by dye injection. An extracellular nerve recording (HE, hook electrode) monitored spike traffic through branches of nerve 1. The sixth abdominal segment is illustrated since it was in the focus of the present study, but the situation is identical in all other segments with unfused abdominal ganglia, and clear similarities exist in the remaining segments. For further details, see text. AG, abdominal ganglia; N, nerve.

movements in distinctly different velocity domains are executed, for instance, postural control and ventilation.

It was our goal, therefore, to ascertain whether or not inhibitory motor neurones innervate the body wall muscles of the insect abdomen, and to identify and describe possible inhibitory motor neurones. We combined extra- and intracellular electrophysiology, labelling of motor neurones by peripheral nerve backfills, and immunocytochemistry directed against the inhibitory transmitter γ-aminobutyric acid (GABA) to identify inhibitory motor neurones in the abdominal ganglia of the locust, *Locusta migratoria* L. The general findings were verified in all segments between the last subesophageal and the last unfused abdominal ganglia, although the fifth and sixth abdominal ganglia were studied in particular detail. We discuss segmental homology (homonomy) of inhibitory body wall motor neurones in the locust ventral nerve cord, and homologies among the orthopteran insects.

We demonstrate that in the locust there are two (in the prothoracic ganglion, three) body wall inhibitors per segment. Their morphologies are consistent throughout the ventral nerve cord, with cell bodies located in the anteriorly adjacent ganglion and contralateral to the nerve of axon exit, posterior and ventral in the soma cortex, with looping primary neurites and bilateral dorsal arborisations. Their targets are several ventral and dorsal intersegmental muscles, most of which have segmental homologues (homonoms) throughout abdomen and thorax. These inhibitory motor neurones of body wall muscles are distinctly different from the limb muscle inhibitors examined previously (Pearson and Fourtner, 1973; Hale and Burrows, 1985; Watson et al., 1985; Wiens and Wolf, 1993). In the thoracic ganglia, both types of inhibitory motor neurones coexist and innervate the leg and body wall muscles, respectively.

Materials and methods

Animals and preparation

Adult locusts *Locusta migratoria* L. from a breeding colony at the University of Ulm were used for all experiments. Both genders were taken at ages between 1–3 days after the imaginal moult.

Locusts were opened by a dorsal midline incision, and gut, fatty tissue and dorsal and ventral diaphragms were removed. Usually head and thorax were discarded and the abdomen pinned open on a Petri dish coated with Sylgard (®, Dow Corning) (only in experiments where a normal respiratory rhythm was desirable were the more anterior body segments left attached). Where necessary for intracellular recording, the attachment sites of intersegmental muscles were fixed with minuten pins. The connectives were severed to isolate abdominal ganglia (AG) 4–7, and all nerves of AG 4, 5 and 7 were cut. This allowed ganglion 5 to be turned upside down, without strain to the remaining nerves, such that the ventral side was uppermost on a Sylgard-platform, with the nerve

stumps immobilised with minuten pins. After digestion treatment with Protease (Sigma) for about 30 s the ventral ganglion surface was accessible for intracellular recording from neuron cell bodies. The experimental arrangement is outlined in Fig. 1.

The preparation was immersed in hypotonic locust saline, containing (in mmol l^{-1}): NaCl 140, KCl 10, $CaCl_2.2H_2O$ 2, $NaH_2PO_4.H_2O$ 4, $Na_2HPO_4.2H_2O$ 6, during preparation and experiments.

Electrophysiology

Extracellular *en passant* nerve recordings were made with single hook electrodes, placed on the nerve of interest and insulated with Vaseline.

Intracellular neuronal recordings were made from motor neuron somata in the preparation described above. Electrodes made from thick-walled borosilicate glass were used and had tip resistances of 80–160 MΩ (tip solution, 3% neurobiotin (Vector) in 1 mol l^{-1} KCl; shaft solution, 1 mol l^{-1} KCl). The neurobiotin was injected with pulses of positive current (2–5 nA, 1 Hz and duty cycle 50% for 30 min, or continuous current for 5–10 min). After fixation of the ganglion, neurobiotin was revealed using Cy3-conjugated streptavidin (Sigma).

Intracellular muscle recordings were performed with flexible electrodes according to Pearson and Iles (1971). Thin-walled borosilicate glass electrodes with tip resistances of 20–40 MΩ were filled with 3 mol l^{-1} potassium acetate. Shafts were shortened to about 1 cm length and mounted on Ag/AgCl-wires (100 μm diameter) with silicone elastomer (Kwik-Cast, WPI). Recordings were made near the middle of the muscle fibre.

In experiments where just the presence of inhibitory muscle innervation was to be examined, the preparation was usually left at 4–8°C for 1–2 h before experiments. During this time, the resting membrane potential of the muscle fibres stabilised at between –20 and –40 mV, depending on muscle fibre type. This was probably due to reduced oxygen supply after removal of the main tracheae. In this situation, inhibitory postsynaptic potentials (IPSPs) were clearly discernible. The activity of excitatory motor neurones was reduced and often absent, minimising muscle movement, while the inhibitory motor neurones showed increased spontaneous activity.

Immunocytochemistry

The immunocytochemical procedures were adapted after Stevenson et al. (1992, 1994) and are outlined only briefly, except where they were different from standard procedures. The nervous system was dissected from the animal and immediately fixed in glutaraldehyde/picric acid/acetic acid solution (GPA; Boer et al., 1979) for 2 h at room temperature. Following dehydration and rehydration in an ethanol series (50, 70, 90, 96 and 100%), the tissue was washed in 0.1 mol l^{-1} phosphate-buffered saline (PBS) containing 0.1 mol l^{-1} phosphate buffer, pH 7.4, 0.1 mol l^{-1} NaCl; washing steps were also interspersed between the following incubations. To saturate double bonds (Stevenson et al., 1994) the ganglia were incubated in 0.5% $NaBH_4$ in PBS for 30 min. Then they were digested in a collagenase/dispase, hyaluronidase mixture (1 mg ml^{-1}, Roche and Sigma, respectively) for 30 min to 2 h, depending on the preparation, at 37°C to improve access for antibodies. Following 1 h incubation in 10% normal goat serum (NGS) in PBS with 0.5% Triton-X-100 (TrX, Sigma), the nervous system was incubated in 1:10 000 diluted GABA antiserum (polyclonal antibody raised in rabbit, SFRI, France) in PBS with 0.1% TrX and 1% NGS for 48 h at 37°C. Incubation with the secondary antibody (Cy2-conjugated goat-anti-rabbit IgG, or biotinylated goat-anti-rabbit IgG, both from Dianova) was in PBS with 0.1% TrX for 12 h at 37°C, and at 1:100 dilution. After washing for 24 h at 37°C, the nervous system was dehydrated, cleared in methylsalicylate and embedded in DPX (Fluka) for subsequent microscopic examination. The specificity of the primary antibody was tested at regular intervals according to standard protocols (mainly omitting the primary antibody, testing different fixatives, etc.).

Backfills and histology

The motor neuron supply of selected muscles was labelled by backfilling with 3% neurobiotin in distilled water. The motor nerve was immersed in the filling solution for about 12 h at 4–8°C. The marker substance was contained in a small vaseline well located close to the muscles in question. Individual motor neurones were filled with neurobiotin by intracellular impalement and current injection (see above). Following selective labelling by nerve backfill or intracellular injection, neurobiotin was coupled to Cy3-conjugated streptavidin. As for the immunocytochemical preparations, the backfills were first examined and photographed under a confocal epifluorescence microscope (DMRE/TCS SP, Leica). Further histological processing was conventional, and ganglia were sectioned at 8–10 μm for reconstructions (e.g. Fig. 7).

Results

Our aim was to examine the possible innervation of abdominal body wall muscles by inhibitory motor neurones in the locust. Several approaches, namely, extra- and intracellular electrophysiology, motor neuron backfills, and immunocytochemistry directed against GABA, were combined to identify and characterise such inhibitory motor neurones.

Electrophysiology

A combination of extra- and intracellular electrophysiology was employed for the initial examination of a possible inhibitory innervation of body wall muscles in the locust abdomen. Fig. 1 illustrates the experimental situation. Prominent inhibitory postsynaptic potentials (IPSPs) were recorded in several body wall muscles, namely, dorsal, pleural and ventral intersegmental muscles. In abdominal segment 6, these were muscles 212a,b,c,d, 213a,b (dorsal intersegmental muscles), 214 (pleural intersegmental muscle), 217 and 218 (ventral intersegmental muscles), nomenclature according to

Table 1. *Innervation pattern of body wall muscles by inhibitory motor neurones, segmental homology (homonomy)*

Neuromere	Neuron	Innervated muscles
S3	SI_{54}	**54?**
	SI_{55}	**55?**
T1	CIa	59/81/82
	CIb	59/60
	SI_{60}	60
T2	SI_{87}	87
	CIb	87/88/116
T3	CIa	141/142
	CIb	**117?**/143/144
A1	CIa	149/150/152
	CIb	**144?**/154/155
A2	CIa	167/168/169
	CIb	**155?**/172/173
A3	CIa	182/183/184
	CIb	187/188
A4	CIa	197/198/199
	CIb	202/203
A5	CIa	212/213/214
	CIb	217/218
A6	CIa	227/228/229
	CIb	232/233
A7	CIa	242/243/244
	CIb	247/248

SI, specific inhibitors; CI, common inhibitors. a and b designate the segmental homologues (homonoms) of CIa and CIb identified in the present study; SI neurons are specified by subscripts according to the muscle they innervate.

Homonomy of SI_{87} in T2 and CIa in abdominal ganglia is predicted on morphological grounds.

Innervation data for T1 (prothoracic ganglion), P. Bräunig, personal communication (see also Bräunig, 1993; Bräunig et al., 2002).

Innervation data for T2 (mesothoracic ganglion) through A3 (last abdominal neuromere fused to T3) are predictions based on the present study, verified only in selected cases (data not shown; T. Müller, personal communication).

Muscles marked by question marks and bold print are predictions based on homonomy.

Snodgrass (1935). In the other abdominal segments with unfused ganglia these were the segmentally homologous (homonomous) muscles (see Table 1). The amplitudes of IPSPs markedly decreased with more negative membrane potentials imposed by current injection, attesting to the inhibitory innervation of these muscles (reversal potentials were not determined, however). Simultaneous extracellular nerve recordings demonstrated that the axons of the inhibitory motor neurones left the ventral nerve cord through nerve 1 of the segmental abdominal ganglion. Note that muscles 219, 220, 221, 222 and 224, and their segmental homologues (homonoms) in other segments, did not receive inhibitory innervation according to all our experiments (see also below).

Intracellular recordings from motor neuron somata were guided by the results of combined motor neuron backfills of nerve 1 and immunocytochemistry directed against the transmitter of insect inhibitory motor neurones, GABA (see below). Fig. 2 shows an intracellular recording from the cell body of an inhibitory motor neuron (CIa), a simultaneous extracellular *en passant* recording from nerve 1 of the segmental ganglion (N1AG6), and an intracellular recording from a dorsal intersegmental muscle (M212a). Five sample traces are superimposed, illustrating the 1:1 correspondence of action potential recordings in the motor neuron soma and nerve 1, respectively, and IPSPs in the muscle fibre. This recording provides proof of inhibitory innervation of muscle 212a by motor neuron CIa. Inhibitory innervation of every muscle shown in Fig. 1 was tested and verified in this way. The time delay between centrally recorded motor neuron spikes in CIa and IPSPs in muscle 212 was approximately 20 ms, equivalent to a conduction velocity of approx. $0.7\,m\,s^{-1}$.

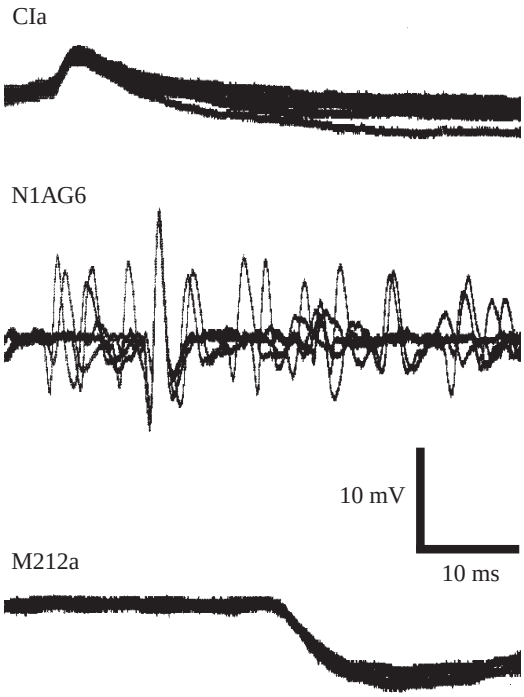

Fig. 2. Electrophysiological identification of muscle supply by inhibitory motor neurones. Top trace, intracellular soma recording from common inhibitor a (CIa); middle trace, extracellular recording from nerve 1 close to its exit from the sixth abdominal ganglion (N1AG6); bottom trace, intracellular recording from a muscle fibre in the dorsal intersegmental muscle 212a (M212a). Five sample recordings are superimposed, taking the largest spike in the nerve recording as reference. Note correspondence of, and constant time delay between, spikes in motor neuron and nerve recordings, and IPSP in muscle fibre recording.

In other body segments, inhibitory muscle innervation was often examined in a more simple way. IPSPs were recorded in one muscle (usually a readily accessible dorsal intersegmental muscle) and the supply of other muscles by the same inhibitory motor neuron was sought by examining recordings from nerve 1 and from the nerve branch supplying a particular muscle, and

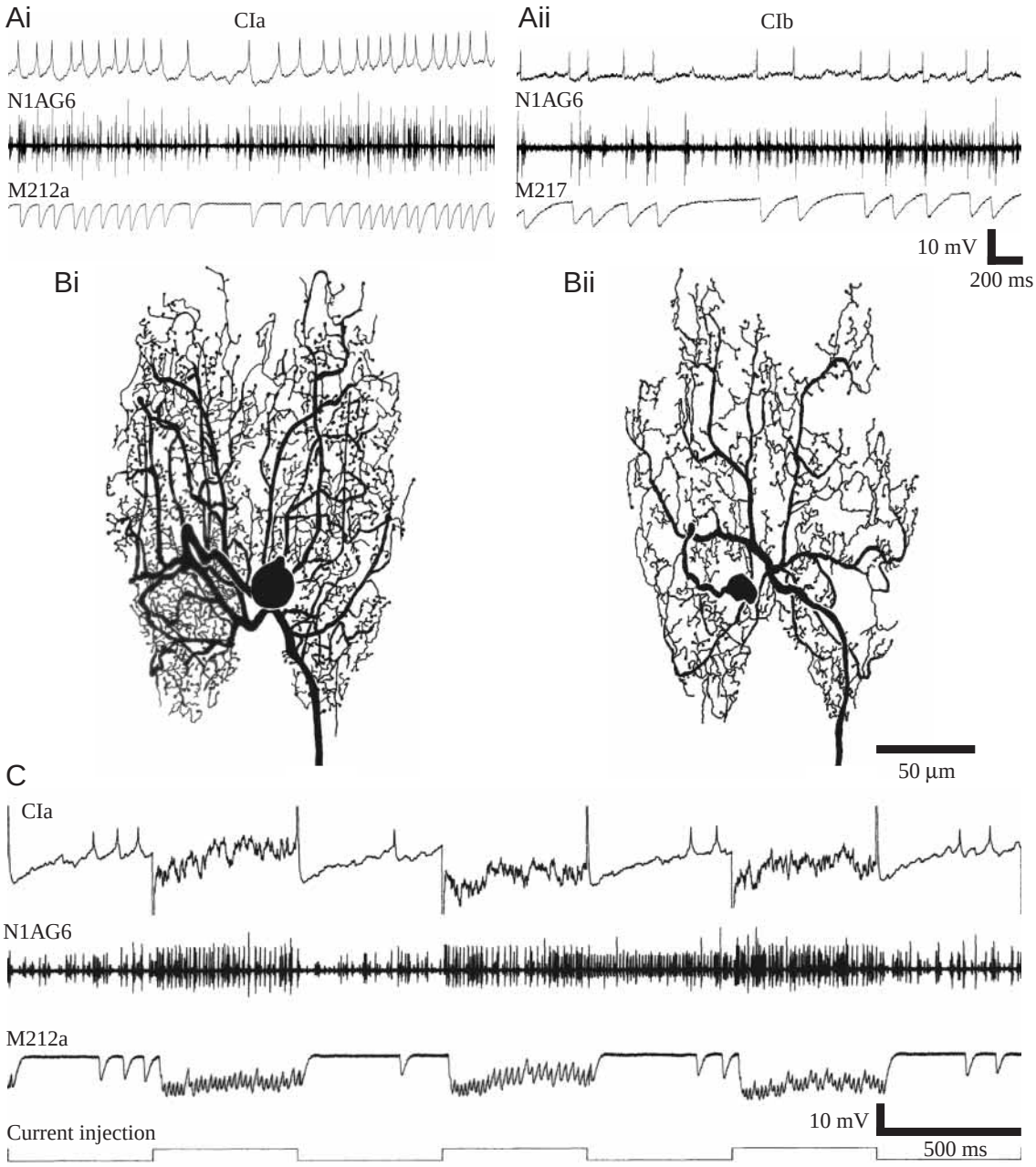

Fig. 3. Identification of inhibitory motor neurones CIa (i) and CIb (ii). (A) Intracellular recording from an inhibitory motor neuron's cell body (top traces) was combined with an extracellular recording from nerve 1 (or one of its branches; middle traces) and an intracellular recording from a target muscle (bottom traces) (experimental situation outlined in Fig. 1). 1:1 correspondence of spikes in motor neuron soma and nerve 1 with IPSPs in the muscle fibre recording indicated that the soma of an inhibitory motor neuron that supplies the muscle had been impaled (compare Fig. 2). (B) Dye was injected into the motor neuron soma to allow staining and morphological identification (see Materials and methods). (C) Current injection (bottom trace, e.g. in the context of staining CIa; other traces as in A) further verified the 1:1 correspondence of CI spikes and IPSPs noted above.

sometimes also by a second intracellular muscle recording. A 1:1 correspondence of spikes and IPSPs was taken as strong indication of inhibitory innervation by the same neuron. Immunocytochemical data (see below) supported these electrophysiological results. In summary, the homonoms of all the muscles (shown in Fig. 1 for the sixth abdominal segment) were found to be supplied by their respective inhibitory motor neurones in abdominal segments 1–7. In the thorax, too, a comparable innervation pattern was observed. The data are summarised in Table 1 and discussed in detail below.

Identification of abdominal inhibitory motor neurones CIa and CIb

The combined recording from nerve supply, muscle fibre

and inhibitory motor neuron described above (Fig. 2) was used to identify neurones subsequently injected with neurobiotin to reveal their morphologies (Fig. 3B; CIa, *N*=4; CIb, *N*=7). Fig. 3A demonstrates the 1:1 correspondence of spikes in the cell body recordings (top traces) and IPSPs in the impaled muscle fibres (bottom traces) (nerve recordings, middle traces). Fig. 3C illustrates that, prior to labelling, identification of the inhibitory motor neuron cell body was substantiated by depolarising current injection, which allowed both a clearer assessment of the 1:1 correspondences noted above, and direct control of IPSP generation in the muscle fibre. Note, for instance, the summation of IPSPs in muscle 212a (third trace from top) due to the high spike frequency in CIa (most clearly seen in the recording from nerve 1, second trace).

In each abdominal segment, two inhibitory motor neurones that supplied the body wall muscles could always be identified. Since both innervated two or more muscles (Fig. 1), they are Common Inhibitory motor neurones. Hence, we termed them CIa (Fig. 3Ai,Bi) and CIb (Fig. 3Aii,Bii), to provide a clear distinction (see below) from the thoracic common inhibitors, $CI_{1\text{-}3}$, described previously (e.g. Hale and Burrows, 1985). The cell bodies of both motor neurones resided in the ganglion anterior to the position where their axons exited the central nervous system in nerve 1 (Fig. 1). They were located in the posterior ventral cortex of the ganglion, usually just contralateral of the midline, although the exact soma positions were quite variable. Occasionally, CIa soma was on, or slightly ipsilateral to, the midline. The soma of CIa was between 25 and 30 μm in diameter, and thus distinctly larger than the soma of CIb, which measured about 20 μm across. The path of the primary neurite made a very characteristic loop, running dorsolaterally from the soma towards dorsal commissure V, (DCV; Watson and Pflüger, 1987), CIa with a sharp turn, and CIb far into the contralateral hemiganglion with a rounded loop (Figs 3Bi,ii, 7). The dendritic arborisations covered nearly the complete dorsal neuropil area of the ganglion and were almost bilaterally symmetrical. Only CIa had a few minor dendrites extending ventrally. The two halves of the bilateral arborisations were connected at the ganglion midline, close to where the primary neurite joined them. Usually three or four prominent secondary neurites were discernible per hemiganglion (Fig. 3Bi). CIa always exhibited more profuse and dense arborisations than CIb. The main neurites of CIa and CIb entered dorsal commissure V, and their axons left the segmental ganglion through the posterior connective to exit the ventral nerve cord through nerve 1 of the next more posterior ganglion (arriving there from the dorsal intermediate tract, DIT) (Fig. 7). In keeping with its larger cell body, CIa also had the larger axon, even in the peripheral nerves.

Activity patterns of the abdominal inhibitors

Once CIa and CIb had been identified, their target areas (see above) and activity patterns could be characterised in more detail. Fig. 4 illustrates the discharge patterns of both inhibitors, by means of recordings from muscle 212, supplied by CIa, and muscle 217, supplied by CIb (a recording from nerve 1 monitored overall neuronal activity). The IPSPs generated in the muscle fibres by the inhibitory motor neurones are clearly visible, reflecting the inhibitors' spike activities (compare Fig. 2). The (larger and briefer) depolarisations produced by excitatory input are also visible; however, they were not studied here (see e.g. Thompson et al., 1999). It is evident that both CIs discharged their highest spike frequencies just at the beginning of the inspiration phase, perhaps actively terminating muscle contractions needed for expiration. Spike activity in CIb was restricted to the beginning of the inspiration phase, while CIa also produced action potentials during the later part of the inspiration and the beginning of the expiration phases, albeit at much lower frequencies. Spike activity in CIa was, on average, higher and also reached higher peak frequencies than the discharges in CIb. CIa activity ceased reliably only around the middle of inspiration.

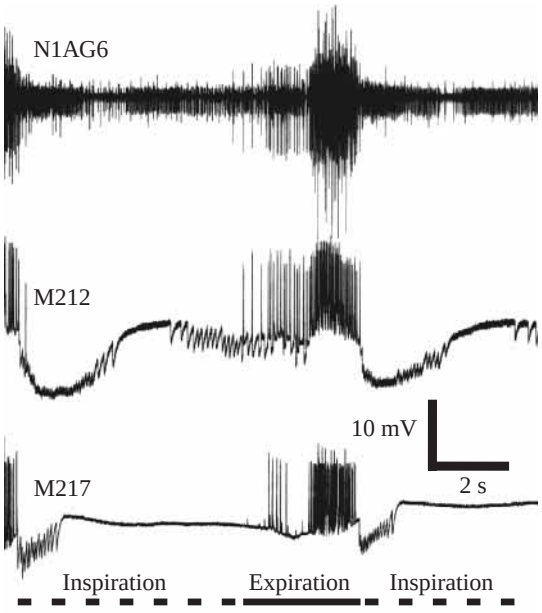

Fig. 4. Activity pattern of inhibitory motor neurones in the ventilatory cycle. First (top) trace, extracellular recording from nerve 1 close to its exit from the sixth abdominal ganglion (N1AG6); middle trace, intracellular recording from a fibre in dorsal intersegmental muscle 212 (M212); bottom trace, intracellular recording from a fibre in ventral intersegmental muscle 217 (M217); below, expiration and inspiration phase of the ventilatory cycle obtained by optical monitoring. Activity of excitatory motor neurones is signified by prominent depolarisations during the expiration phase, activity of inhibitory motor neurones by smaller IPSPs, which are particularly evident due to summation at the beginning of the inspiration phase. Muscle 212 is supplied by CIa, muscle 217 by CIb (see Fig. 1).

Immunocytochemistry

Anti-GABA immunocytochemistry was employed to mark the cell bodies of putative inhibitory neurones in the ganglia of the ventral nerve cord, and putative inhibitory axons in the peripheral nerves (see Materials and methods). Combining anti-GABA immunocytochemistry with backfills of nerve 1

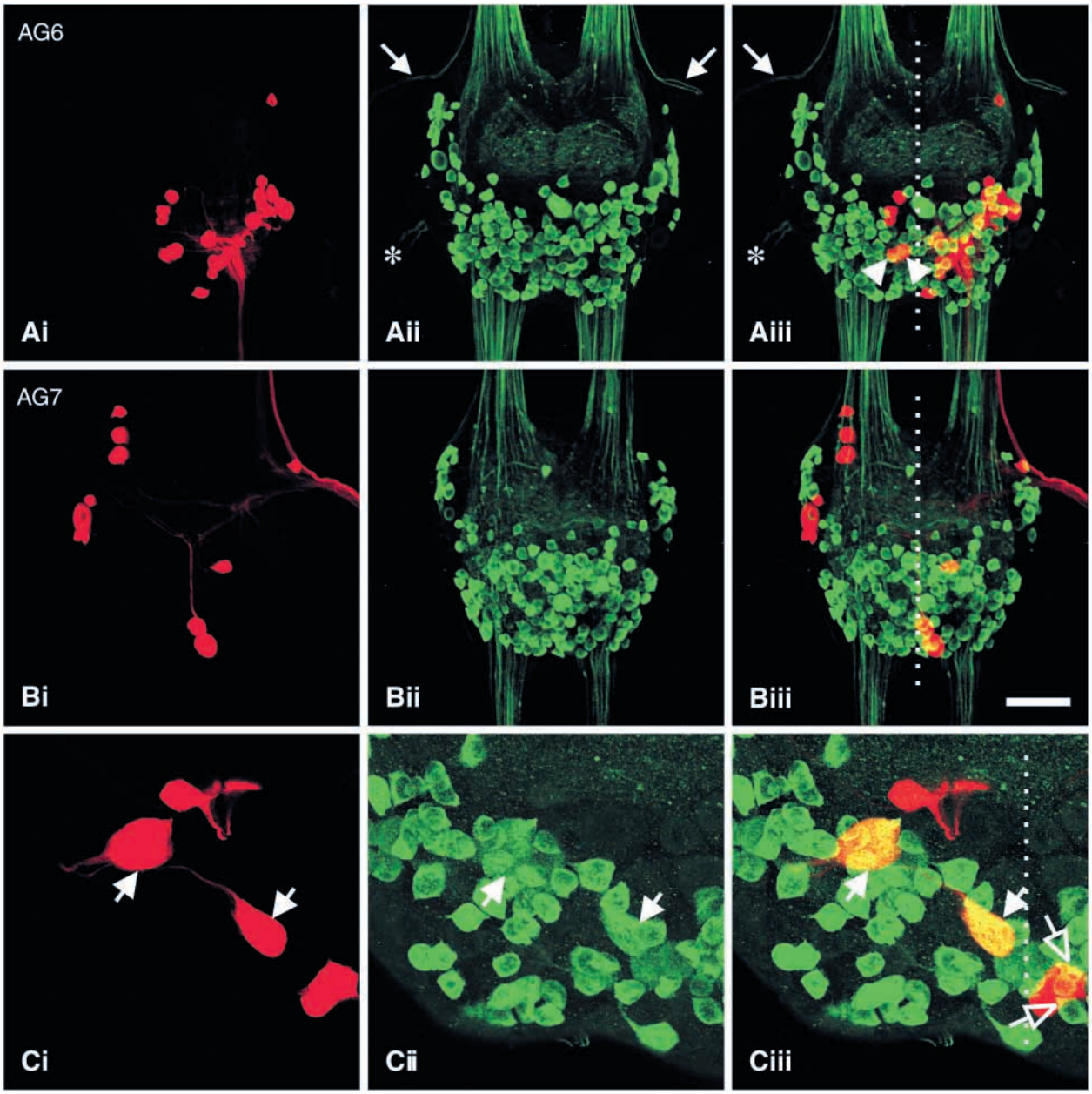

Fig. 5. Confocal microscopic images of abdominal ganglia 6 (A) and 7 (B) of the locust after backfilling of the right nerve 1 in abdominal ganglion 7 (red fluorescence, Ai,Bi,Ci) and GABA-immunocytochemistry (green fluorescence, Aii,Bii,Cii). Superposition of both labels is shown in Aiii,Biii,Ciii. There are only two somata in abdominal ganglion 6 that show the double label; they are located posteriorly, ventrally and contralaterally, and belong to CIa and CIb (arrowheads in Aiii). (C) Detail from a different preparation; abdominal ganglion 5, backfill in abdominal ganglion 6 (arrows indicate somata of CIa and CIb; ostensible double-labelling due to partial overlap of neighbouring red and green cells marked by open arrows). Two GABA-immunoreactive motor axons project to the periphery (arrows in A) through nerve 1 of each of the unfused abdominal ganglia. Asterisks in Aii and Aiii mark unspecific staining in nerve 2 of the sixth abdominal ganglion. Dotted lines indicate ganglion midlines. Scale bar, 100 μm (A,B); 20 μm (C).

provided another method to identify inhibitory motor neurones CIa and CIb (Fig. 5). The backfill technique labelled motor neurones with axons in nerve 1 (Fig. 5Ai,Bi,Ci), while immunocytochemistry labelled the cell bodies of GABA-immunoreactive, and thus putative inhibitory, neurones (Fig. 5Aii,Bii,Cii), including motor neurones. Combined staining thus identified inhibitory motor neurones with axons in nerve 1 (Fig. 5Aiii,Biii,Ciii). There were always (N=58) two inhibitory motor neuron somata labelled in the abdominal ganglion anterior to the nerve 1 backfill. According to the electrophysiological data described above these were unequivocally identified as the cell bodies of CIa and CIb. Soma size and location were in agreement with those given in the anatomical description above.

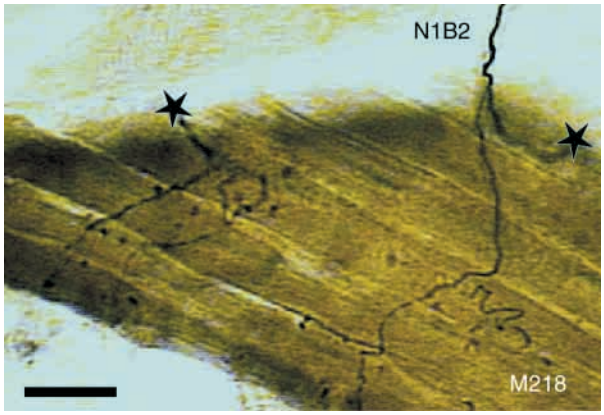

Fig. 6. Immunocytochemical identification of muscle supply by inhibitory motor neurones. Muscles of the abdominal body wall (muscle 218 is shown) were subjected to anti-GABA immunocytochemistry, which stained axons and terminals of the inhibitory motor neuron supply; branches of nerve 1B2 are shown here. Asterisks mark the disconnected ends of an originally continuous nerve branch. Scale bar, 50 μm.

In the nerves 1 of all unfused abdominal ganglia there were two immunoreactive axons, which obviously entered the segmental ganglion from the ipsilateral anterior connective and again left through the nerve root before making contact with any structures in the ganglion itself (arrows in Fig. 5Aii, Aiii). In a few cases (*N*=11), it was confirmed through intracellularly recorded and dye-injected inhibitory motor neurones that in abdominal ganglion 6, the thicker of the two axons was that of CIa, while the thinner axon belonged to CIb. In segments where abdominal neuromeres are fused to each other (A8–10, A11 not yet clear) or to the metathoracic ganglion (A1–3), and also in the thoracic ganglia (T1–3) and the third subesophageal neuromere (S3), corresponding immunoreactive axons were observed in the nerve roots, which contain the axons of neurones homonomous to those running through abdominal nerve roots 1 (Steffens and Kutsch, 1995) (summarised in Fig. 8).

When the two immunoreactive axons in abdominal nerve 1 were followed into the periphery, they were observed to innervate the muscles noted in Fig. 1 for abdominal segment 6. This is illustrated for muscle 218 and the axon of CIb in Fig. 6. Even synaptic boutons were visible in many immunocytochemistry preparations, usually on the more central fibres of a given muscle, and in agreement with the electrophysiological muscle fibre recordings reported above, where inhibitory innervation was often absent in the superficial fibre layers of a muscle. These observations are in agreement with previous accounts of arthropod muscle structure (e.g. Müller et al., 1992), stating that CI innervation parallels innervation by ‘slow’ motor axons, and that the muscle fibres supplied by ‘slow’ axons usually occupy the central portion of a muscle (Rathmayer and Erxleben, 1983). Above all, these observations confirmed the results of the electrophysiological experiments described above, namely, inhibitory innervation of muscles 212, 213, 214, 217 and 218 in abdominal segment 6 (Fig. 1), and of the homonomous muscles in the other abdominal segments. Inhibitory innervation was evidently absent in muscles 219, 220, 221, 222 and 224, and their homonoms.

Immunocytochemistry also indicated that a number of body wall muscles in the thorax receive inhibitory innervation from motor neurones homonomous to CIa and CIb (summarised in Table 1), although CIa and CIb were rigorously identified by intracellular electrophysiology only in the sixth abdominal segment.

Discussion

Inhibitory motor neurones of locust abdominal body wall muscles

In each abdominal hemiganglion, there are two inhibitory motor neurones, CIa and CIb, which supply the dorsal intersegmental muscles 212, 213 and 214, and their homonoms (CIa), and the ventral intersegmental muscles 217 and 218, and their homonoms (CIb) (Fig. 1). They were identified by (i) intracellular dye injection during parallel electrophysiological recordings from the inhibitory motor neuron and a muscle fibre supplied by it, and (ii) by combined labelling through anti-GABA immunocytochemistry and backfills of motor axons in nerve 1 of the abdominal ganglion (Fig. 5). The cell bodies of both inhibitors are located in the ganglion anterior to where the axons leave the ventral nerve cord *via* nerve 1 of the segmental ganglion. Their morphology in the ganglion is very characteristic, with contralateral, posterior and ventral somata, looping primary neurites and bilateral dorsal arborisations.

At a first glance, the presence of inhibitory motor neurones in the abdominal segments of the locust may appear surprising. Considering the absence of appendages (except genitalia and cerci in the most posterior segments), and the fact that almost all insect inhibitory motor neurones described so far subserve the control of appendicular musculature, one might have expected the absence of inhibitory muscle innervation in the abdomen (see Introduction). Walking legs (Hale and Burrows, 1985), antennae (Honegger et al., 1990), and even crustacean mandibles (Ferrero and Wales, 1976) are all supplied by common inhibitory motor neurones. Whether there is a similar inhibitory supply of body wall muscles was as yet unknown, except for a few cursory reports for locust (Yang and Burrows, 1983; Bräunig, 1993; Bräunig et al., 2002; Schmäh et al., 2001; Schmäh and Wolf, 2002), cricket and bushcricket (Consoulas and Theophilidis, 1992; Consoulas et al., 1993). The body wall inhibitors, CIa and CIb, are not, however, homologues of the common inhibitors which supply the leg muscles in the thorax, $CI_{1\text{-}3}$. This is most clearly demonstrated by the coexistence of both types in the prothoracic segment (Bräunig et al., 2002; Schmäh, 2002; Schmäh and Wolf, 2002), where they supply body wall and leg muscles, respectively. The two classes of inhibitors probably have different developmental origins, since the neuroblast, which gives rise to two of the leg inhibitors, neuroblast 5.5 (Wolf and Lang, 1994), is absent in the abdominal ganglia. They nevertheless share several common

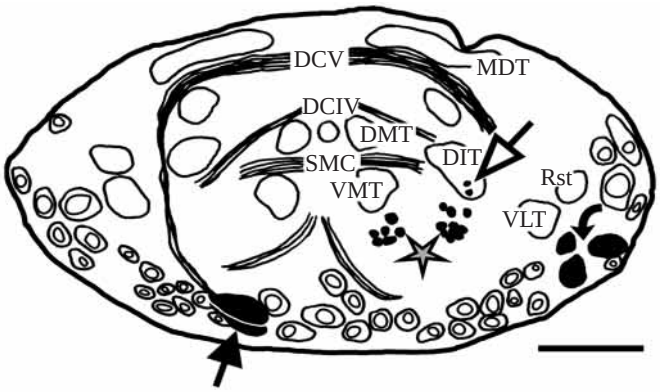

Fig. 7. Reconstruction in the frontal plane of a serially sectioned abdominal ganglion 5. Backfill of motor neurones through nerve 1 of abdominal ganglion 6 and subsequent GABA-immunocytochemistry identified the somata of CIa and CIb (black, straight arrow); the curved arrow indicates motor neuron somata without GABA immunoreactivity and the asterisk marks their primary neurites. The primary neurites of CIa and CIb leave their somata to loop dorso-laterally, crossing the ganglion midline in dorsal commissure V (DCV), and their axons project into the posterior connective *via* the dorsal intermediate tract (open arrow). Tracts and commissures are termed according to the nomenclature of Watson and Pflüger (1987). Dorsal is to the top. Scale bar, 50 μm.

features, namely, the innervation by each neurone of several muscles, the contralateral, posterior and ventral position of the motor neuron somata, and some characteristics of the trajectories of the primary neurites within the neuropil (Fig. 7).

It is not clear at present whether or not these similarities extend to the inhibitors' function. Innervation of several muscles by a single motor neuron would indicate a global function in setting muscle performance, as demonstrated for the limb inhibitors (see Wiens, 1989). The data available on the activity patterns of body wall inhibitors (Fig. 4) may support this interpretation. CIa and CIb are most active just at the beginning of the inspiration phase. This is also observed in the bushcricket (Consoulas and Theophilidis, 1992). Although the intersegmental muscles supplied by CIa and CIb are not involved in the main (dorso-ventral) respiratory movements of the abdomen, the inhibitors might still contribute to the termination of muscle contractions supporting expiration, or to the fast execution of the initial inspiration movements. As yet unknown are the activity patterns of CIa and CIb during other abdominal motor tasks, namely, postural control at rest, walking, flight and flight steering (Dugard, 1967; Baader, 1988), and during copulation and oviposition.

Homonomy (segmental homology)

Apparently, there are segmental homologues (homonoms) of the abdominal inhibitory body wall motor neurones in other segments of the locust body (summaries in Fig. 8, Table 2), as indicated by anti-GABA immunocytochemistry, and selected nerve (partly P. Bräunig, personal communication) and muscle (partly T. Müller, personal communication) recordings. When these data are combined with morphological descriptions in previous studies (Bräunig, 1993; Bräunig et al., 2002; Kutsch and Heckmann, 1995; Steffens and Kutsch, 1995; Schmäh et al., 2001; Schmäh, 2002; Schmäh and Wolf, 2002), they provide an unequivocal identification of inhibitory motor neurones in the following sections of the ventral nerve cord: (i) the third subesophageal neuromere, (ii) the pro- and mesothoracic ganglia, (iii) the three abdominal neuromeres fused with the metathoracic ganglion, and (iv) all unfused abdominal ganglia. The presence of two homonomous inhibitory motor neurones in the metathoracic ganglion and in the most anterior neuromeres of the terminal ganglion are predicted only on the basis of the present immunocytochemical data.

In their backfill study of ventral body wall muscles, Steffens and Kutsch (1995) provided detailed anatomical descriptions of 'common contralateral A-type cells' in all ganglia and neuromeres between the third subesophageal neuromere and seventh abdominal ganglion, except the metathoracic neuromere. These 'common contralateral A-type cells' exhibit all the characteristics of CIb, including common innervation of ventral intersegmental muscles, contralateral and posterior soma location, and looping primary neurite. Since the backfills

Table 2. *Summary of inhibitory body wall motor neurones in the locust*

	Neuron(s)	
Neuromere*	This study	Other authors
S3	**SI_{54}[2], SI_{55}[2]**	LC, CLAC
T1	CI_a[3],CI_b[3], SI_{60}[3]	LC, CLAC
T2	SI_{87}[1], CI_b[1]	CLAC
T3	**CI_a[2], CI_b[2]**	CLAC, IC
A1	**CI_a[2], CI_b[2]**	CLAC
A2	**CI_a[2], CI_b[2]**	LC, CLAC
A3	**CI_a[2], CI_b[2]**	CLAC
A4	CI_a[1], CI_b[1]	LC, CLAC
A5	CI_a[1], CI_b[1]	CLAC
A6	CI_a[1], CI_b[1]	LC, CLAC
A7	CI_a[1], CI_b[1]	
A8	**CI_a[2], CI_b[2]**	
A9	**CI_a[2], CI_b[2]**	

*Neuromere refers to soma location, as opposed to that of nerve exit.

SI, CI, specific and common inhibitory motor neurones; subscripts a and b designate different identified cells, and putative homonoms of CI_a and CI_b identified in the present study; SI neurons are specified by subscripts according to the muscle they innervate.

CLAC, 'common contralateral A-type cell' of Steffens and Kutsch (1995).

LC, 'loop cell' of Kutsch and Heckmann (1995).

IC, inhibitory cell of Yang and Burrows (1983).

[1]Identified in present study.

[2](in bold), predicted from present study, i.e. observed in backfill preparations or anti-GABA immunocytochemistry, but no electrophysiology or co-localisation experiments were performed.

[3]After Bräunig (1993), Bräunig et al. (2002), Schmäh (2002).

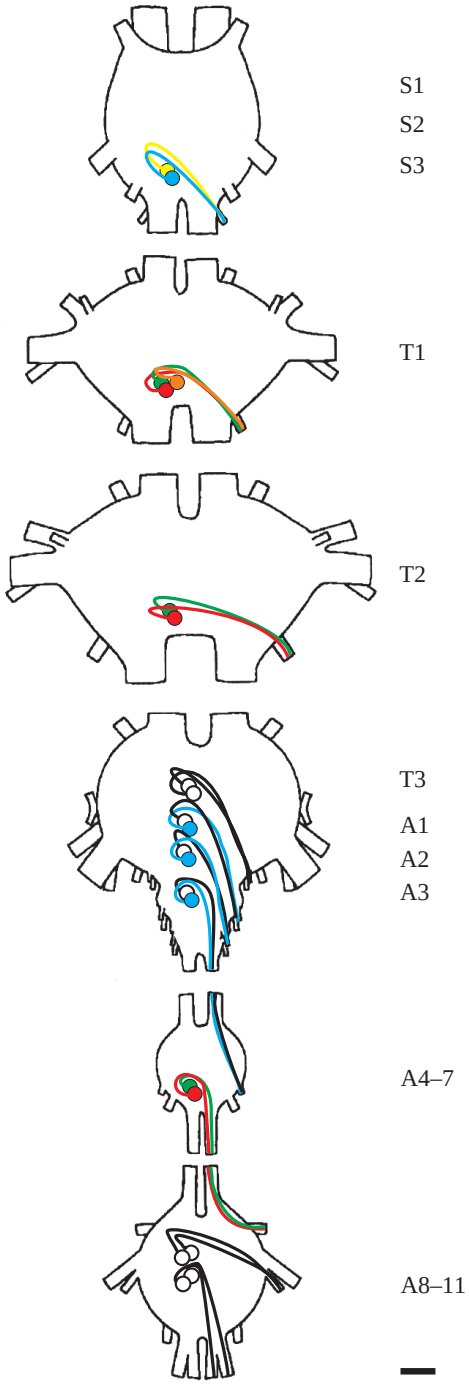

Fig. 8. Summary of inhibitory body wall motor neurones in the locust, incorporating data from other authors and possible homonomies. From the top, subesophageal (S1–3), thoracic (T1–3), fused (A1–3, A8–11) and unfused (A4–7) abdominal neuromeres and ganglia. Segmentally homologous (homonomous) inhibitory motor neurones are shown as outline sketches. Colour coding: red, CIa; green, CIb; yellow, 'loop cell' of Kutsch and Heckmann (1995) (putative homologue of CIb); blue, 'common contralateral A-type cell' of Steffens and Kutsch (1995) (putative homologue of CIa); orange, neuron after Bräunig (1993) and Bräunig et al. (2002); open circles, neurones predicted from present study. Scale bar, 200 µm.

of Steffens and Kutsch (1995), and our own backfills of nerves 1 (Fig. 5), marked the full complement of motor neurones that supply the respective ventral body wall muscles, comparison of these data with the immunocytochemical labelling identifies homonoms of CIb in the segments named above. A similar line of argument holds for the 'loop cell' described by Kutsch and Heckmann (1995), which innervates dorsal body wall muscles. This cell has been examined in the third subesophageal neuromere, the prothoracic ganglion, the mesothoracic ganglion, and abdominal ganglia 2, 4 and 6. The 'loop cell' is homonomous to CIa by the criteria just noted.

In the case of the terminal ganglion, there are no previous studies that would allow homonomisation of equivalents of the 'loop cells' or 'contralateral A-type cells' with CIa or CIb. The present immunocytochemical data are strongly suggestive, however. There is the expected pattern of immunoreactive neurones in abdominal neuromeres 8 and 9, i.e. two axons each leave through the third nerve root of the terminal ganglion (tergal nerve of neuromere 9) and through the epiproct nerve (corresponding to nerve 1 of neuromere 10), and two immunoreactive motor neuron somata per hemineuromere exist in the appropriate regions of the soma cortex (indicated in Fig. 8). No inhibitory motor neurones appear to exist in neuromeres 10 and 11, which carry the genitalia, and no immunoreactive axons were observed in the corresponding nerves. Despite this consistent general pattern, there was sometimes variability, even asymmetry, in the courses of the inhibitory axons and even in the number of axons per nerve.

These findings are in good agreement with the immunocytochemical study of Watson and Pflüger (1987), except that these authors occasionally observed thin immunoreactive structures in the cercal nerve and even in nerves 2 of the eigth neuromere and the free abdominal ganglia. Considering that two species of locust were examined, different antisera were used in the two studies, and that GABA immunocytochemistry is prone to false positive as well as false negative results, this difference is not too surprising, and the issue awaits further scrutiny.

There are two immunoreactive axons in nerve 6 of the metathoracic ganglion and two immunoreactive somata with the appropriate morphological characteristics in the metathoracic neuromere, indicating a normal complement of two body wall inhibitors in the metathoracic neuromere. In the prothoracic ganglion, a third inhibitory motor neuron of body wall muscles is present, in addition to the CIa and CIb homonoms, according to Bräunig (1993), Bräunig et al. (2002), Schmäh and Wolf (2002), and the present immunocytochemical data. The morphology of this third inhibitor is strongly reminiscent of that of the two other body wall CIs, suggesting a close relationship.

Species comparison and evolutionary considerations

The presence of three body wall inhibitors in the locust prothoracic segment raises the question of whether or not there might be three inhibitory body wall motor neurones in other

insects, and what the original, evolutionary plesiomorphic complement of body wall inhibitors might be (Schmäh, 2002). The situation in the abdominal ganglia of phasmids is similar to that in the locust, and thus allows no further conclusions. In the abdominal ganglia of dragonflies, by contrast, there appear to be three inhibitory motor neurones, all with their major features reminiscent of CIa and CIb in the locust (Schmäh, 2002). Data from crickets and bushcrickets point in a similar direction (Consoulas and Theophilidis, 1992; Consoulas et al., 1993; Böser, 1999). Böser (1999) reports three inhibitory motor axons, and associated cell bodies, which supply body wall muscles in the pro-, meso- and metathoracic ganglia of the cricket *Acheta domesticus*. As in the locust prothorax, these body wall inhibitors coexist with the leg inhibitors, $CI_{1\text{-}3}$. Although preliminary, these observations, taken together, suggest that the original, plesiomorphic situation in pterygote insects is indeed a complement of three body wall inhibitors, a condition that is reflected in the locust prothoracic ganglion.

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