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# MAPS OF THE SOMATA OF EFFERENT NEURONES WITH AXONS IN THE LATERAL NERVES OF LOCUST ABDOMINAL GANGLIA

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## Summary

**We used the cobalt-backfilling method to map the somata of neurones with axons that project in the two paired lateral nerves of the abdominal neuromeres of the locust *Schistocerca gregaria* with the objective of expanding and bringing together the incomplete and scattered information on these efferent neurones. We compared somata sizes and positions, and the pathways of primary neurites, with information from previous studies on individual, or groups of, abdominal neurones and we identify many of the somata we mapped.**

**The stained somata belong to paired motor neurones and paired neurosecretory neurones, to unpaired neuromodulatory neurones (dorsal unpaired median, DUM, neurones) and unpaired bilaterally projecting neurones. In different neuromeres, the total number of somata with axons in these lateral nerves ranges from 73 to 106. Within an individual segmental neuromere, approximately 25 % of the somata belong to neurones with axons in nerve 1 (N1) and 35 % to those with axons in nerve 2 (N2) of that segment, while the remaining 40 % belong to neurones with axons in N1 of the next posterior segment. This basic pattern is repeated in all abdominal neuromeres, with differences in the percentages depending on whether the neuromeres are pregenital fused, pregenital unfused or genital.**

**Nerve 1 contains the axons of 26–37 neurones with central somata in different neuromeres, of which 40 % are in the segmental neuromere and 60 % in the next anterior neuromere. In the segmental neuromere, 15 % of somata are ipsilateral to the nerve, 30 % are at the midline and 55 % are contralateral, whereas in the next anterior neuromere, 70 % are ipsilateral, 10 % are at the midline and 20 % are contralateral.**

**Nerve 2 contains the axons of 11–28 neurones in different neuromeres, all of which have somata in the same segmental neuromere from which the nerve projects. Of these, approximately 70 % are ipsilateral, 30 % at the midline and none contralateral, except for the first abdominal and eighth male abdominal neuromeres, where one and two somata, respectively, are contralateral.**

Key words: motor neurone, neurosecretory neurone, abdominal innervation, locust, *Schistocerca gregaria.*

## Introduction

Maps of the distribution of identified neurones have greatly aided our understanding of the organisation of neuronal networks. In many invertebrates, functional maps of the larger neurones are an integral aid to analyses of the mechanisms responsible for the generation of movement. Such maps of identified somata also provide evidence for neuronal homologies across species as, for example, in the abdominal ganglia of *Aplysia* spp. (Blankenship and Coggeshall, 1976; Kandel, 1979). Comparisons of the central morphology and the numbers of neurones can also illustrate how neuronal anatomy can be conserved in evolution despite changes in behaviour, as in the distal leg motor neurones of different species of decapod crustacean (Faulkes and Paul, 1997).

Studies on the organisation and action of the central nervous system of locusts have largely concentrated on the brain, suboesophageal and thoracic ganglia. For the metathoracic neuromere in particular, there is a detailed map of the motor neurones that innervate the muscles of the hind legs and hind wings (Siegler and Pousman, 1990a,b; Siegler et al., 1991). In contrast, the abdominal ganglia are known in far less neuroanatomical detail, even though the insect abdomen is involved in movements as diverse as ventilation (Lewis et al., 1973; Hustert, 1975), circulation of the blood (Ferber and Pflüger, 1990; Dircksen et al., 1991), steering during flight (Baader, 1988, 1991), digestion (Nässel et al., 1992, Cantera and Nässel, 1992) and reproduction (Yasuyama et al., 1988; Kimura et al., 1989; Kalogianni and Pflüger, 1992; Belanger and Orchard, 1993; Facciponte et al., 1995; Thompson and Roosevelt, 1998). The abdominal central nervous system of the locust consists of 11 neuromeres, the first three of which are fused to each other and to the third thoracic neuromere, forming the metathoracic ganglion. Neuromeres A4–A7 are unfused ganglia, whereas neuromeres A8–A11 are fused to form the terminal ganglion (see Burrows, 1996). Each

neuromere has two pairs of lateral nerves and an unpaired median nerve, except neuromere A10, which lacks a median nerve, and neuromere A11, which in the adult is present only in rudimentary form. Abdominal neuromeres anterior to the seventh abdominal neuromere (A7) do not show sexual dimorphism, whereas those of A7 and the terminal ganglion do (Pflüger and Watson, 1988).

The abdominal musculature and its innervation have been studied in locusts and other insects to determine whether they conform to a common '*Bauplan*' or basic repeated body plan (Kutsch and Breidbach, 1994; Kutsch and Heckmann 1995a,b; Heckmann and Kutsch, 1995; Steffens and Kutsch, 1995). These reports have usually focused on specific sets of muscles, such as the dorsal longitudinal or the ventral muscles, and their motor neurones. Studies locating biogenic amines and neuropeptides in abdominal ganglia of locusts and other insects have revealed the morphology and action of specific sets of neurones (Dircksen et al., 1991; Stevenson et al., 1992; Nässel, 1996). Other studies in locusts have shown the positions of the dorsal unpaired median (DUM) neurones (Pflüger and Watson, 1988, 1995; Stevenson et al., 1992, 1994) and some identified motor neurones (Lewis et al., 1973; Baader, 1988; Ferber and Pflüger, 1990). The motor neurones in the fourth abdominal ganglion of the hawkmoth *Manduca sexta* have been mapped, and their fate during metamorphosis has been followed to determine whether they continue to innervate the same muscles, die or survive to innervate new targets (Taylor and Truman, 1974; Levine and Truman, 1985).

In locusts, the information on those neurones that project laterally from the abdominal ganglia remains both incomplete and scattered. We sought, therefore, to produce a map of the positions of the somata of neurones in the abdominal neuromeres in the locust that have axons in the lateral nerves, with the aim of aiding future work on the identification of these neurones and the neuropeptides and neurotransmitters they express. We have backfilled the two lateral nerves of selected neuromeres with cobalt to reveal the numbers and positions of somata belonging to neurones with axons in these nerves. We relate these maps to existing descriptions of neurones to identify many of the somata we stained.

## Materials and methods

All experiments were performed on adult male and female locusts, *Schistocerca gregaria* (Forskål), aged 5–10 days post-final moult and taken from our crowded laboratory culture. Both males and females were used for neuromeres anterior to A7, since sex differences are not observed in these neuromeres (Pflüger and Watson, 1988). For neuromeres A7 and A8, males and females were studied separately, since sex differences are present in these neuromeres (Pflüger and Watson, 1988; Stevenson et al., 1994). The locusts were mounted ventral side uppermost, and parts of the ventral cuticle were removed to expose particular ganglia. The selected ganglion and the one immediately anterior to it, still linked by their paired connectives, were then dissected from the locust leaving intact a suitable length of a particular lateral nerve. Ganglia were then placed in saline in one of a pair of wells drilled in a Perspex block and separated by a narrow channel. The end of the nerve to be backfilled was placed in the adjacent well containing 6 % hexammine cobaltic chloride. Vaseline mixed with paraffin oil was present in the channel to act as a barrier between the two wells. The dish was placed in a humidified chamber and left at 4 °C for 4–40 h to allow for the diffusion of cobalt ions along axons of differing diameters and lengths. The ganglia were transferred to a dish containing 15 ml of saline, and the cobalt ions were precipitated by the addition of 60 µl of 20 % ammonium sulphide. After three rinses in saline, the ganglia were fixed for 1 h in 5 % neutral buffered formaldehyde. Most ganglia were silver-intensified (Bacon and Altman, 1977), dehydrated in an ethanol series, cleared in methyl salicylate and mounted in Canada balsam. Neuromeres that were overstained, either because of an over-long incubation period or because of leakage of cobalt, and those that were understained were not included in the analysis.

For each nerve of each neuromere, between six and 15 successful backfills were obtained; 148 fills were included in our analysis. The somata and primary neurites of the backfilled neurones were drawn within the outline of their ganglion with the aid of a drawing tube attached to a Zeiss Axiophot microscope. To produce a composite map of stained somata, the position and size of a particular soma and the pathway of its primary neurite were compared with these features of other stained neurones, in relation to the nerve roots and the outline of the whole ganglion. Small differences in the positions of somata in different locusts mean that the maps represent 'best fits', and a soma drawn in a particular position will not necessarily occur in that position in all locusts. We ascribed identities to a large proportion of the somata we mapped by making meticulous morphological and spatial comparisons (sizes and positions of somata and pathways of the primary neurites) with published accounts containing pictures and descriptions of identified individual neurones or groups of neurones. The numbering of muscles that some of the neurones innervate is based on Snodgrass (1929, 1935).

In the figures and tables, somata referred to as reliably stained (solid filled circles in Figs 1–5) were usually observed in all stains of a particular nerve and always in more than half the stains. A small number of additional somata were stained infrequently from a particular nerve (not in the same locust), and these were treated as observations that we could not verify with certainty. These somata are represented by open circles in Figs 1–5 as ones that were 'observed only once in the position indicated'.

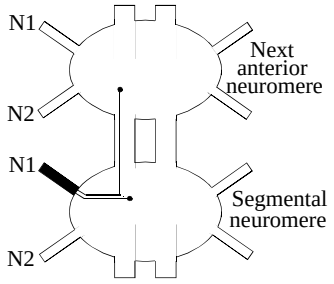
## Results

### *Positions of somata with axons in N1*

Lateral nerve 1 (N1, also called the dorsal or tergal nerve) was backfilled separately in the following neuromeres: T3 (third thoracic neuromere) and A1–A3 (abdominal neuromeres 1–3) of the fused metathoracic ganglion, the

Table 1. *Stained somata with axons in nerve 1*

| Neuromere of | Segmental neuromere | | | | Next anterior neuromere | | | | |
|---|---|---|---|---|---|---|---|---|---|
| N1 backfill | Ipsilateral | Midline | Contralateral | Subtotal | Ipsilateral | Midline | Contralateral | Subtotal | Total |
| T3 (T2.N6) | 1 | 2 | 7(10) | 10 | 7(9) | 4 | 2 | 13 | 23 |
| A1 (T3.N6) | 2 | 3(4) | 6 | 11 | 9(11) | 3 | 4 | 16 | 27 |
| A2 | 2 | 2(3) | 6 | 10 | 10(13) | 3 | 3 | 16 | 26 |
| A3 | 2 | 3 | 7 | 12 | 14 | 2 | 2 | 18 | 30 |
| A4 | 2(3) | 8(13) | 7(9) | 17 | 12(13) | 1 | 4 | 17 | 34 |
| A5 | 3 | 8 | 7 | 18 | 13(14) | 2 | 2 | 17 | 35 |
| A7 (female) | 3 | 6(7) | 6 | 15 | 17 | 2 | 3 | 22 | 37 |
| A7 (male) | 2(4) | 3 | 5(6) | 10 | 14 | 1(2) | 5(6) | 20 | 30 |
| A8 (female) | 0(1) | 2 | 5 | 7 | 15(17) | 1 | 3(5) | 19 | 26 |
| A8 (male) | 0(2) | 2 | 5 | 7 | 13 | 2 | 4(5) | 19 | 26 |




Somata of neurones with axons in N1 are present either in the neuromere from which the nerve projects (the segmental neuromere) or in the next anterior neuromere (see diagram at left).

In this and other tables, values represent the number of somata stained reliably at the positions indicated. Numbers in parentheses represent the maximum seen in any one preparation. Subtotals and totals include only those somata stained reliably. T3 (T2.N6) indicates that nerve 1 of T3 is fused with nerve 6 of T2; A1 (T3.N6) indicates that nerve 1 of A1 is fused with nerve 6 of T3.

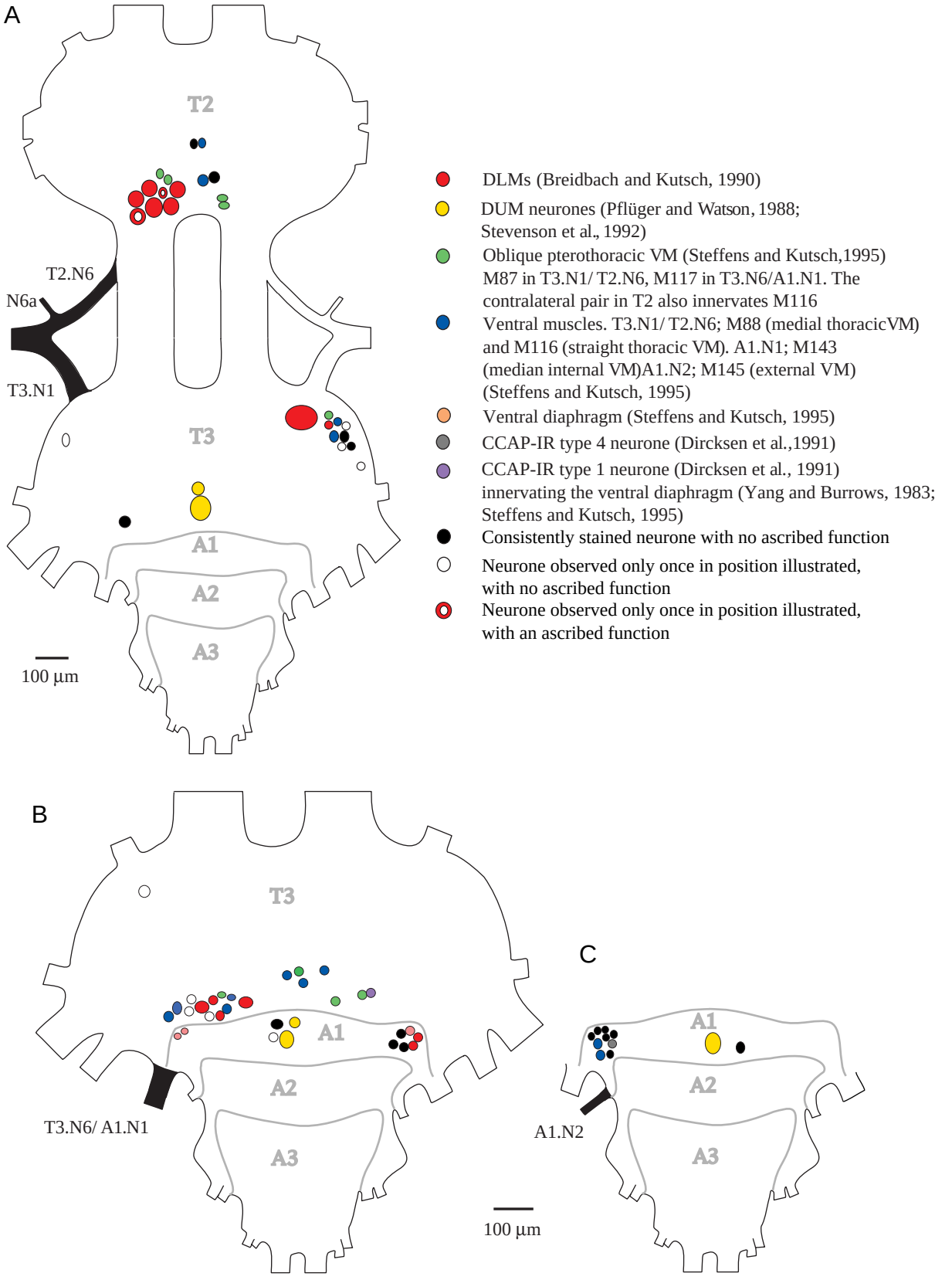
unfused pregenital abdominal ganglia A4 and A5, the unfused genital ganglion A7 and neuromere A8 of the fused terminal ganglion. The number of somata stained in these neuromeres was 23 in T3 and ranged from 26 to 37 in the abdominal neuromeres (Table 1, 'Total'; Figs 1–5). Approximately 40 % of the efferent neurones with axons in N1 had somata in the neuromere from which N1 projects (the segmental neuromere), whereas the remaining 60 % had somata in the next anterior neuromere with their axons projecting through the ipsilateral connective. A variation in this pattern was seen for N1 of unfused pregenital neuromeres A4 and A5, where somata that had axons in N1 were almost equally distributed between the segmental and the next anterior neuromeres. Somata were present in ipsilateral, midline and contralateral positions of both the segmental and the next anterior neuromeres. Overall, segmental neuromeres had approximately 15 % of somata ipsilateral to the nerve, 30 % at the midline and 55 % contralateral (Table 1; Figs 1–5). In the next anterior neuromere, the greatest number of somata (70 %) were ipsilateral, with only 10 % at the midline and 20 % contralateral (Table 1). This general distribution pattern of somata was repeated throughout the abdomen with some variations. These included, for example, the absence of ipsilateral somata in the segmental neuromere in male and female A8, while in the projections of N1 into the segmental neuromeres of A4 and A5, more midline somata than contralateral somata were present.

*Positions of somata with axons in N2*

Lateral nerve 2 (N2, also called the ventral or sternal nerve) was backfilled separately in the same abdominal neuromeres as N1 but not in T3, where the nerve with this name is not homologous to N2 of the abdominal neuromeres and contains only axons from sensory neurones (Bräunig et al., 1981; Hustert et al., 1981; Burrows, 1996). Efferent neurones with axons in N2 had somata only in the segmental neuromere from which the nerve projects (Table 2; Figs 1–5). Each neuromere had fewer somata of efferent neurones with axons in N2 than in N1, but there were greater differences in numbers of somata between different neuromeres (compare Table 1 with Table 2). For example, neuromere A1 had only 11 somata, whereas female A7 had 28. Despite these differences, a basic repeating pattern could still be recognised; the majority of the somata (approximately 70 %) were ipsilateral to the nerve, 30 % were at the midline and none was contralateral. Differences were seen in A1, where one soma was contralateral, in male A8, where two somata were contralateral, and in female A7, where there were more midline than ipsilateral somata. There were also sex-specific differences: females had more midline somata than males in neuromeres A7 and A8 and more ipsilateral somata in A8, whereas males had at least two contralateral somata in A8 that were absent in females (Figs 4, 5; Table 2).

*Bilaterally projecting neurones*

Abdominal neuromeres A1–A7 each contain three




bilaterally projecting DUM neurones, and female A7 contains eight DUM neurones (Stevenson et al., 1994). These unpaired neurones were also identified in our stained preparations (Figs 1–5), as were a number of bilaterally projecting non-octopaminergic neurones (Ferber and Pflüger, 1990) of A4 and female A7. The maximum number of bilaterally projecting

Fig. 1. Maps of somata of neurones with axons in lateral nerves. The nerves stained are shown in black. (A) Positions of somata with axons in nerve 1 (N1) of thoracic neuromere 3 (T3) and in N6 of mesothoracic neuromere T2. (B) Positions of somata with axons in N1 of A1 and in N6 of T3. (C) Positions of somata with axons in N2 of A1. Somata that could be identified have been colour-coded. The approximate outlines of the individual neuromeres (T3, A1–A3) that are fused to form the metathoracic ganglion are indicated. Note the larger scale used in B and C and in all subsequent figures. Abbreviations used in this and subsequent figures are as follows: A, abdominal neuromere; CCAP-IR, crustacean cardioactive peptide-immunoreactive; DLM, dorsal longitudinal muscle; DUM, dorsal unpaired median (neurone); M, muscle; MN, motor neurone; N, nerve; T, thoracic neuromere; VM, ventral muscle.

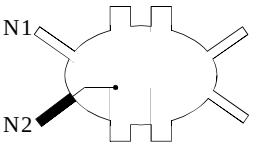
neurones we stained consistently in A4 was eight; four of these had axons in N1, with three somata in an anterior group and one posteriorly (one of the three solid black circles in the posterior group in Fig. 3A; the other two solid black circles in this group could also be bilaterally projecting neurons), and four had axons in N2, with two somata in an anterior group and two in a posterior group (Fig. 3C, solid black circles). The maximum number of consistently stained bilaterally projecting, non-octopaminergic neurones (Kalogianni and Pflüger, 1992) in female A7 was 10 (Fig. 4A, midline, solid green circles).

### *Total number of somata in a neuromere with axons in lateral nerves 1 or 2*

The total numbers of somata of efferent neurones in a neuromere were calculated from consistent stains of N1 and N2 on one side of the segmental neuromere and N1 on the same side of the next posterior neuromere (Table 3). The numbers were doubled to include bilateral homologues on the other side, but excluded the bilaterally projecting neurones. The lowest number of somata, 73, was in the fused neuromere A1, rising to 93 in the unfused neuromere A4, and reaching 106 in female A7. The greatest number of neurones (40 %) had axons in N1 of the next posterior neuromere, whereas 35 % had axons in N2 and 25 % in N1 of the segmental neuromere. The exception was female A7, which had more neurones with axons in N2 of the segmental neuromere than in N1 of the next posterior neuromere.

Table 2. *Stained somata with axons in nerve 2*

| Neuromere | Ipsilateral | Midline | Contralateral | Total |
|---|---|---|---|---|
| A1 | 9 | 1 | 1 | 11 |
| A2 | 9(11) | 2(3) | 0 | 11 |
| A3 | 9(12) | 3 | 0 | 12 |
| A4 | 13(14) | 5(6) | 0(3) | 18 |
| A5 | 11(14) | 6(11) | 0(5) | 17 |
| A7 (female) | 12(16) | 16 | 0 | 28 |
| A7 (male) | 13(18) | 4 | 0(2) | 17 |
| A8 (female) | 15 | 6(7) | 0 | 21 |
| A8 (male) | 10(11) | 2(3) | 2(4) | 14 |



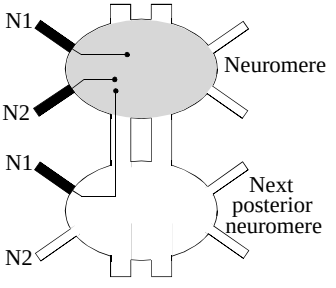

Somata of neurones with axons in nerve 2 (N2) occur only in the neuromere to which the nerve projects (see diagram at left).

### *Identity of laterally projecting neurones*

We ascribed identities to many somata by comparing our results with previous reports (see Materials and methods) and these are colour-coded in Figs 1–5.

Table 3. *Stained somata in a neuromere with axons in nerves 1 or 2*

| Neuromere | N1, left segmental neuromere | N1, left next posterior neuromere | N2, left | Total bilateral neurones | Total, left+right |
|---|---|---|---|---|---|
| A1 | 11 | 16 | 11 | 3 | 73 |
| A2 | 10 | 18 | 11 | 3 | 75 |
| A3 | 12 | 17 | 12 | 3 | 79 |
| A4 | 17 | 17 | 18 | 11 | 93 |
| A7 (female) | 15 | 19 | 28 | 18 | 106 |
| A7 (male) | 10 | 19 | 17 | 3 | 89 |




The total number of somata in one neuromere includes those with axons in nerve 1 (N1) of that neuromere, in N2 of that neuromere and in N1 of the next posterior neuromere that enter through the ipsilateral posterior connective.

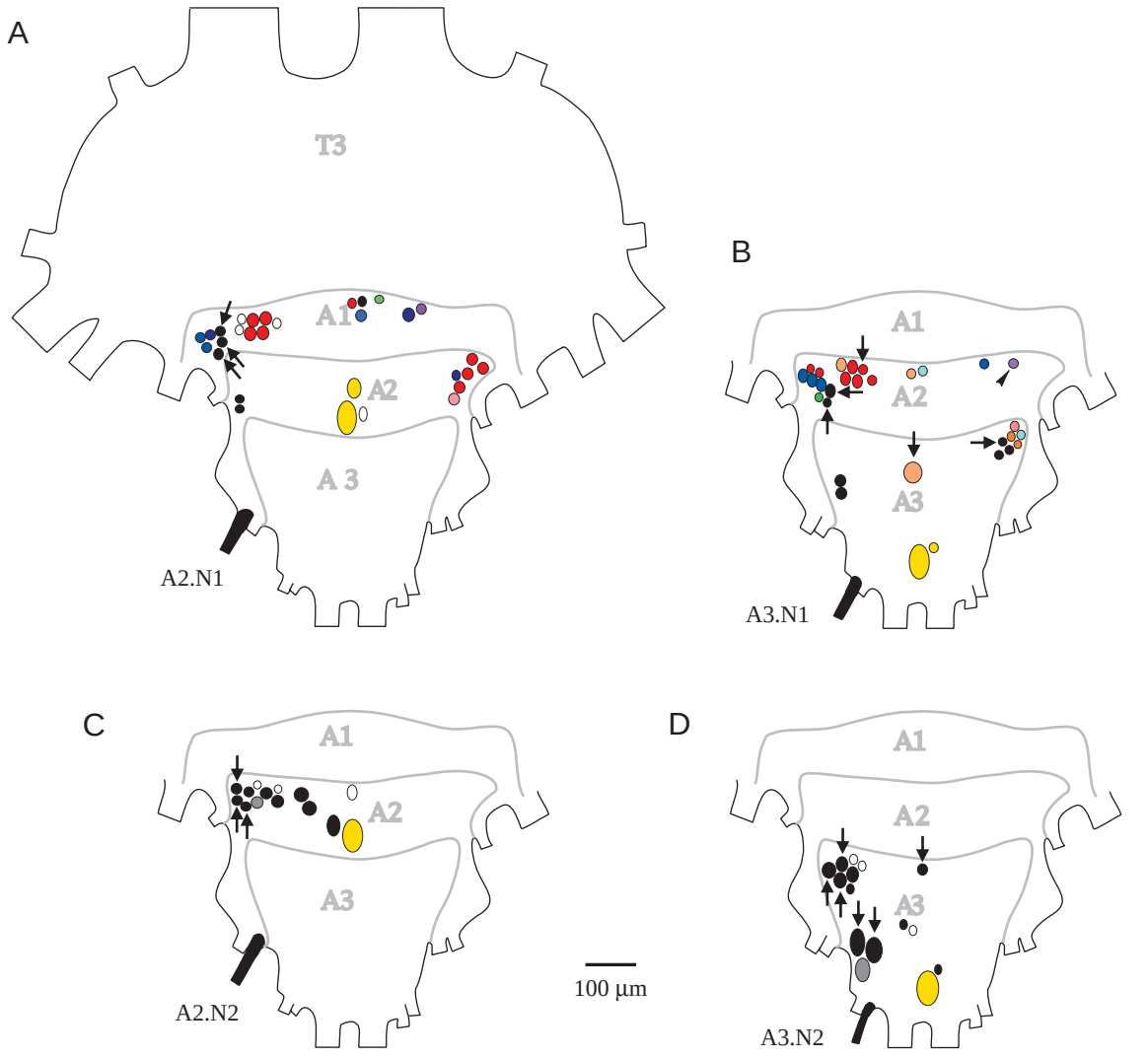



Fig. 2. Maps of somata of neurones with axons in lateral nerves of abdominal neuromeres A2 and A3. (A) Positions of somata with axons in nerve 1 (N1) of A2. (B) Positions of somata with axons in N1of A3. (C) Positions of somata with axons in N2 of A2. (D) Positions of somata with axons in N2 of A3.

Motor neurones that innervate the dorsal longitudinal muscles (DLMs) all have their axons in N1, but their somata are present in two neuromeres (Baader, 1988; Breidbach and Kutsch, 1990; Kutsch and Heckmann, 1995a). Two somata were contralateral in the segmental neuromeres of T3, A1, A3 and A8 (Figs 1A,B, 2B, 5A,B). Four somata were contralateral in A2 (Fig. 2A) and three in A4, A5 and in A7 of both males and females (Figs 3A,B, 4A,B). In the next anterior neuromere,

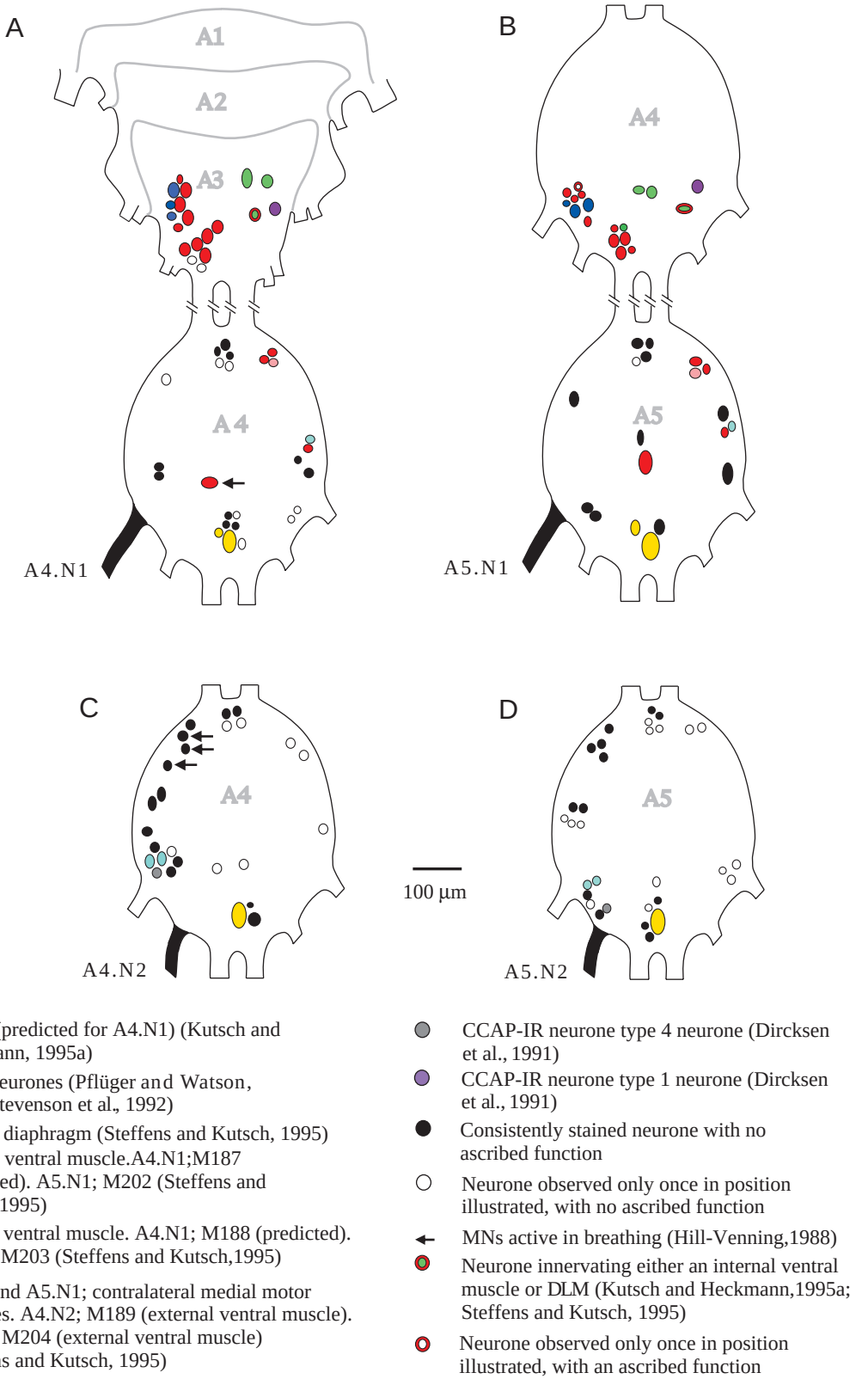



Fig. 3. Maps of somata of neurones with axons in lateral nerves of abdominal neuromeres A4 and A5. (A) Nerve 1 (N1) of A4. (B) N1 of A5. (C) N2 of A4. (D) N2 of A5.

there were between four (T3, Fig. 1B) and 11 (A4 and A5; Fig. 3A,B) somata. The numbers of somata in all other neuromeres lay within these two limits (Figs 1–5). When the nerves of T3 and A1 were stained, the somata in the next anterior neuromere

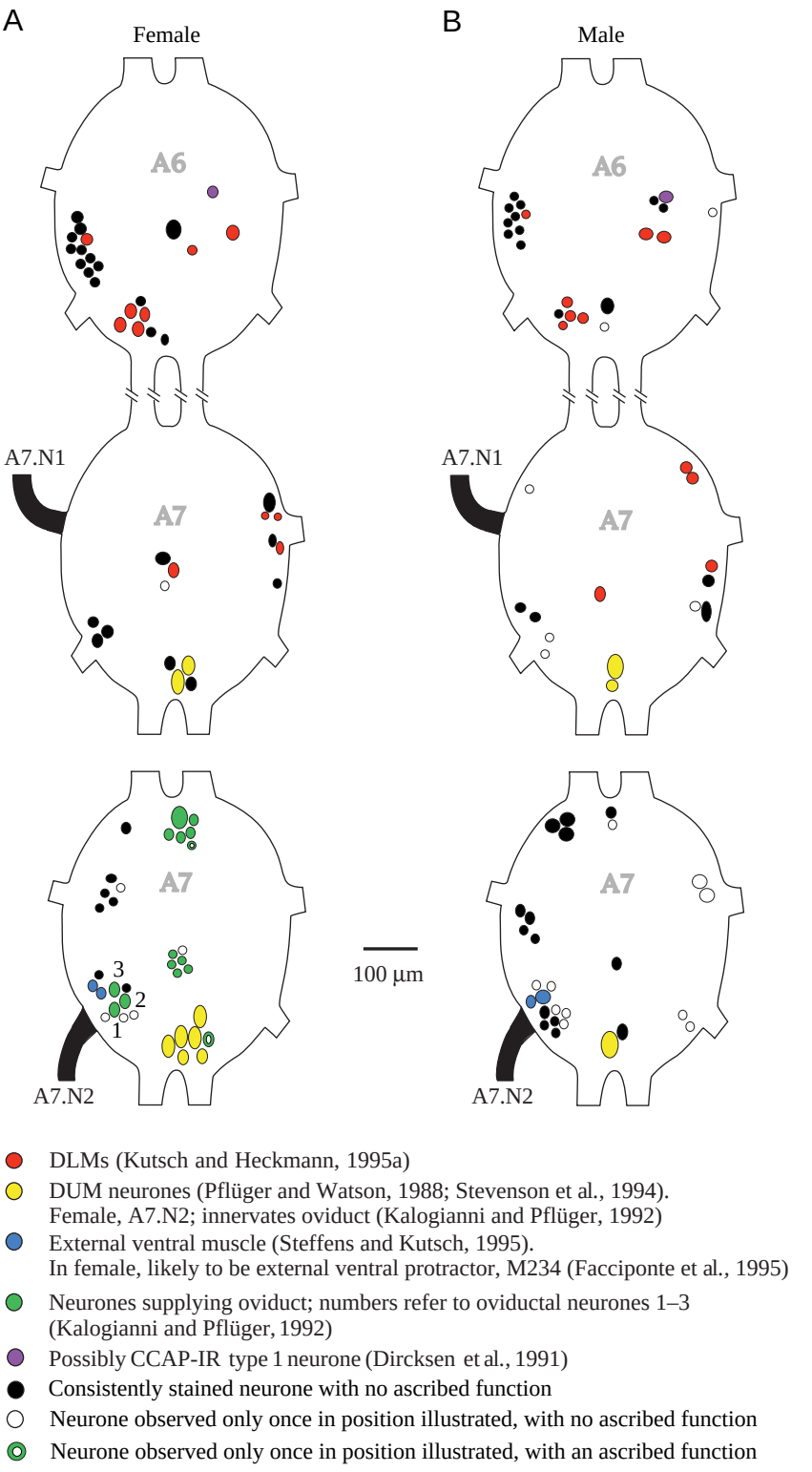
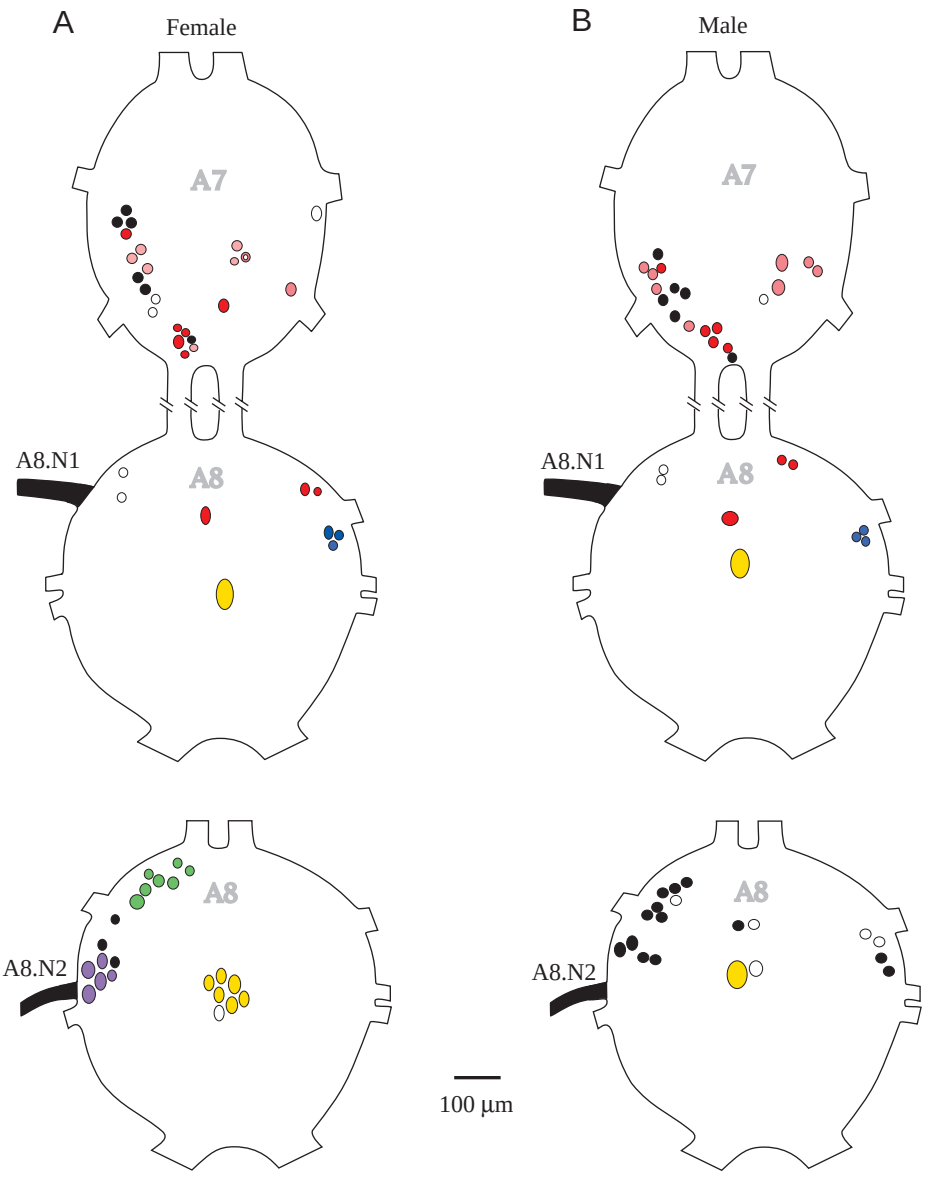



Fig. 4. Maps of somata of neurones with axons in lateral nerves 1 and 2 (N1 and N2) of the seventh abdominal neuromere (A7) in the female (A) and (B) male.

were always ipsilateral; however, for the nerves of more posterior neuromeres, the somata could be ipsilateral, midline or contralateral.

Motor neurones that innervate ventral muscles (VMs) also have axons in N1 (Steffens and Kutsch, 1995). A single soma was located contralaterally in each of the segmental neuromeres A2–A5, and six or seven somata were present in the next anterior neuromere (six in A1–A3, Figs 2A,B, 3A; seven in A4, Fig. 3B). For N1–A7, no published data are available for the motor neurones innervating ventral muscles so that we can only assume that some of our stained somata (solid black circles, Fig. 4) could be motor neurones




DLMs (based on Kutsch and Heckmann, 1995a)

DUM neurones (Pflüger and Watson, 1988; Stevenson et al., 1994)

In females: ventral protractor muscle (M256) of ovipositor valves. In males: paradorsal muscle, M244 (Seymour, 1990; Thompson and Roosevelt, 1998)

Lateral ventral retractor muscle (M248) of ovipositor valves (Seymour, 1990; Thompson and Roosevelt, 1998)

In females: ventral closer muscle (M247) of ovipositor valves. In males: medial and lateral internal ventral muscles, M247 and M248 (Seymour, 1990; Thompson and Roosevelt, 1998)

Ventral opener ovipositor motor neurones (Thompson and Roosevelt, 1998)

Consistently stained neurone with no ascribed function

Neurone observed only once in position illustrated, with no ascribed function

Neurone observed only once in position illustrated, with an ascribed function

Fig. 5. Maps of somata of neurones with axons in lateral nerves 1 and 2 (N1 and N2) of the eighth abdominal neuromere (A8) in the female (A) and (B) male.

innervating ventral muscles. In neuromeres T3 and A1, there was a less consistent pattern of VM motor neurone somata, in which three (T3, Fig. 1A) or none (A1, Fig. 1B) were located in the segmental neuromere and six (T2, Fig. 1A) or 11 (T3, Fig. 1B) in the next anterior neuromere. Two motor neurones innervating external ventral muscles (Steffens and Kutsch,

1995) have axons in N2 and ipsilateral somata in the segmental neuromeres of A1, A4, A5 and A7 (Figs 1C, 3C,D, 4A,B). Homologous motor neurones are likely to be present in A2 and A3, but there are no published data to substantiate this.

The ventral diaphragm (VD) is innervated by neurones with axons in N1 (Yang and Burrows, 1983; Dircksen et al., 1991; Steffens and Kutsch, 1995). One contralateral soma in the segmental neuromere was present in neuromeres A1–A5 (Figs 1B, 2A,B, 3A,B). Two somata were ipsilateral to N1 in which their axons project in the segmental neuromere A1 (Fig. 1B).

Two small contralateral somata in T3 revealed by staining N1 of that segment (solid black circles, Fig. 1A) could innervate the lateral oblique intersegmental, the spina-pleural or the pleural subalar muscles (Siegler and Pousman, 1990a). The latter two are larval muscles that degenerate soon after the adult moult, but their motor neurones could be present in some of the young adults that we used.

Nerve 2 contains the axons of motor neurones that innervate longitudinal inspiratory and dorso-ventral expiratory muscles active during ventilation (Lewis et al., 1973). Motor neurones innervating the longitudinal inspiratory muscle (M189) of A4 are indicated in Fig. 3C. The two more posterior of the three somata indicated as active in breathing (arrows, Fig. 3C) could be motor neurones innervating dorso-ventral expiratory muscles (Lewis et al., 1973), whereas the more anterior motor neurone is inspiratory (Hill-Venning, 1988). Motor neurones with axons in N2 and active during ventilation are also indicated for A2 and A3 (Fig. 2C,D), although it was not possible to identify their target muscles. Indeed, the targets of most motor neurones with axons in N2 of the abdominal neuromeres are unknown.

Three DUM neurones were stained in the abdominal neuromeres, except for female A7 in which there were eight (Figs 1–4). Nerve 1 contains the axons of two DUM neurones (Goodman and Bate, 1981; Stevenson et al., 1992; Bräunig et al., 1994). In neuromeres A1–A7, the larger of the two somata is DUM1a (also called DUMDL), which innervates both dorsal longitudinal and ventral muscles (Stevenson et al., 1992). The smaller soma is DUM1b (called DUMheart in the abdominal neuromeres) (Goodman and Bate, 1981; Stevenson and Pflüger, 1994). Both DUM1a and DUM1b were present in T3 (Fig. 1A), but only one DUM neurone with an axon in N1 was present in A8 (Fig. 5) (Stevenson et al., 1994). The only DUM neurone with an axon in N2 was DUM2 (Figs 1–5), except for neuromeres A7 and A8 of females, which both have six DUM neurones with axons in N2 (Figs 4A, 5A) (Stevenson et al., 1994).

Neurones known to be immunoreactive (IR) to crustacean cardioactive peptide (CCAP) were also backfilled. The CCAP-IR type 1 neurone has its axon in N1, and its soma is contralateral in the next anterior neuromere (Dircksen et al., 1991) and was stained after backfilling N1 of neuromeres A1–A7 (Figs 1–4). The CCAP-IR type 4 neurones are probably neurosecretory cells and have axons in N2 (Dircksen et al., 1991). When N2 of neuromeres A1–A5 was backfilled, the somata of these neurones were stained ipsilaterally in each segmental neuromere (Figs 1–3).

We identified several neurones known to be involved in reproduction (Figs 4, 5). When N2 of female A7 was backfilled (Fig. 4A), the somata of oviductal neurones 1–3 (Kalogianni and Pflüger, 1992) were stained. Twelve other somata innervating the oviduct were also stained, six of which were located anteriorly and six posteriorly in A7. Two somata of motor neurones innervating the ventral protractor (Facciponte et al., 1995) of the ovipositor valves were also stained after backfilling N2 of female A7. In neuromere A8, we stained the contralateral somata of three motor neurones with axons in N1 (Fig. 5) that innervate the short protractor muscle (M256) of the ovipositor valves in females and the paradorsal muscle (M244) in males (Seymour, 1990; Thompson and Roosevelt, 1998). We stained eight somata in the next anterior neuromere (Fig. 5) that innervate M247 of the ovipositor valve ventral closer in females and muscles M247 and M248 in males (Seymour, 1990; Thompson and Roosevelt, 1998). Backfilling N2 of female A8 revealed the anteriorly located somata of seven motor neurones (Fig. 5A) that innervate the lateral ventral retractor muscle (M248) of the ovipositor ventral valves and five somata of the ventral opener ovipositor motor neurones (Thompson and Roosevelt, 1998).

## Discussion

We backfilled the axons of neurones in the two lateral nerves of the abdominal neuromeres to map the position of their somata in the central nervous system. Within one neuromere, the stained somata belong to neurones with axons in N1 and N2 of that segment and in N1 of the next posterior segment. We were able to identify many of the somata we mapped by comparing their size and location and the pathway of primary neurites with other published accounts. Most of the somata belonged to motor neurones, and only a few to either DUM neurones or paired neurosecretory neurones. It is possible that some somata with no ascribed function may belong to strand receptors because, in the thoracic segments, sensory neurones associated with strand receptors have cell bodies within a segmental ganglion (Bräunig, 1982). To date, however, such sensory structures have not been described in the abdomen, and all other known proprioceptors, such as the abdominal muscle receptor organ, have their somata in the periphery at the organs themselves (Ferber and Hustert, 1996).

### *Number of efferent neurones in an abdominal neuromere*

The number of efferent neurones we stained in an abdominal neuromere ranged from 73 in A1 to 106 in female A7. Generally, there is good agreement between the numbers and positions of somata we have stained and those described by others for particular nerve branches or for neurones innervating a single muscle or group of related muscles (e.g. Kutsch and Heckmann, 1995a; Steffens and Kutsch, 1995; Thompson and Roosevelt, 1998). Kutsch and Briedbach (1994) estimated there to be approximately 70 neurones with endings on

muscles, but did not specify whether this value was for a specific neuromere or a mean value for all abdominal neuromeres. This value is close to ours for the fused neuromeres A1–A3, but the unfused neuromeres A4 and A7 have more somata, reflecting the greater number of neurones with axons in N2. Perhaps some of the N2 neurones we stained do not innervate muscle, but end on sites such as Malpighian tubules (see below).

Differences could, however, be attributable to the use of a backfilling method that has been recognised to result in variation (Lewis et al., 1973; Ferber and Pflüger, 1990). For example, Ferber and Pflüger (1990) reported large variations (0–4 in both anterior and posterior groups, with the most common value of one in each) in numbers of somata of midline, bilaterally projecting cells in A5, possibly because of the small diameter of their axons. Our largest variations were also for the midline somata of A4 (with axons in segmental N1) and A5 (axons in N2). The ipsilateral somata with axons in N2 of male and female A7 also showed large variations in the number of somata filled. Perhaps these neurones also have narrow axons, or they may die during maturation (Levine and Truman, 1985; Siegler and Pousman, 1990a). We minimised differences attributable to the staining method by backfilling the same nerve in many (6–15) locusts and the problem of post-eclosion death by using adult locusts of similar ages. Kalogianni and Pflüger (1992) report eight anterior and 12 posterior midline somata in female A7 resulting from backfilling N2, whereas we consistently found five anterior and 11 posterior. Furthermore, our stained preparations have not revealed some of the midline, bilaterally projecting neurones in neuromere A5 reported by Ferber and Pflüger (1990). They used bilateral backfilling with both nickel chloride and cobaltous chloride and found that a dye concentration of 6 % (the same concentration as we used) often failed to stain many of these neurones, whereas 3 % was more successful. Kalogianni and Pflüger (1992) used dye concentrations of 1.5–6 %. We might expect, therefore, that our protocol would be less effective in staining these midline cells, but it did stain all the other ipsilateral and contralateral cells described in these two reports. Our protocol was selected to maximise staining of somata in both the segmental neuromere and the next anterior neuromere, which the reports of Ferber and Pflüger (1990) and Kalogianni and Pflüger (1992) did not address.

### *Repeating pattern of somata distribution*

The numbers of somata in ipsilateral, midline and contralateral positions showed a basic repeating pattern throughout the abdominal neuromeres (Tables 1, 2). There were some exceptions. In A4, A5 and female A7, midline and contralateral somata with axons in N1 were similar in number because of the greater number of midline, bilaterally projecting neurones, but in all other neuromeres the number of contralateral somata was larger.

Repeating patterns were also observed for the sets of motor neurones innervating the dorsal longitudinal and ventral muscles (Figs 1–5) (Briedbach and Kutsch, 1990; Kutsch and Heckmann, 1995a; Steffens and Kutsch, 1995). This may indicate common developmental origins for each group of motor neurones from individual, segmentally repeated neuroblasts and thus parallel the organisation of different motor neurones in neuromere T3 (Siegler and Pousman, 1990a). Whether the same sets of motor neurones innervate the same sets of muscles in different segments is a basic question when considering the concept of a '*Bauplan*' or basic repeated body plan (Kutsch and Breidbach, 1994; Kutsch and Heckmann, 1995a,b; Heckmann and Kutsch 1995; Steffens and Kutsch, 1995). Detailed investigations have revealed that this concept is not straightforward. A basic innervation pattern seems to exist for dorsal longitudinal motor neurones across different species, despite variations in the periphery, where some insects have wings (moved by the dorsal longitudinal muscles) and others do not (Heckmann and Kutsch, 1995). Similarly, for the different segments within one species, the dorsal longitudinal muscles and their motor neurones show a clear repeating pattern (Kutsch and Heckmann, 1995a), but for the ventral muscles in *Schistocerca gregaria* the supply of apparently homologous muscles shifts from one set of motor neurones to another (Steffens and Kutsch, 1995). Thus, putatively equivalent motor neurones of different neuromeres can supply morphologically different muscles, suggesting that the muscles and motor neurones have to be considered separately when searching for a basic segmental *Bauplan.*

### *Identification of the neurones*

Most of the somata that were stained belong to motor neurones, with a small number belonging to neurosecretory neurones, most notably the DUM neurones. We were also able to recognise the CCAP-IR type 4 neurones that have axons in N2. By analogy with other insect species, some of the posterior lateral somata with axons in N2 may be neurosecretory neurones involved in the regulation of the heart, breathing and fluid secretion. In each abdominal neuromere of the cockroach *Leucophaea maderae*, the neuropeptide leucokinin I (LK I) is present in two pairs of neurones with axons in N2 (Nässel et al., 1992). In *Locusta migratoria*, locustakinin (LomK), which is a neuropeptide related to LK I (Schoofs et al., 1991), is co-expressed with *Locusta*-diuretic peptide (*Locusta*-DP) (Kay et al., 1991) in posterior lateral abdominal neurones (Thompson et al., 1995). LomK and *Locusta*-DP act additively on Malpighian tubules to regulate fluid secretion, and the neurones expressing these two peptides are likely to be homologous with those of *Leucophaea maderae* showing LK I-immunoreactivity.

The maps we have produced give the most complete and accessible picture of laterally projecting neurones of the locust abdomen. They reveal neurones that can be identified and should enable those cells whose function is unknown to be identified.

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