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Serotonergic and Octopaminergic Systems in the Squat Lobster *Munida quadrispina* (Anomura, Galatheidae)

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ABSTRACT

Immunocytochemical mapping of serotonergic and octopaminergic neurons in the central nervous system of the squat lobster *Munida quadrispina* reveal approximately 120 serotonin-immunoreactive cell bodies (distributed throughout the neuromeres except in abdominal ganglion 5) and 48 octopamine-immunoreactive cell bodies (in brain and thoracic neuromeres but none in the circumesophageal or abdominal ganglia). Immunopositive neuropils for both amines are distributed in multiple areas in each neuromere and overlap extensively. Serotonergic and octopaminergic neurons have extensive bilateral projections in abdominal ganglia, whereas the majority of projections in thoracic and subesophageal ganglia are unilateral (contralateral to soma). This difference correlates with typical differences between abdominal and thoracic motor system coordination. Processes of immunoreactive cells for both amines form extensive, peripheral, neurosecretory-like structures. Serotonin seems to be released peripherally in more segments, and from more nerves per segment, than octopamine. *M. quadrispina* has fewer serotonergic and octopaminergic immunoreactive cells, in particular, fewer segmentally repeated cells, than other species studied to date. Nevertheless, the general organization of the aminergic systems is similar, and several aminergic cells have locations and morphologies that strongly suggest homology with identified aminergic cells in other crustaceans. Among these are segmentally repeated neurons that, in *M. quadrispina*, form serotonin-immunopositive tubular structures in the thoracic hemiganglia innervating pereiopods 1–3 that are unlike anything reported previously for any species. Comparisons of immunocytochemical maps within one species and between species exhibiting different behaviors provide insights into possible sites of action, functional differences between, and evolution of biogenic aminergic systems. J. Comp. Neurol. 439:450–468, 2001.

Indexing terms: biogenic amine; modulation; evolution; crustacean

Serotonin (5-HT) -like immunoreactivity has been proposed as a potentially very useful tool for studying evolutionary relationships among the neuroendocrine systems of crustaceans (Thompson et al., 1994). Octopamine (OCT) has not received as much research attention but is believed to have physiologically antagonistic actions to 5-HT in some systems (Beltz, 1999). Immunocytochemical mapping of OCT-like immunoreactivity may also prove a powerful tool for elucidating evolutionary relationships among species. In addition, coordinated analyses of serotonergic and octopaminergic systems may provide insights into aminergic functions in a wide variety of physiological processes.

Among the many neural systems and behaviors in crustaceans that are influenced by the biogenic amines serotonin and octopamine are agonistic posture and behavior (Livingstone et al., 1980; Harris-Warrick and Kravitz, 1984; Huber et al., 1997a,b; Antonsen and Paul, 1997; Doernberg et al., 2001), the neural systems controlling the heart and stomach (Florey and Rathmayer, 1978; Flamm and Harris-Warrick, 1986), the sensitivity of sensory systems (Pasztor and MacMillan, 1990; Rossi-Durand, 1993), and the strength of synaptic outputs of sensory fibers to other neurons (Yeh et al., 1996; for a review see Beltz,

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1999). Most work on aminergic systems and the influences of 5-HT and OCT has been done in American lobsters and several species of crayfish, although some studies have been done in crabs, spiny lobsters, and a few nondecapod crustaceans.

The distribution of serotonergic neurons is known in only a few crustacean species and octopaminergic neurons in only two. Complete or nearly complete maps of serotonergic neurons in the central nervous system are available for lobsters (Beltz and Kravitz, 1983, 1987; Langworthy et al., 1997), crayfish (Elofsson, 1983; Sandeman and Sandeman, 1987; Sandeman et al., 1988; Real and Czternasty, 1990), a crab (Harzsch and Dawirs, 1995), several species of isopods (Thompson et al., 1994), and *Anaspides tasmaniae* (Harrison et al., 1995), a syncarid crustacean that retains many primitive malacostracan features. Johansson (1991) compared the distribution of serotonergic neurons in the olfactory lobes of several species, including two shrimp and a galatheid. Biochemical analyses have demonstrated OCT in discrete regions or structures in the nervous systems of lobsters (Evans et al., 1975; 1976a,b; Livingstone et al., 1981), but antibodies to OCT that produce highly specific and reproducible immunolabeling have not been widely used. The American lobster is the only crustacean species for which an immunocytochemical map of octopaminergic neurons has been published (Schneider et al., 1993, 1996). Comparable data for crayfish have been presented in poster form (Spörhase-Eichmann et al., 1998).

The positions, morphologies, and projections of many serotonergic neurons are conserved among crustaceans, even between distantly related species. Equally interesting are the many differences among species. No attempt has been made to use such comparative data to understand serotonergic function. Furthermore, without diverse comparative information on the octopaminergic systems, we can have no insight into whether concordant evolutionary changes occur in these two aminergic systems, as might be expected if their actions are antagonistic.

The structure and physiology of the central nervous system and neuromusculature involved in producing many of the behaviors modulated by 5-HT and OCT differ among decapod species. For example, 5-HT and OCT influence the lateral giant escape circuit in crayfish (Glanzman and Krasne, 1983; Yeh et al., 1996, 1997) and 5-HT–containing neurons are excited by both lateral and medial giant escape neurons (Hörner et al., 1997). However, there are anomuran species that lack both giant neuron escape circuits yet are behaviorally sensitive to 5HT and OCT (Antonsen and Paul, 1997). Their escape and swimming tailflips are mediated by nongiant tailflipping circuitry, which in crayfish is responsible for swimming by repetitive tailflipping but can also initiate escape responses (Wine and Krasne, 1982; Sillar and Heitler, 1985; Wilson and Paul, 1987; Paul, 1989, 1991). Furthermore, brachyuran crabs and some anomurans have lost the ability to tailflip at all. Studying the changes in aminergic systems associated with these modifications or loss of motor circuits or loss of tailflipping behavior could inform us about how aminergic systems function and have evolved. Toward the goal of using comparative methods to study evolution and function of aminergic systems, we describe and compare the distributions of serotonergic and octopaminergic neurons in the central nervous system of the galatheid anomuran *Munida quadrispina*.

METHODS

Animals and dissection

M. quadrispina between 0.4 and 1.1 cm carapace length were collected by trawl from Saanich Inlet near Victoria, B.C., and kept in recirculating, 10°C seawater aquaria in the laboratory. Animals were used within 4 months of collection, although we found no evidence that long periods of confinement in the laboratory changed patterns of immunoreactivity. Most of the results presented in this study were obtained from whole-mount material, but thick sections were also made to confirm positions of some structures. Animals were anesthetized in sea water ice, and nerve cords were removed under cold physiological saline (460 mM NaCl, 13.7 mM $CaCl_2$, 12.7 mM KCl, 10 mM $MgCl_2$, 14 mM Na_2SO_4, 5 mM maleic acid, 10 mM Tris base, pH 7.4). Pretreating nerve cords with 10^{-5} M 5-HT or OA in physiological saline, or injecting animals with 10^{-4} M 5-HT or OA just before dissection, improved the strength of the label. However, these techniques also resulted in labeling of somata and some processes that did not label otherwise, presumably due to uptake mechanisms in nonserotonergic cells (Musolf et al., 1997). For this reason, pretreated nerve cords were used only for facilitating the tracing of processes that were identified in untreated animals, and no illustrations from pretreated animals are presented here. Likewise, the descriptions in the results are, except where noted, from strongly labeled nerve cords which had not been pretreated. Nerve cords were placed in fixative as quickly as possible after dissection, or after a 30-minute rinse, if they had been pretreated with an amine solution. Careful desheathing of the cords was done after fixation; some damage to the immunolabeled structures in some nerve cords was unavoidable, due to the brittleness of the fixed tissue, but desheathing before fixation resulted in noticeably diminished label. We did not look at the optic lobes or the cardiac or stomatogastric ganglia. All antibody washes were done in the same buffer solutions: 0.1 M phosphate-buffered saline (PBS), and PBS containing 1% Triton X-100 (PBS-Triton, pH 7.4).

Serotonin immunolabeling

Sixty-seven whole-mounts and four sectioned nerve cords were analyzed. Nerve cords were fixed in fresh 4% paraformaldehyde in 0.1 M phosphate buffer, pH 6.5, overnight at room temperature and then washed in several changes of PBS-Triton. *M. quadrispina* tissues proved particularly difficult to penetrate; therefore, several steps were taken before antibody application to improve permeability. Without these additional steps, antibody labeling was consistently quite good on structures very close to the surface, but quickly faded within 50–100μm of tissue depth. After several additional washes in PBS, permeability of the tissues was improved by treatment with 0.1 mg/ml Pronase (Sigma) or collagenase-dispase (Boehringer Mannheim) for 5–10 minutes, followed by two to four, 10-minute washes in freshly made 0.5% (w/v) sodium borohydride in PBS. The tissues were dehydrated through a graded ethanol series, washed for 10 minutes in either propylene oxide or Dent's solution (80% methanol, 20% DMSO), and rehydrated. After several washes in PBS-Triton, tissues were treated overnight in a blocking solution of 5% normal goat serum in PBS-Triton then incubated in a 1:5,000 dilution of a polyclonal

antibody to 5-HT raised in rabbit (Incstar). The primary antibody incubation buffer (pH 7.4) was prepared as follows: to PBS was added 1% sodium metabisulfite, 0.25% Triton X-100, 0.25% BSA, 3% dry milk powder; the solution was homogenized, then centrifuged, and the supernatant was filtered through no. 1 filter paper (Whatman). After several more rinses in PBS-Triton, the tissues were incubated with a 1:500 dilution of a carboxyfluorescein-conjugated secondary antibody raised in goat against rabbit IgG (Vector Laboratories). Both antibody solutions were incubated with the tissue for 3–4 days at 4°C. The tissue was dehydrated, cleared in methyl salicylate, and mounted in either methyl salicylate or Cytoseal (VWR). For thick sections, the tissue was fixed as above, washed in PBS, embedded in 5% agarose, and 100-μm sections were cut on a Vibratome. The tissue was washed twice for 20 minutes in 0.5% sodium borohydride, then the blocking solution and the same series of antibody incubations were applied as above, except that each was left overnight. The sections were dehydrated, cleared with toluene, and mounted in Cytoseal. Specificity controls were done by omitting the primary antibody or by preadsorbing the primary antibody in an excess (1 mg/ml) of 5-HT creatinine sulfate (Sigma). Both methods eliminated 5-HT immunoreactivity in the tissues. Positive controls were done alongside the *M. quadrispina* nerve cords on nerve cords from crayfish (*Procambarus clarkii*) and showed patterns similar to 5-HT–like immunoreactivity, as have been published (Real and Czternasty, 1990). *P. clarkii* nerve cords also showed identical patterns of immunolabeling regardless of whether they were subjected to the strong penetration increasing protocol necessary for *M. quadrispina* nerve cords. This finding suggests that the use of this rather harsh protocol did not change the immunolabeling patterns in *M. quadrispina*.

Octopamine immunolabeling

Forty-one whole-mounts and two sectioned nerve cords were analyzed. Because glutaraldehyde fixatives were used, we chose the smallest adult animals available, which were between 0.3- and 0.8-cm carapace length. There were two reasons for this approach: first, glutaraldehyde cross-links tissues very tightly, which tends to inhibit antibody penetration, and second, glutaraldehyde causes much more autofluorescence in these animals than does paraformaldehyde, which can obscure weakly labeled structures in larger animals. Two fixation procedures were used: (1) 1.5% glutaraldehyde in 0.1 M cacodylate buffer containing 1% sodium metabisulfite, pH 6.8 for 2 hours and pH 4.0 overnight at room temperature; or (2) a mixture of 10 ml of 25% glutaraldehyde, 30 ml of saturated aqueous picric acid, 0.2 ml of acetic acid, and 1% sodium metabisulfite (Schneider et al., 1993) overnight. Nerve cords fixed in the picric acid fixative had very weak or no immunolabeling. The procedure after fixation for both whole-mounts and sections was the same as for the 5-HT immunolabeling, with the following antibodies. The blocking solution was 10% normal goat serum, the monoclonal primary antibody was raised in mouse against octopamine conjugated to thyroglobulin, used at 1:10 000 dilution (BIOMAR Diagnostic Systems, Inc.) (Schneider et al., 1993; Spörhase-Eichmann et al., 1998), and the secondary antibody was biotinylated goat anti-mouse (Vector Laboratories) (1:250 dilution). CY3-conjugated streptavidin (1:250 dilution) was reacted with the biotinylated secondary antibody for visualization (Jackson ImmunoResearch). Specificity controls omitted the primary antibody or preadsorbed the primary antibody in 1 mg/ml DL-octopamine, dopamine, or 5-HT creatinine sulfate. The first two procedures eliminated and the latter two did not affect immunolabeling.

Imaging and reconstruction

Epifluorescent photographs and camera lucida drawings were made by using a Leitz Aristoplan microscope. Confocal images were obtained by using Zeiss LSM 410 and Zeiss LSM 510 laser scanning confocal microscopes. Although confocal microscopy could, in general, produce clearer images with a much greater depth of field than epifluorescence, the size of the whole-mounted tissue prevented the confocal microscopes from being used to their full advantage. Therefore, individual processes were followed and most of the line drawings in the results were obtained by using epifluorescent illumination and the camera lucida. Reconstructions of confocal images were done by using Adobe Photoshop software on Apple Power Macintosh computers.

RESULTS

Anatomy of the central nervous system of *M. quadrispina*

The central nervous system of *M. quadrispina* is typical of decapods in having an anterior brain; a pair of circumesophageal (hemi-) ganglia (CEG); a subesophageal ganglion (SEG) comprising six fused ganglia (three head and three thoracic neuromeres); five thoracic ganglia (embryonic thoracic neuromeres 4–8), which are often called T1-T5 because they innervate the five pereiopod-bearing segments; and six abdominal ganglia (A1–A6). Typical of anomurans, the connectives between the SEG and T1 and between adjacent ganglia T1–T5 are virtually absent; the first abdominal ganglion is fused against the posteromedial part of thoracic neuromere 8 (T5) and, hence, lies in the thorax. The sternal artery passes vertically through a conspicuous orifice on the midline of this ganglionic mass between the sixth and seventh thoracic neuromeres.

The organization of the segmental nerves leaving the thoracic and abdominal ganglia is similar to that in crayfish and lobsters. The first segmental nerve of each thoracic ganglion T1–T5 leaves the ganglion as several separate nerves, the largest of which are the two distal leg nerves and the two thoracico-coxal nerves. In T5, the thoracico-coxal nerves leave the ganglion dorsal to the distal leg nerves, as they do in crayfish (Elson, 1996). However, the thoracico-coxal nerves of the more anterior pereiopod ganglia are displaced increasingly to the posterior relative to the distal leg nerves (Antonsen and Paul, 2000). The second segmental nerves of these ganglia emerge just posterior to the distal leg nerves, and the third emerge dorsally from the posterior region of the ganglia or from the remnant of the connectives just posterior to the ganglion (Antonsen and Paul, 2000). In A2 through A5, the first and second segmental nerves leave laterally, but due to the position of A1, these nerves leave this ganglion posteriorly. The third segmental nerves branch from the connectives dorsally, posterior to each ganglion (Sillar and Heitler, 1985; Wilson and Paul, 1987). Seven nerves leave from each side of A6, although in *M.*

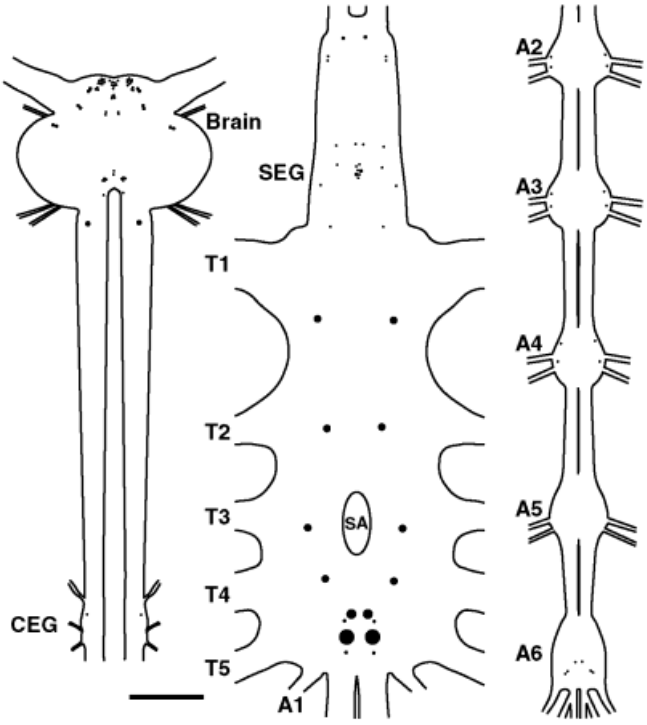

Fig. 1. Distribution of serotonin (5-HT) -immunoreactive somata. The size of each dot roughly corresponds to the size of the cell body. Most of the lengths of the connectives between ganglia are not shown. A1–A6, abdominal ganglia 1–6; CEG, circumesophageal hemiganglia; SEG, subesophageal ganglion; SA, sternal artery; T1–T5, thoracic ganglia innervating pereiopods 1–5. Scale bar = 500 μm.

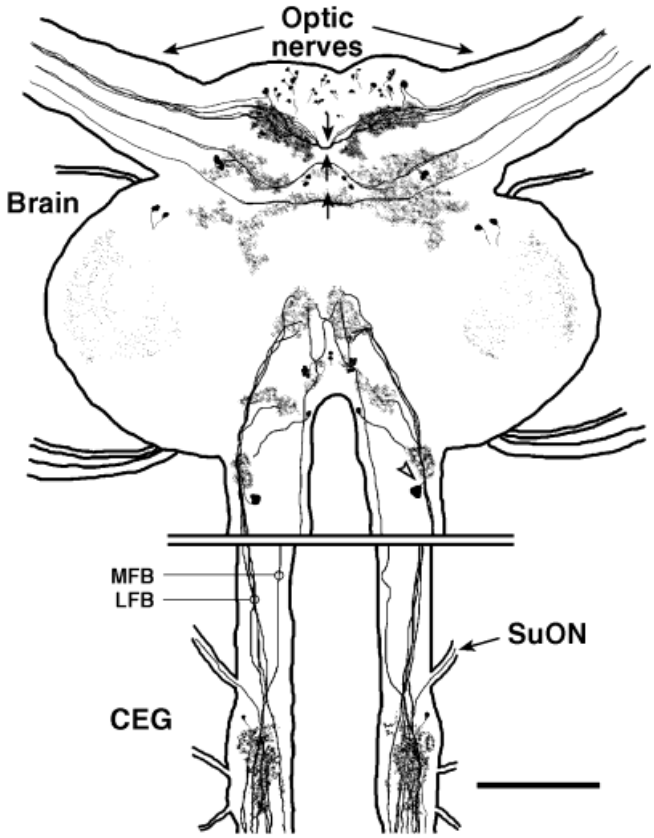

Fig. 2. Serotonin (5-HT) immunoreactivity in the brain and circumesophageal (hemi-) ganglia (CEG). This is a composite drawing of a single nerve cord constructed from camera lucida drawings and traces of confocal photomicrographs. Most of the length of the connectives between the brain and CEG has been omitted. The solid arrows in the brain indicate the three tracts of fibers crossing the midline in the rostral brain; the open arrowhead points to one of the large immunoreactive cell bodies in the connectives. The stippled areas indicate immunoreactive neuropil; the density of the stippling corresponds to the intensity of the label. LFB, lateral fiber bundle; MFB, medial fiber bundle; SuON, supraesophageal nerve. Scale bar = 500 μm

quadrispina, these nerves are initially fused at their exit from the ganglion into two or three (depending on the branch points) combined nerves (Paul et al., 1985).

Distribution of 5-HT–like immunoreactivity

Approximately 120 neurons with 5-HT–like immunoreactivity were found in the central nervous system of *M. quadrispina* (Fig. 1); their soma diameters range from 10 to 85 μm. Of these, approximately 60 are located in the brain, two in the CEG (one in each hemiganglion), approximately 24 in the SEG, two each in T1–T4 (thoracic neuromeres 4–7), four each in T5 and A1–A4, and six in A6. There may be some unpaired medial cells in the anterior parts of the brain and possibly in the SEG, but there are none in the abdominal ganglia. All of the 5-HT–immunoreactive cell bodies are in the ventral half of the ganglia, except in the brain where they are distributed throughout the dorsoventral axis. Large areas of dense 5-HT–immunoreactive neuropil exist in all ganglia. We detected no differences in immunoreactivity between the sexes or at different times of year.

Three distinct bundles of immunolabeled fibers extend the length of the nerve cord. These bundles appear similar in organization to similar bundles described in the American lobster (Beltz and Kravitz, 1983); we have followed a similar naming convention. The medial and lateral bundles are continuous from the base of the brain to the last abdominal ganglion, although individual fibers enter and leave them throughout their length (Figs. 2, 4, 7). They maintain their relative positions in the tracts of ascending and descending axons, which are quite medial in the fused thoracic ganglionic mass. The third, the central fiber bundle, extends from thoracic neuromere 4 (T1) through neuromere 8 (T5) between the medial and the lateral bundle (Fig. 4). Within and between the neuromeres of the fused thoracic mass, all three fiber bundles are in the dorsal third. In the long connectives between other ganglia, they are generally found in the dorsal half, but individual fibers or occasionally whole bundles were seen in the ventral half of some nerve cords.

In the brain, many small (10–25 μm) immunoreactive cell bodies are located in the anterior medial cell cluster. All but two of these have posterior processes that could not be followed far from the somata (Figs. 2, 3A). The large, lateral somata of the remaining pair send strongly labeled processes out the optic nerves. At least 14 additional, strongly immunoreactive processes could not be traced to their cell bodies. Eight additional im-

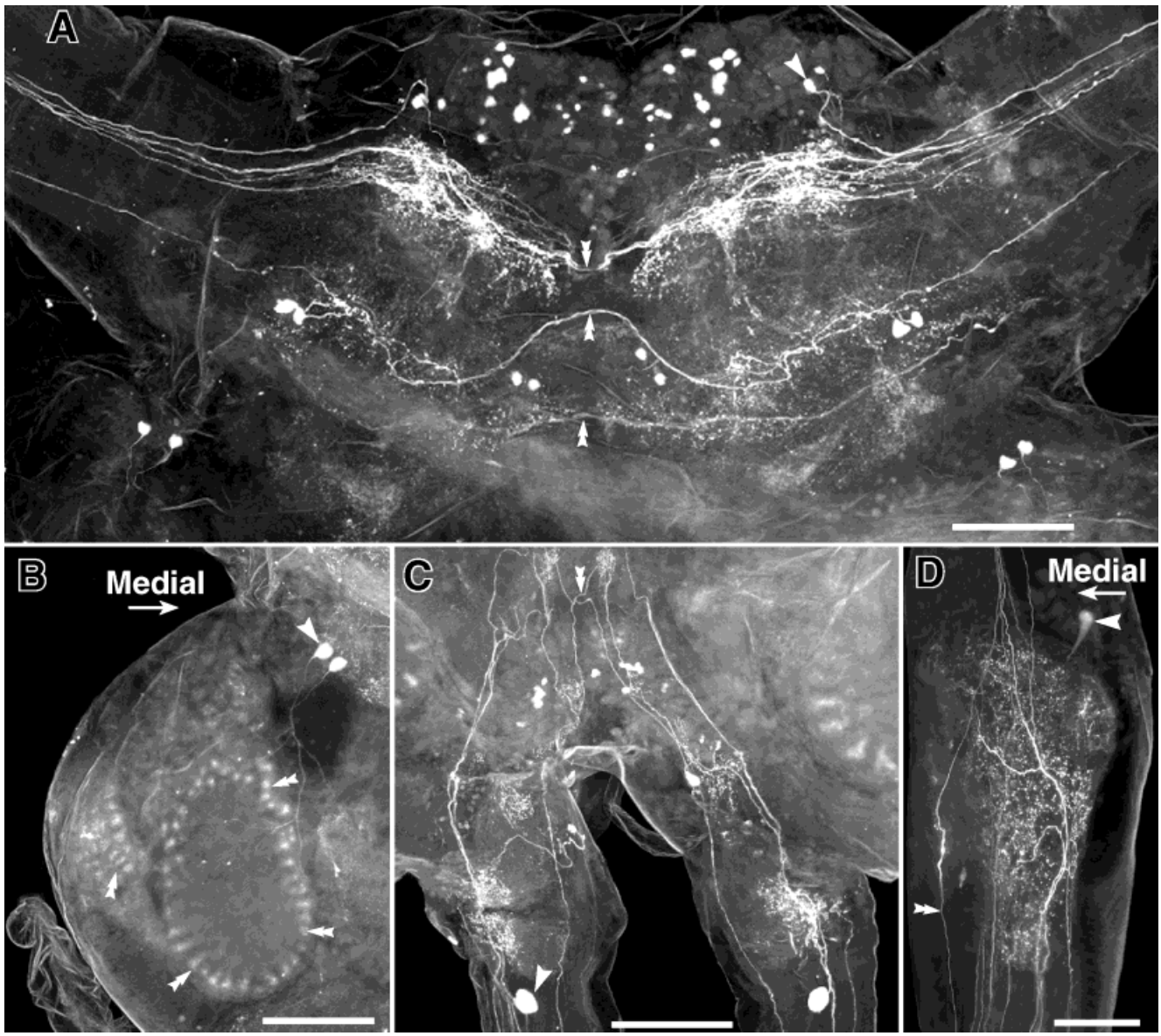

Fig. 3. Confocal photomicrographs of serotonin (5-HT) immunoreactivity in the brain and one circumesophageal (hemi-) ganglia (CEG). Anterior is up. **A:** The anterior part of the brain. The arrowhead indicates one of the cell bodies whose axon was followed into the optic nerve; the double arrowheads indicate the three tracts of decussating fibers in the rostral brain. **B:** The lateral deutocerebrum, showing the immunolabeling in glomerular-like structures in the olfactory lobes (double arrowheads) and the two large cell bodies which send processes into the olfactory lobes. For clarity of the glomerular label, only a thin slice of the total thickness of the brain is shown; no structures other than the remainder of the glomeruli are excluded in this view. **C:** The base of the brain. The arrowhead points to one of the bilateral pair of large immunoreactive somata in the connective just posterior to the brain; the double arrowheads indicate the branches from fibers in the lateral fiber bundle that cross the midline. **D:** A circumesophageal hemiganglion. The arrowhead indicates the weakly immunoreactive cell body; the double arrowhead indicates the single fiber that runs medially through the ganglion. Scale bars = 200 μm in A–C, 100 μm in D.

munoreactive cell bodies, three commissures with immunoreactive axons, and several areas of neuropil are also in the anterior brain. We cannot specify the anatomic locations of the immunolabeled structures of the anterior brain as precisely as we would like, or compare the fine structure with that in crayfish, because the detailed cytoarchitecture of *M. quadrispina*'s brain has not been described.

Glomerular-like structures with 5-HT–like immunoreactivity occur around the periphery of the olfactory lobes (Fig. 3B). Two fairly large (45–50 μm) cells on each side of the brain lie dorsally between the olfactory lobe and the base of the optic nerve. They may be homologues or analogues of the giant neurons associated with the olfactory and accessory lobes in crayfish (Sandeman et al., 1988); processes of these cells could be traced into the center of

the olfactory lobes, but no further branches or arborizations were evident. We could not discern any immunolabel in the accessory lobes, which are very small in anomurans (Sandeman et al., 1992).

Sixteen cell bodies with 5-HT–like immunoreactivity are in the posterior brain. Four of these, including one pair of very large (70–80 μm) cells in the connective, send processes into the connective neuropil. Processes of the rest of the posterior brain cells could not be followed individually. Five axons are in each connective posterior to the brain, one medial and four in the lateral bundle. Each of these axons branches into one or more of the three distinct areas of immunoreactive neuropil in the ipsilateral posterior brain, and the medial axon also sends a process into the most anterior contralateral of these neuropils (Fig. 3C).

Each CEG contains one weakly immunoreactive cell body (15μm diameter) and a very dense, strongly immunoreactive neuropil (Figs. 2, 3D). The soma is ventral in the rostrolateral part of the (hemi-) ganglion and projects posteriorly into the neuropil, where we were unable to follow it farther. The connective posterior to each CEG contains nine immunoreactive axons: six in the lateral bundle and three in the medial bundle, of which five could be followed through to the brain (Fig. 3D). Two axons in the lateral bundle and one in the medial bundle were followed between the CEG neuropil and cells in the SEG (see below), but the cell bodies of the remaining six axons have not been identified. Eight of the axons are distributed roughly in the center of the CEG, within the dense neuropil, and at least five contribute to this neuropil. The ninth axon passes through the ganglion medially, and has a short enlarged region surrounded by a dense plexus of beaded immunoreactive fibers (Fig. 3D). This axon is part of the lateral fiber bundle in the connectives anterior and posterior to the ganglion. One strongly immunoreactive fiber, whose cell body of origin has not been identified, enters each of the supraesophageal nerves, which lead to the stomatogastric ganglion.

Approximately 10 small (10–20 μm), ventral immunoreactive cell bodies are located along or close to the midline in the posterior half of the SEG (Fig. 4). The fibers leaving these cells initially project anteriorly in the medial fiber bundle, but none of them could be followed as far as the next rostral neuromere. A single pair of lateral cell bodies in each of the second, third, and fourth neuromeres sends processes across the midline. In neuromere 2, these processes project anteriorly in the contralateral medial fiber bundles. The pairs in neuromeres 3 and 4 send con-

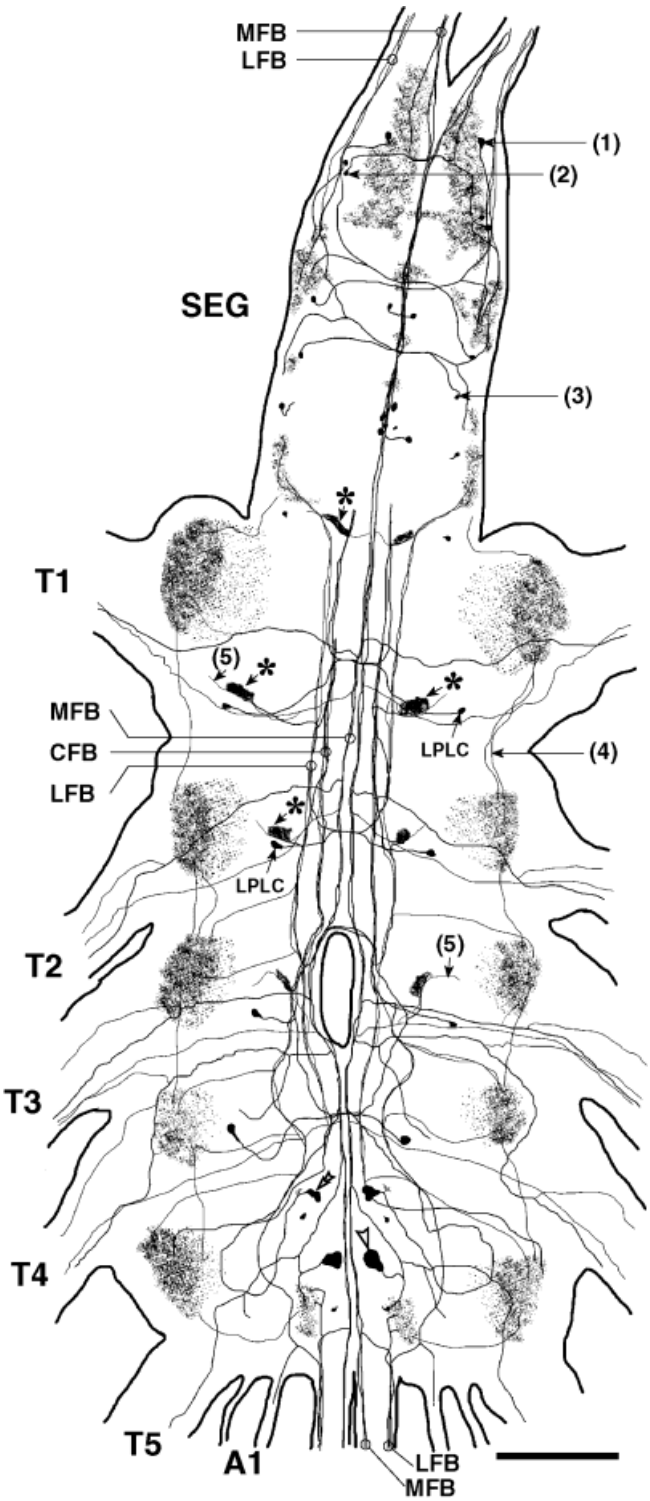

Fig. 4. Camera lucida drawing of serotonin (5-HT) immunoreactivity in the subesophageal, thoracic, and first abdominal ganglia. Areas of immunoreactive neuropil are represented by stippling; the density of the stippling corresponds to the intensity of the label. In the subesophageal ganglion (SEG), note the lateral cell bodies with caudal projections (1), with contralateral rostral projections in the medial fiber bundle (2), and with contralateral rostral and caudal projections in the lateral fiber bundle (3). Note also how the lateral fiber bundle turns laterally upon entering the SEG from T1. One of the large anterior medial cells (LAMCs) in each of A1 and T5 is indicated by the open arrowhead and double arrowhead, respectively. The lateral (LFB) and central (CFB) fiber bundles are distinct in the anterior thoracic ganglia but dispersed and difficult to follow through the posterior thoracic ganglia and A1, whereas the medial fiber bundle (MFB) remains distinct. Some of the lateral fibers that may be part of the lateral fiber bundle (4). Large posterior lateral cells (LPLCs) in thoracic ganglia 1–4 form the unusual putative neurosecretory structures (asterisks) in the contralateral caudal part of the next anterior ganglion (see Fig. 5B). Close to these structures are branches of the LAMCs of T5, which project into segmental nerve 3 (5). Scale bar = 500 μm.

tralateral branches both anteriorly and posteriorly along the lateral edges of the SEG. The posterior branches project to lateral immunoreactive neuropils in the next posterior neuromere, whereas the anterior branches extend at least to the neuropils of the CEG as part of the lateral fiber bundles. Additional pairs of lateral cell bodies in neuromeres 2 and 3 have ipsilateral, posteriorly projecting fibers that extend through the next two posterior neuromeres. But, the fibers leaving the two pairs of lateral immunoreactive cell bodies in neuromeres 5 and 6 could not be followed.

One pair of large, ventral, immunoreactive cell bodies occurs in each of T1 through T4 (thoracic neuromeres 4 through 7) (Figs. 4, 5A). Real and Czternasty (1990) called similar, presumably homologous cells in crayfish "large posterior lateral cells" (LPLC), and we have adopted their terminology. In *M. quadrispina*, the LPLCs increase in size from the anterior to the posterior ganglia. They project contralaterally, the processes then splitting into anterior and posterior branches. The anterior branch joins the contralateral lateral fiber bundle, then crosses to the central fiber bundle in the next anterior ganglion and continues into the posterior end of the SEG, where the immunolabel invariably became too faint to follow. The posterior branch enters the contralateral central fiber bundle directly (Fig. 6A); its terminations have also not been found.

Each of the LPLCs in *M. quadrispina* forms a putative neurosecretory structure unlike anything described in other species (Fig. 5B). These tubular structures are oriented dorsoventrally in the posterior part of the contralateral hemiganglion anterior to each cell, where they are angled along a mediposterior to lateroanterior axis. They are slightly anterior and ventral to the base of the third segmental nerves, except in the fourth leg ganglion (neuromere 7), where they are immediately ventral to the base of the third nerve. Dye injected into the circulatory system revealed small hemolymph vessels in the area of these structures (not shown), but their relationship to the neurosecretory structures is uncertain. Branches of the large anterior medial cells of the last thoracic ganglion (see below) pass close to these putative neurosecretory structures and may contribute to them before leaving the ganglia through the third segmental nerves (Fig. 5B). Fibers from the LPLCs do not enter these nerves.

The third major longitudinal fiber bundle, the central fiber bundle, is present only in and between the pereiopod ganglia and appears to contain mostly, if not exclusively, fibers from the LPLCs of T 1–4. A fourth, discontinuous fiber bundle that was never seen to contain more than three fibers is present laterally on each side of these ganglia (T1-5) (Fig. 4); it passes between the lateral neuropils of the pereiopod ganglia, but individual fibers could not be followed through the lateral neuropils. Fibers from this bundle join with the continuous lateral fiber bundle at the posterior edge of the SEG, forming the most posterior part of the lateral fiber bundle in this ganglion. Fibers lead from the continuous lateral fiber bundle to the lateral neuropils, but none appeared to be continuous with any fibers of this discontinuous fiber bundle. It is not clear whether the latter's fibers are branches of fibers in the continuous lateral fiber bundles, and therefore part of them, or should be considered a separate, regionally restricted serotonergic tract. None of the cell bodies of origin of any of the fibers in the lateral bundle has been identified. With the exception of the possible division of the lateral fiber bundle in the thoracic ganglia, the fibers constituting each bundle are, in general, very closely associated with one another in the dorsal part of the ganglia.

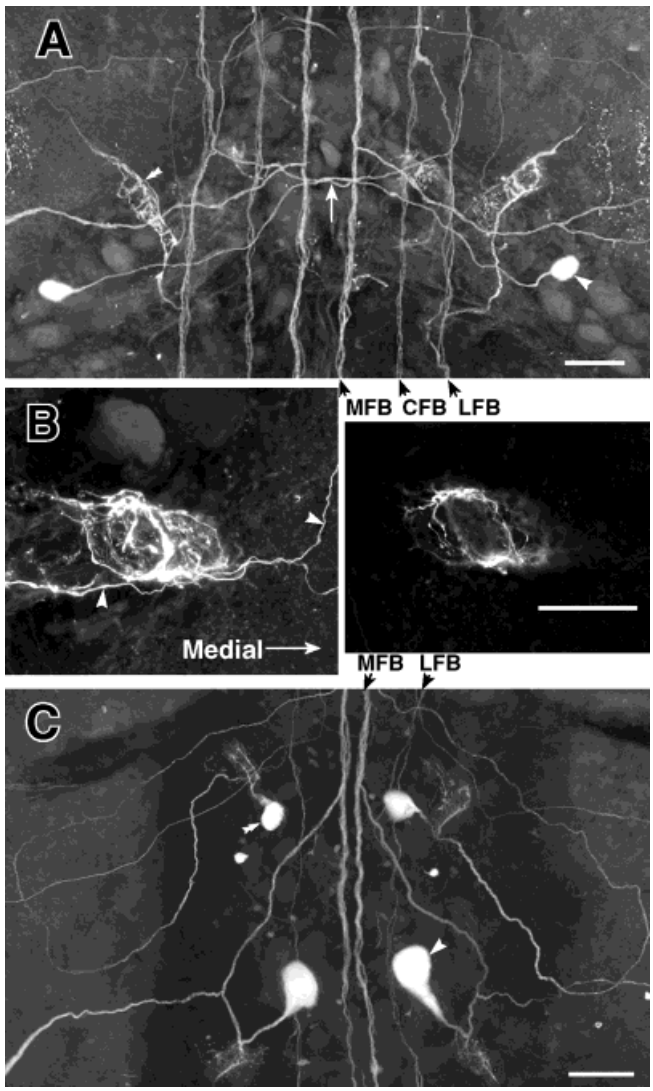

Fig. 5. Confocal photomicrographs of serotonin (5-HT) immunoreactivity in the thoracic and first abdominal ganglia. Anterior is up for all parts of the figure. **A:** The posterior region of T2, showing the large posterior lateral cells (LPLCs; arrowhead) that send processes contralaterally (arrow). The putative neurosecretory structures (double arrowhead) in this ganglion are formed by the cells in T3. The lateral (LFB), central (CFB), and medial (MFB) fiber bundles are tight. **B:** Higher magnification photomicrograph of one of the putative neurosecretory structures formed by the LPLCs. This example is in T2; its cell body is contralateral in T3. Note the branch of the T5 LAMC (arrowheads) that passes close to this structure and may contribute to it. On the right is a single confocal slice that clearly shows the circular cross-section of this structure. **C:** The large anterior medial cells of A1 (arrowhead) and T5 (double arrowhead). The medial fiber bundle (MFB) is distinct in these ganglia, and one or two fibers of the lateral fiber bundle (LFB) can be seen, but the central fiber bundle cannot be distinguished. The small pair of cell bodies in A1 is posterior to this image. Scale bars = 100 μm in A,C, 50 μm in B.

Two pairs of ventral 5-HT–immunoreactive cell bodies occur medially in each of T5 (the last thoracic neuromere)

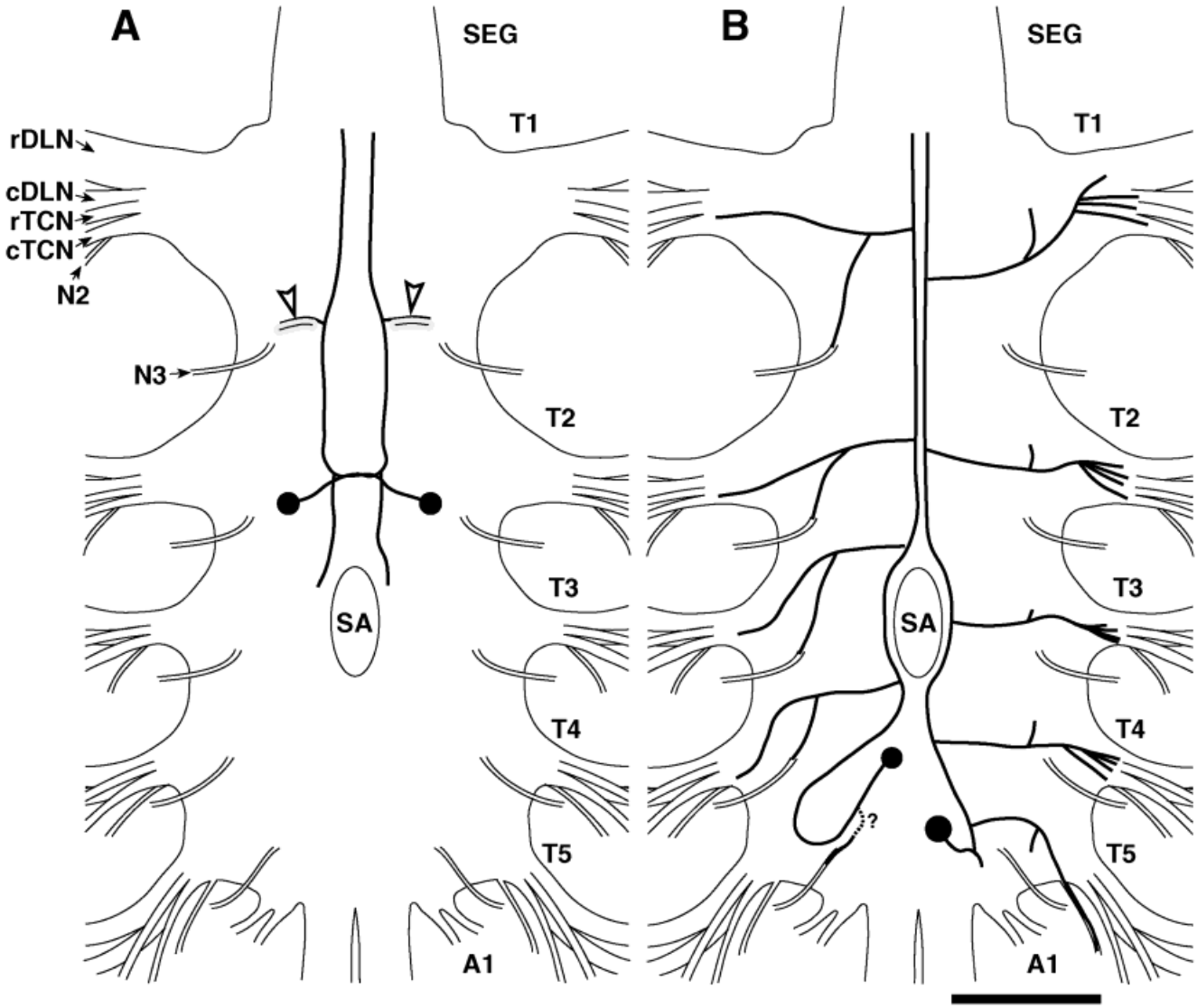

Fig. 6. Schematic drawings of the large serotonin (5-HT) -immunoreactive cells of the thoracic and first abdominal ganglia. **A:** The large posterior lateral cells of T2 project contralateral processes both rostrally and caudally; a branch of the rostral projection forms the putative neurosecretory structures (arrowheads) in the caudal part of T1. **B:** The left large anterior medial cells (LAMC) of T5 and the right LAMC of A1. The anteriorly projecting fibers of these cells join the medial fiber bundle in the next anterior ganglion. See Table 1 for a summary of the peripheral projections of these cells. SA, sternal artery; rDLN and cDLN, rostral and caudal distal leg nerves, respectively; rTCN and cTCN, rostral and caudal thoracico-coxal nerves, respectively; N2, nerve 2; N3, nerve 3. The arrangement of nerves is essentially similar for all thoracic ganglia. Scale bar = 500 μm in B (applies to A,B).

and A1 (Figs. 4, 5C). The cells of one pair in each ganglion are small (10–15 μm) and have processes that project medially and anteriorly, but could be followed no more than a few micrometers. The cells in the other pair are large (50–60 μm in T5, 70–85 μm in A1) and, based on their morphology and position, are likely to be homologues of the large anterior medial cells (LAMC) described in several other species (Beltz and Kravitz, 1983, 1987; Real and Czternasty, 1990; Harzsch and Dawirs, 1995) (Fig. 6B). The fibers of the LAMCs in *M. quadrispina* are the largest (7–8 μm) and most strongly labeled in the medial bundles and are, therefore, easy to follow. These two pairs of cells give rise to all of the identified fibers that enter the segmental nerves of the thoracic ganglia (Table 1), where they form dense plexuses of fine beaded fibers around the periphery of the nerves, as do the LAMCs in lobsters and crayfish.

Each LAMC in T5 sends a large process posteriorly and laterally through that hemiganglion's neuropil without apparent branching or contribution to that neuropil and then loops anteriorly to enter the medial fiber bundle (Figs. 5C, 6B). Immunolabeled fibers in nerves 2 and 3 of T1–4 were traced to these cells. One weakly immunoreactive fiber in nerve 3 of T5 could not be traced to its origin. The only branches of these cells seen in T5 lead to a small area of neuropil just anterior to the cell bodies. Each LAMC of A1 sends a process laterally, then anteriorly to join the medial fiber bundle in T5. From the ante-

TABLE 1. Sources of 5-HT–Immunoreactive Fibers in the Segmental Nerves of the Pereiopod Ganglia[1]

Ganglion	Nerve	Source of 5-HT–immunoreactive fibers
T1 (claw)	1	A1LAMC
	2	A1LAMC, T5LAMC
	3	T5LAMC
T2	1	A1LAMC
	2	A1LAMC, T5LAMC
	3	T5LAMC
T3	1	A1LAMC
	2	A1LAMC, T5LAMC
	3	T5LAMC
T4	1	A1LAMC
	2	A1LAMC, T5LAMC
	3	T5LAMC
T5	1	None
	2	A1LAMC
	3	?

[1]Immunoreactive fibers entering nerve 1 project branches into both distal leg nerves and both thoracico-coxal nerves. An immunoreactive fiber projects into nerve 3 of T5, but we have been unable to trace it to its cell body of origin. LAMC, large anterior medial cells, 5-HT, serotonin.

rior turn point, small branches lead to a small area of neuropil just posterior to the LAMC somata in A1 (Figs. 5C, 6B). Large branches of LAMC enter both the first and second nerves of T1–T4 but only nerve 2 of 5. The extensive lateral immunoreactive neuropils in each of the pereiopod ganglia appear to be formed of branches of the A1 LAMC and one or two branches of unidentified cells from the lateral fiber bundles.

The distribution of neuropils and the positions and branches of fibers in the medial and lateral fiber bundles are similar in A1 to those in A2–A4, although the somata and their projections in the more posterior abdominal ganglia are very different. There are two pairs of 5-HT–immunoreactive cell bodies ventrally in each of A1 through A4, and three in A6, but none in A 5. Unlike lobsters and crayfish, which have several paired or unpaired medial 5-HT–immunoreactive cells distributed through their abdominal ganglia (Beltz and Kravitz, 1983; Real and Czternasty, 1990), *M. quadrispina* have no unpaired medial serotonergic cells in their abdominal ganglia, and only one medial pair, in abdominal ganglion 6. All are small (10–15 μm) and give rise to anteriorly projecting fibers, none of which we could follow to any areas of neuropil or out of the ganglia (Figs. 7, 8). The anatomy and distribution of 5-HT–immunoreactive somata is basically identical in A2 through A4 (Figs. 7, 8A). Both pairs of cell bodies are located laterally, one pair anteriorly and the other about mid-way back in each ganglion. This is similar to positions of putative homologues in lobsters (Beltz and Kravitz, 1983) and crayfish (Real and Czternasty, 1990), although in lobsters, the cell bodies are located more medially. Four of the immunoreactive cell bodies in A6 are located posteriorly and the remaining two are close to the center of the ganglion (Figs. 7, 8D). The initial projections of one pair of posterior cell bodies were often followed into the vicinity of a lateral area of immunoreactive neuropil in A6 before becoming too faint. They were never seen to contribute to the neuropil (Figs 7, 8D).

We could not identify individual fibers as ascending or descending in the abdominal portion of the medial and lateral fiber bundles, because none of their cell bodies of origin could be determined. Each lateral fiber bundle contains three immunoreactive fibers from A1 to A5 and two from A5 to A6. Each medial fiber bundle contains two

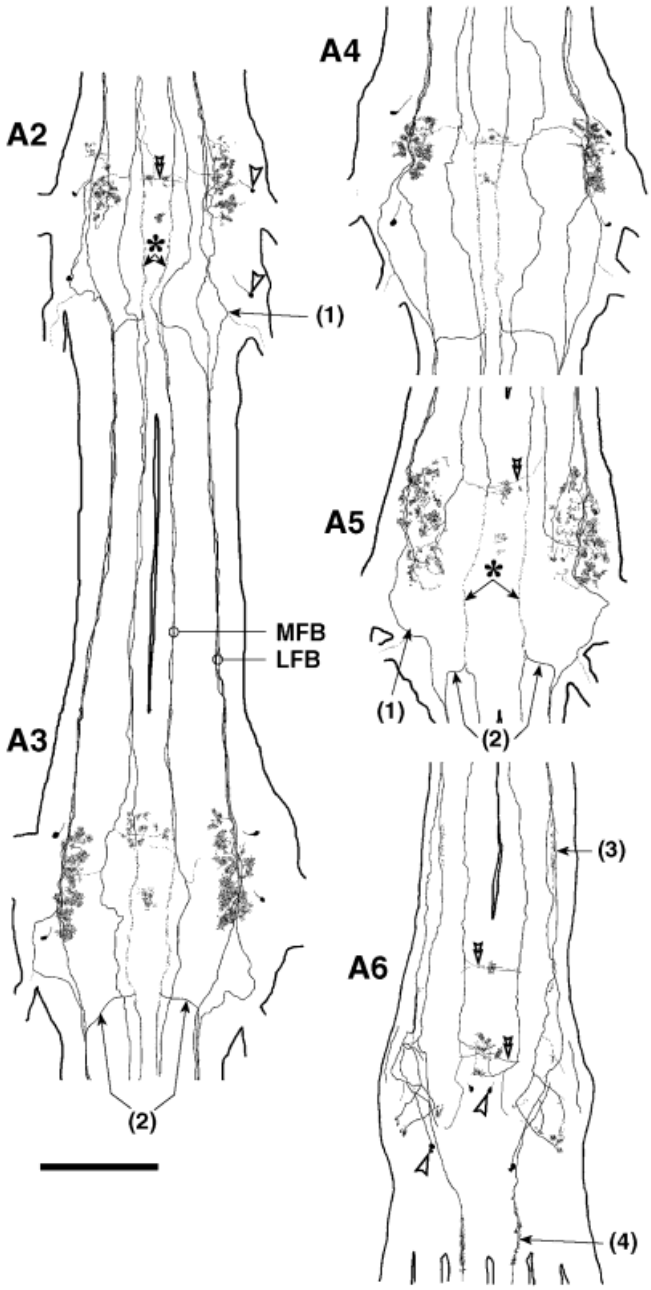

Fig. 7. Serotonin (5-HT) immunoreactivity in abdominal ganglia 2 through 6. These drawings are composites of camera lucida drawings and traces of confocal photomicrographs from a single nerve cord. Features are identified in A2 (similar in ganglia 3 and 4), 5, and 6. Note the small lateral cell bodies (open arrowheads, identified in A2), fine processes that form the medial fiber bundles (MFB) in the ganglia (asterisk, identified in A5), the branches of an MFB fiber that cross the midline (open double arrowhead), the fine branches of the most lateral fiber which project into nerve 2 (1), and the fibers which cross between the lateral fiber bundles (LFB) and MFBs in the caudal part of the ganglia (2, identified in A2). In A6, note the fiber from the lateral bundle that forms dense plexuses of beaded fibers in the connective (3) and in the caudal part of the ganglion and out nerve 7 (4). Scale bar = 200 μm.

fibers from A1 through A5 and one from A5 to A6. Some fibers are not continuous throughout the abdominal cord.

In A1 through A4, one of the fibers from the lateral fiber bundle of the anterior connective terminates in the lateral neuropil, whereas the other two branch within it before exiting into the posterior connective. One makes a lateral turn and sends a fine (1 μm), faintly immunoreactive, unbeaded branch out the second nerve, which always became too faint to follow beyond 20–40 μm out the nerve. The fibers of the lateral bundles are only very faintly immunoreactive in the middle of the connectives, so we could neither confirm that the fiber which sends the branch out nerve 2 is the same in each ganglion nor that the two fibers which pass through each ganglion are continuous through the abdominal cord. In A5, two of the three fibers in the lateral bundle of the anterior connective terminate in the lateral neuropil. The third passes through the ganglion, turning laterally and sending a fine branch out the second nerve on the way, a pattern similar to that in A1–4.

The two fibers of the medial fiber bundle separate within each of A1 through A5, one staying medial and the other diverging slightly lateral. One branch of the more lateral fiber, which was followed from A1 to its termination in the ipsilateral neuropil of A5, enters the ipsilateral lateral neuropils of A1–4, whereas another branch passes contralaterally where, in well-labeled ganglia, it could be followed to approximately halfway between the midline and the contralateral lateral neuropil before becoming too faint. Along the midline are two small areas of immunoreactive neuropil: one in the area of the branches from the medial fiber bundle that cross the midline and the other slightly more posterior. The source of the fibers contributing to these immunoreactive neuropils could not be traced. A dense plexus of fine processes and varicosities in the middle part of each ganglion surrounds the more medial fiber (Fig. 8B) and makes it impossible to follow this fiber's course. We were not able to determine, therefore, whether this fiber is continuous through the ganglia or whether the fibers anterior and posterior to each ganglion arise from different cells. A similar feature has been described in lobsters (Beltz and Kravitz, 1983). Near the posterior edge of each ganglion, but within the plexus of fine fibers, one fiber of the medial bundle passes laterally to join the lateral bundle posterior to the ganglion. This fiber appears to arise (or end) within the plexus of fine fibers; therefore, its source is not known.

All three pairs of fibers that enter A6 from the connective (one in the medial "bundle" and two in the lateral bundle) contribute to the fairly sparse, compared with other ganglia, lateral neuropil. The medial fiber sends a branch contralaterally at two points, in contrast to the

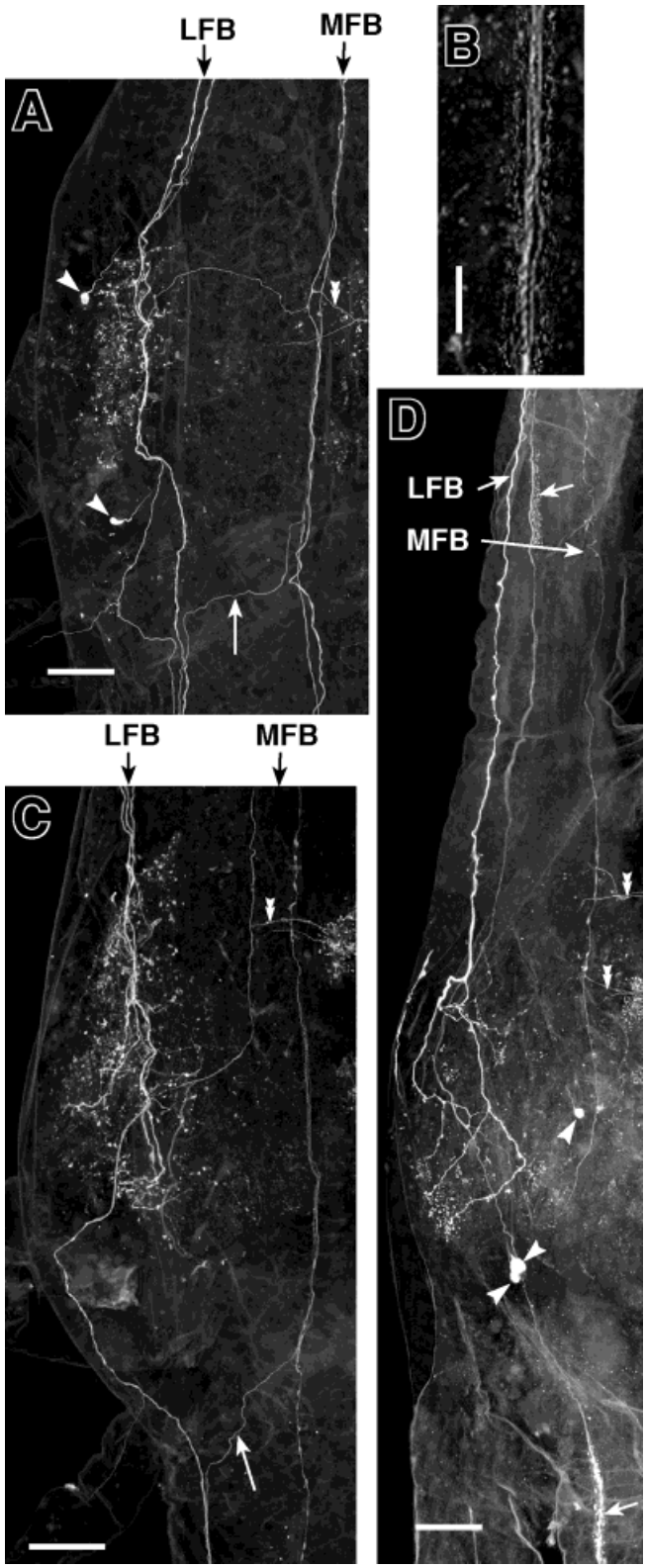

Fig. 8. Confocal photomicrographs of serotonin (5-HT) immunoreactivity in the abdominal ganglia. Anterior is up and medial is right for all parts of the figure. **A:** One hemiganglion of A4. A2 and A3 are essentially identical. Note the small lateral cell bodies (arrowheads), the branches of the fiber from the medial fiber bundle (MFB) that cross the midline (double arrowhead) and the fiber that crosses between the medial and lateral bundles (LFB; arrow). There is not much separation between the more lateral and medial fibers of the medial fiber bundle within the ganglion. **B:** A high magnification view of the fine, dense fibers of the medial fiber bundle in the ganglion. **C:** A hemiganglion of A5. Conventions as in A. **D:** A hemiganglion of A6. The small cell bodies are indicated by the arrowheads. The same fiber from the lateral fiber bundle forms dense plexuses of fine beaded fibers in the connective rostral to the ganglion and in the caudal part of the ganglion at the exit of nerve 7 (arrows). Scale bars = 100 μm in A,C,D, 50 μm in B.

single decussations in A1 through A5, the more posterior branch contributing to the more posterior of two medial immunoreactive neuropil areas (Figs. 7, 8D). We were not able to determine the sources of the more anterior medial neuropil. One of the lateral fibers produces two dense plexuses of fine beaded processes: one is in the connective anterior to A6, the other begins in the posterior part of the ganglion and continues into the seventh nerve and into its branches, which innervate the posterior gut (Figs. 7, 8D). This arrangement is different than in crayfish, where no immunoreactive fibers are seen in the seventh nerve of abdominal ganglion 6 unless the nerve cords are presoaked in 5-HT or adjacent sensory nerves are stimulated for 1 hour after dissection (Musolf et al., 1997). Two large (7–8 μm), very strongly immunoreactive fibers (one per side) were consistently seen along the lateral surfaces of A6, just below the sheath, but the source of these fibers could not be traced, and they did not send off any visible branches.

Distribution of OCT-like immunoreactivity

Forty-eight OCT-immunoreactive cells were seen in the central nervous system of *M. quadrispina* (Fig. 9). Fourteen of these are in the brain, 22 in the SEG, and 12 in the thoracic ganglia. The largest cells (60–70 μm diameter) are the pairs located medially and anteriorly in T1 and T2 (thoracic neuromeres 4 and 5) which, by their positions and anatomy, may be homologues of the anterior "crotch" cells described in lobsters (Schneider et al., 1993). OCT immunolabeling was variable between animals. In most nerve cords, we could find all or most of the structures described below, although the intensity of the label differed considerably. In some animals, only very weak labeling of cell bodies and a few processes could be distinguished. This variability did not correlate with molting cycle, season, feeding, or anything else we could determine. Additionally, with the exception of a few structures, such as the somata of the putative crotch cell homologues, some cells in the brain, and some large fibers in immunoreactive neuropil, the intensity of the OCT immunolabeling was much lower than of 5-HT immunolabeling. The relative intensities and variability of OCT immunoreactivity were consistent between whole-mounts and thick sections.

Distinct bundles of OCT-immunoreactive fibers similar to the serotonergic fiber bundles were seen only within and between the abdominal ganglia. The lateral and medial fiber bundles in the abdomen, which are not associated with the similarly named serotonergic fiber bundles, consist of two closely associated axons each. In the thorax, OCT-immunoreactive fibers appeared to follow individual paths. Nevertheless, fibers identified by their cell bodies of origin appeared in similar positions bilaterally and in different animals. Although not organized into distinct bundles, the fibers are loosely split into lateral and medial groups, with most of the dispersion along the dorsoventral axis. Therefore, the term *fiber bundle* is not particularly appropriate for OCT-immunoreactive fibers in thoracic ganglia, and simply describing a fiber's relative position as lateral or medial may be more accurate.

Fourteen OCT-immunoreactive cell bodies, between 15 and 30 μm in size, are located at the base of the brain between the roots of the connectives (Figs. 10, 11A). The processes of the more lateral cells project anteriorly into areas of immunoreactive neuropil, but the processes of the more medial cells could not be followed. In well-labeled nerve cords, four or five OCT-immunolabeled fibers were present in each optic nerve, one of which was consistently traced posteriorly to the SEG (Fig. 10). The cell bodies of origin of these fibers were not identified. The distribution of OCT-immunoreactive neuropil in the brain overlaps extensively but not completely that of 5-HT–immunoreactive neuropil (Figs. 2, 10). In addition to the projections of the relatively lateral cell bodies at the base of the brain, immunoreactive fibers in the optic nerves and the connectives posterior to the brain were traced to areas of immunoreactive neuropil. Weak immunolabel was seen in glomerular-like structures in the olfactory lobes (Fig. 10). The only immunoreactive fibers seen to enter the olfactory lobes were branches of one of the fibers present in each connective posterior to the brain. Two areas of fairly strongly immunoreactive neuropil extend across the midline of the anterior brain through approximately the middle third of the dorsoventral depth of the brain. A diffuse, weakly immunoreactive neuropil extends through most of the posteromedial portion of the brain, from the dorsal surface approximately three-fourths of the way ventrally. Finally, areas of rather weakly immunoreactive neuropil occur laterally in each connective just posterior to the brain. The fine, labeled processes in these neuropils could not be easily followed. Neuropil appeared as a pattern of unconnected beads of immunoreactivity with occasional larger, more strongly immunoreactive fibers passing among the beads. A similar appearance of OCT-immunoreactive neuropil has been described in lobsters (Schneider et al., 1993).

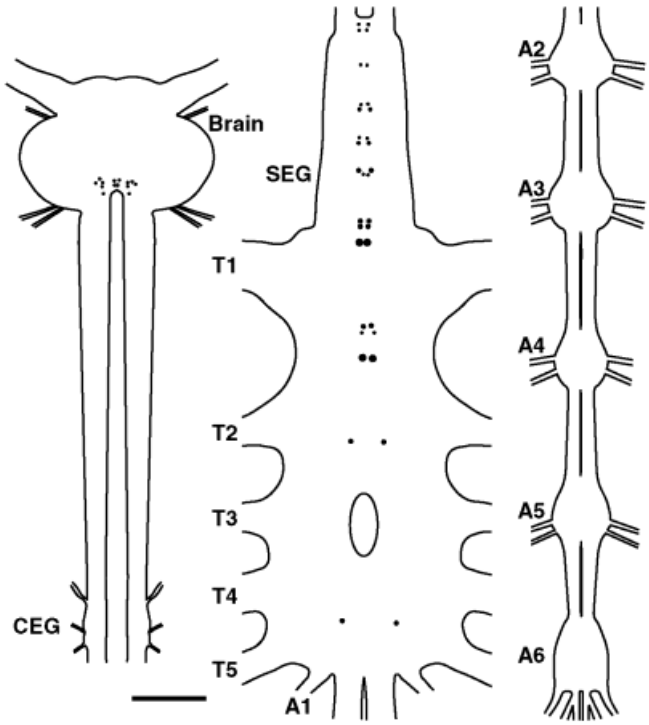

Fig. 9. Distribution of OA-immunoreactive neurons. The size of each dot roughly corresponds to the size of the cell body. The lengths of the connectives between ganglia have been shortened. SEG, subesophageal ganglion, CEG, circumesophageal (hemi-) ganglion. Scale bar = 500 μm

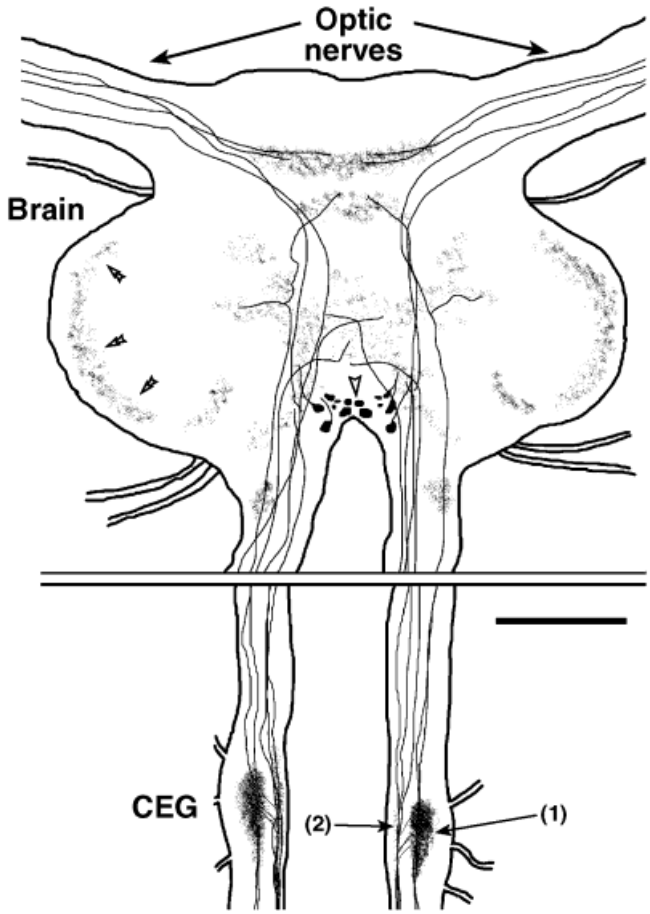

Fig. 10. Camera lucida drawing of OA immunoreactivity in the brain and circumesophageal (hemi-) ganglion (CEG). Most of the length of the connectives is not shown. The 14 immunoreactive somata in the brain are clustered at the caudal edge (arrowhead). Weakly immunolabeled neuropil areas are distributed through the brain, including at the edges of the olfactory lobes (double arrowheads). Note the two areas of dense immunoreactive fibers in each CEG, one in the lateral neuropil (1), and one surrounding a group of fibers in the medial part of each hemiganglion (2). Scale bar = 200 μm.

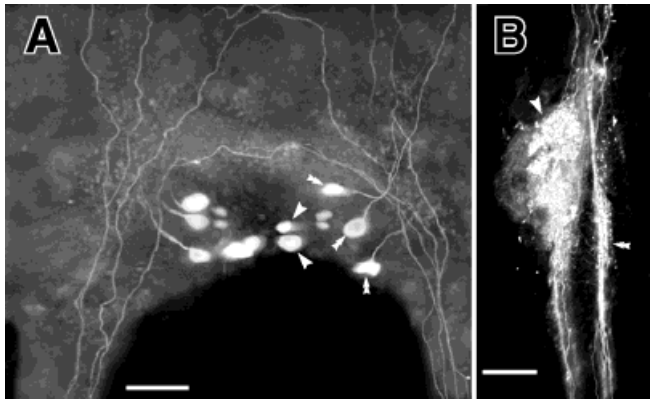

Fig. 11. Confocal micrographs of OA immunoreactivity in the brain and a circumesophageal ganglion (CEG). **A:** The cluster of immunoreactive somata at the base of the brain. The processes of the largest and most lateral cells project rostrally into areas of immunoreactive neuropil (arrowheads). **B:** Two areas of dense immunoreactive fibers occur in each CEG; one in the lateral neuropil of the ganglion (arrowhead) and one surrounding a group of medial immunoreactive fibers (double arrowhead). Scale bars = 50 μm in A, 25 μm in B.

All five of the OCT-immunoreactive fibers seen in the connectives between the brain and CEG were traced to the SEG, but none of their cell bodies was located. The CEGs contain the most intensely labeled plexuses of fibers in the entire central nervous system (Figs. 10, 11B), but no OCT-immunoreactive cell bodies. Eight immunoreactive fibers are arranged in a medial and a lateral fiber bundle in the connective posterior to each ganglion, three of which appear to be ascending fibers that terminate in the CEG neuropils. The remaining five immunoreactive fibers also contribute to the CEG neuropils as they pass through the ganglion to the brain. In each hemiganglion, six of the fibers are located medially and the other two more laterally. Two distinct areas of dense immunoreactive neuropil surround these regionally very localized fiber bundles, the larger around the lateral and the other a plexus of beaded processes surrounding the medial fibers. Fine processes pass between the two areas of neuropil, at least some of which appeared to arise from the medial fibers. We could not determine whether any of the lateral fibers send branches to the medial neuropil. We saw no immunolabeled fibers entering any of the segmental nerves of the CEG.

Twenty-two OCT-immunoreactive cell bodies, most of fairly uniform size (20–30 μm), labeled along or close to the midline in the SEG (Fig. 12). Longitudinal immunoreactive fibers occur in lateral and medial tracts, too loosely arranged to be called bundles. In addition to sparse, bilateral immunoreactive neuropil in each of the six neuromeres, the midline neuropil in each neuromere also labeled very weakly.

Two pairs of OCT-immunoreactive cell bodies in the first SEG neuromere and one pair in the second neuromere did not label at all other than in the soma (Fig. 12). These three pairs of cells are all located anteriorly and in the dorsal half of their respective neuromeres. All of the remaining immunoreactive cell bodies in the SEG, in neuromeres 3 through 6, are ventral. The processes from one of immunoreactive pairs of cells in each of neuromeres 3 and 4 remain ipsilateral, projecting anteriorly, then laterally, and finally posteriorly to A6 through the lateral tract of immunoreactive fibers (Fig. 12, 13A).

These axons branch into the ipsilateral neuropil of their own neuromere and all neuromeres posterior to them. In the abdominal ganglia, these cells also send branches to contralateral neuropils. Each branch followed into an area of immunoreactive neuropil quickly thinned, and the label became too faint to follow; none was seen to pass beyond the neuropil or out any segmental nerve. A second pair of immunoreactive cell bodies in each of the third and fourth SEG neuromeres project contralaterally, then anteriorly to the next anterior neuromere, where they branch and appear to contribute to the contralateral immunoreactive neuropil of these neuromeres (Fig. 13B). Neuromeres 5 and 6 each contain two pairs of immunoreactive cell bodies. One pair in each neuromere project ipsilateral fibers medially and anteriorly through two anterior neuromeres without giving off any visible branches before becoming

too faint to follow. The second pairs of cell bodies in the fifth and sixth neuromeres project fibers contralaterally out segmental nerves of their own neuromeres. These cells are slightly larger (30–50 μm) than the rest of the immunoreactive cells in the SEG and may be, based on their anatomy, segmental homologues of the immunosecretory posterior crotch cells described in lobsters (Schneider et al., 1993). Their cell bodies are variably positioned; in some neuromeres they are located on top of one another along the midline, whereas in others they are separated and located as much as one-third of the way laterally in the neuromere (e.g., the cells of neuromere 5 in Fig. 12).

Cells that appear from their position and anatomy to be homologues of the crotch cells described in lobsters are present in *M. quadrispina* but in far fewer numbers (Fig. 12). Anterior crotch cells are located dorsally in T1 and T2 (thoracic neuromeres 4 and 5) (Fig. 12). Each of these cells projects laterally before bifurcating, one branch entering the lateral neuropil, the other projecting to the next anterior segment (Figs. 13C, 14A). Here, the axon turns laterally and enters the second segmental nerve, where it contributes to a dense plexus of peripheral immunoreactive fibers within the second nerve. Whether the projections of these cells are ipsilateral or contralateral is unclear because, as in lobsters, the somata are large (60–70 μm) and directly overlie one another along the midline. Two distinct types of posterior crotch cells labeled in T1 and T2 (Fig. 12). The cell bodies of one type, represented by a single pair in T1 are ventral and close to the midline (Figs. 12, 13C, 14A). In some animals, they are almost on top of one another along the midline, but in others, where they were slightly off the midline, it was evident that they project contralaterally without any apparent branching and enter the contralateral nerve 2 of T1 (Figs. 12, 13C, 14A). These two cells may be segmental homologues of the two pairs of cells with similar projections in the posterior two neuromeres of the SEG. One pair of somata of the second type of posterior crotch cell is located slightly off the midline in each of T1 and T2 (Fig. 12). The somata in the T1 are slightly smaller than those in T2 (15–25 μm vs. 30–40 μm). These cells also project contralaterally, where they bifurcate and send one terminal branch into the contralateral neuropil of their own ganglion and one branch anteriorly (Figs. 12, 13B, 14A). The anterior branches enter the SEG, but were always too faint to follow any further. Despite the lateral placement of these cells in *M. quadrispina* in comparison to crotch cells described in lobsters (Schneider et al., 1993), their projections suggest that they are homologous.

The most caudal pair of OCT-immunoreactive cell bodies in *M. quadrispina* is located ventrally in T5 (Figs. 12, 13C, 14B). These cells project anteriorly and send branches into the second segmental nerves of T2–T5, where they form putative neurosecretory structures consisting of peripheral plexuses of fine fibers. Immunoreactive fibers entering segmental nerve 2 of T1 may also be branches of these cells, but we were unable to confirm this.

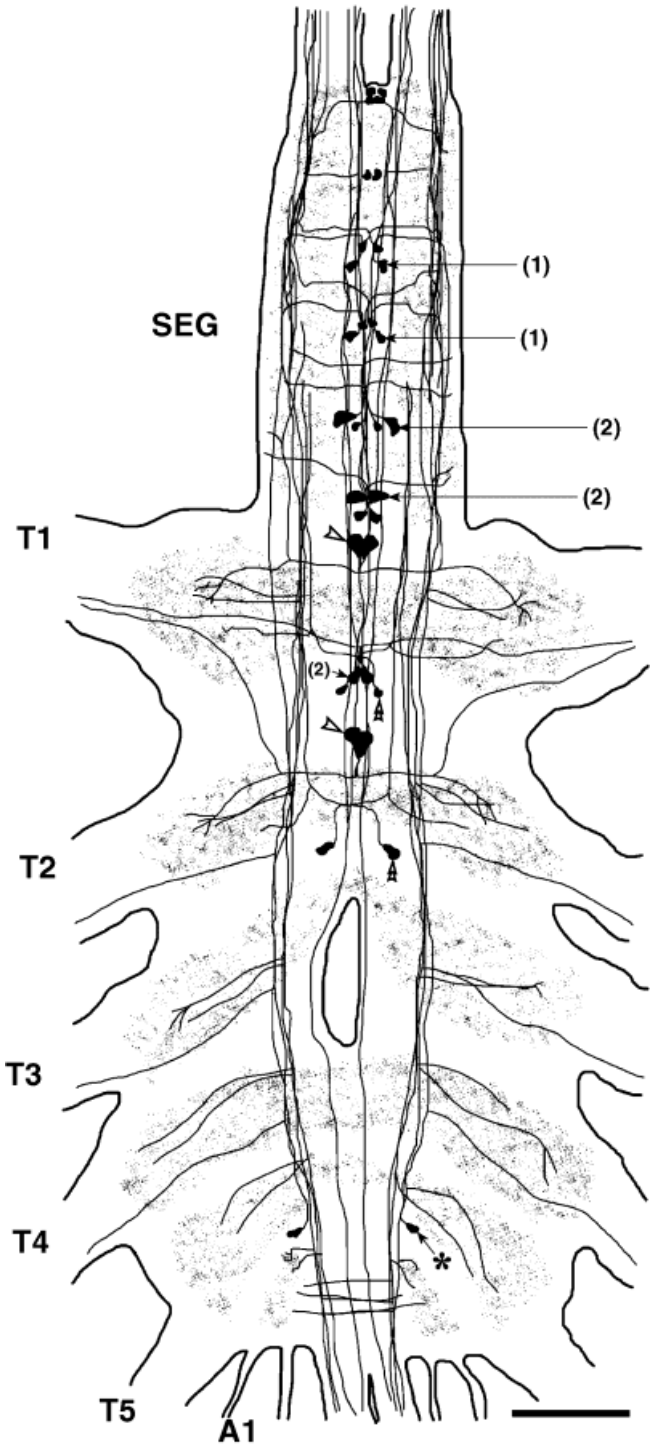

Fig. 12. Camera lucida drawing of OA immunoreactivity in the subesophageal ganglion (SEG), T1–5 (thoracic neuromeres 4–8), and A1. (1) indicates the cells in the third and fourth subesophageal neuromeres that send processes caudally to the sixth abdominal ganglion. (2) indicates the cells in the fifth and sixth subesophageal (second and third thoracic) neuromeres and T1 (thoracic neuromere 4) that send contralateral processes into the second segmental nerves. The open arrowheads indicate the anterior crotch cells; the open double arrowheads indicate the posterior crotch cells with contralateral rostral projections. The asterisk indicates the cells in T5 that send branches into the second nerves of each T2 through T5 and may also be the source of the fiber in the second nerve of T1, which was not traceable to its cell body of origin (not visible on this drawing). Scale bar = 200 μm.

This pair of cells appears to be responsible for most of the peripheral putative octopaminergic fibers from the thoracic ganglia.

There are no OCT-immunoreactive cell bodies in the abdomen of *M. quadrispina* (Figs. 13, 15), which is also the case in crayfish (Spörhase-Eichmann et al., 1998), but not in American lobsters, which have a pair of immunoreactive cell bodies in both abdominal ganglia 4 and 5 (Schneider et al., 1993). A total of eight descending axons, four on each side, are arranged into tightly grouped medial and lateral fiber bundles. The two fibers in each lateral fiber bundle were traced to cells in the third and fourth neuromere of the SEG (see above; Fig. 13A). The two fibers in the medial fiber bundle were not traced to their cell bodies of origin, although in well-labeled nerve cords, one of them was traced to the brain and the other to the lateral region of the second neuromere of the SEG. Each of the descending fibers contributes to the lateral neuropil of every abdominal ganglion, but we were unable to determine whether individual fibers had distinct innervation fields. No OCT-immunoreactive processes were seen to enter any of the segmental nerves in the abdomen.

Octopamine-immunoreactive processes and neuropils are distributed throughout each abdominal ganglion (Fig. 15). The lateral neuropils in each ganglion label fairly intensely, but medial neuropil label strongly only in A6 and very weakly in the other abdominal ganglia. A strik-

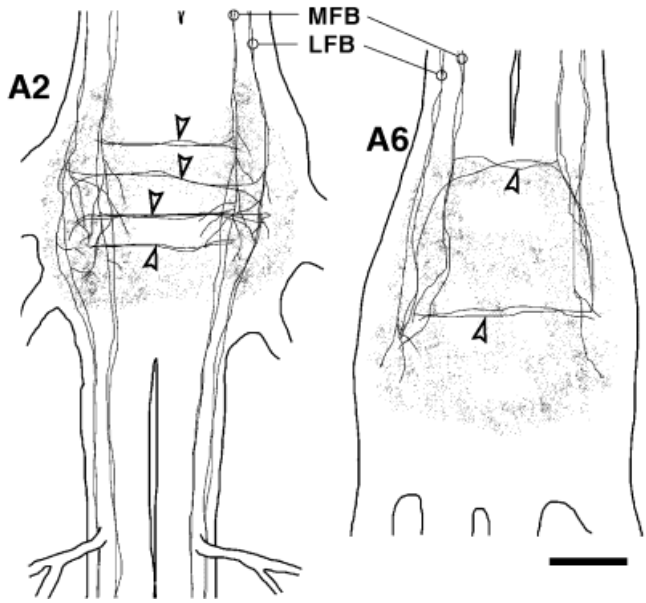

Fig. 15. Cameral lucida drawings of OA immunoreactivity in A2 and A6. Immunoreactivity is basically identical in A2 through A5. The four descending fibers are arranged into medial (MFB) and lateral (LFB) fiber bundles. Note the many immunoreactive fibers crossing the midline (arrowheads). Scale bar = 50 μm.

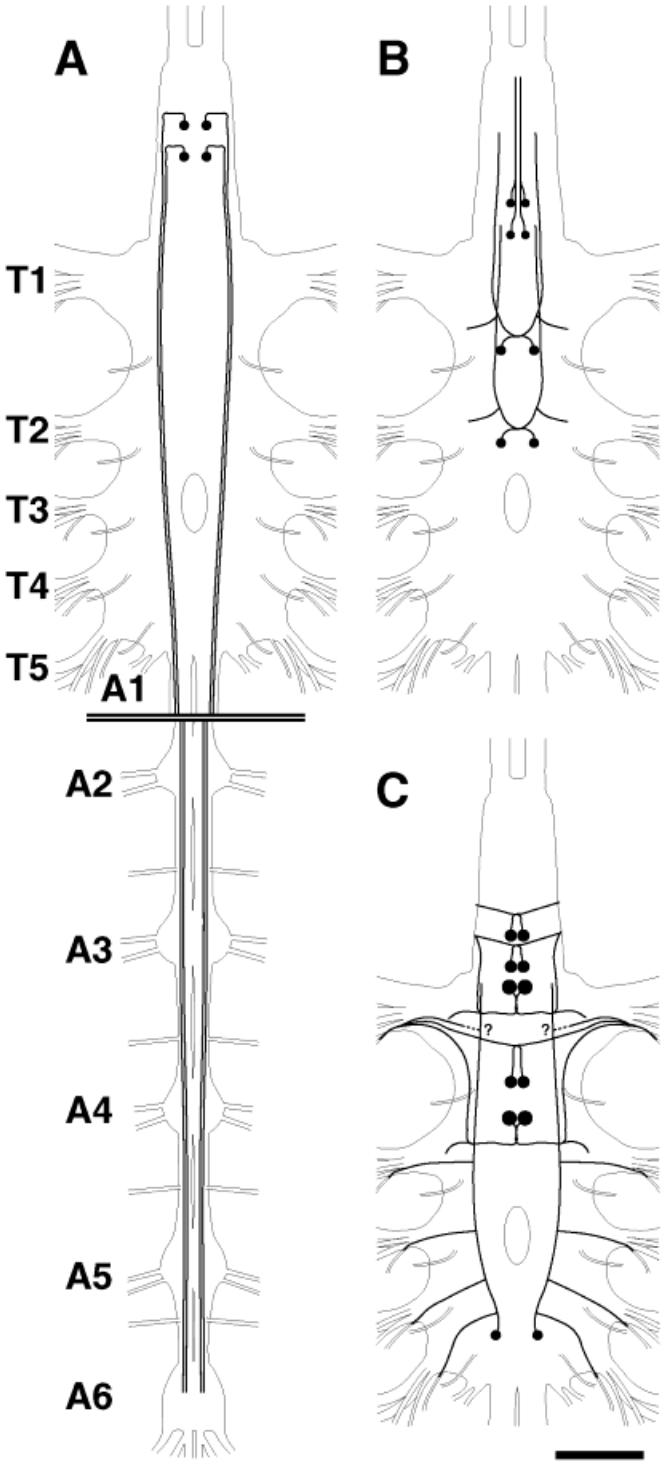

Fig. 13. Schematic drawings of OA-immunoreactive neurons. **A:** The descending interneurons in the third and fourth subesophageal neuromeres that send ipsilateral processes caudally to A6. Most of the connectives between A1 and A2 are not shown. **B:** Cells with anterior projections. The anterior fibers of all of these cells faded without visible terminal arborizations. **C:** The putative OA-neurosecretory system. Six pairs of cells in the caudal two neuromeres of the subesophageal ganglion and T1, T2, and T5 contribute to this system. All have branches that enter second segmental nerves and form dense peripheral plexuses. Note that three different fibers enter the second nerve of T1, two of which are from cells in T1, and one of which was not traced to its cell body of origin, but may have come from the cells in T5. Scale bar = 250 μm in C (applies to A–C).

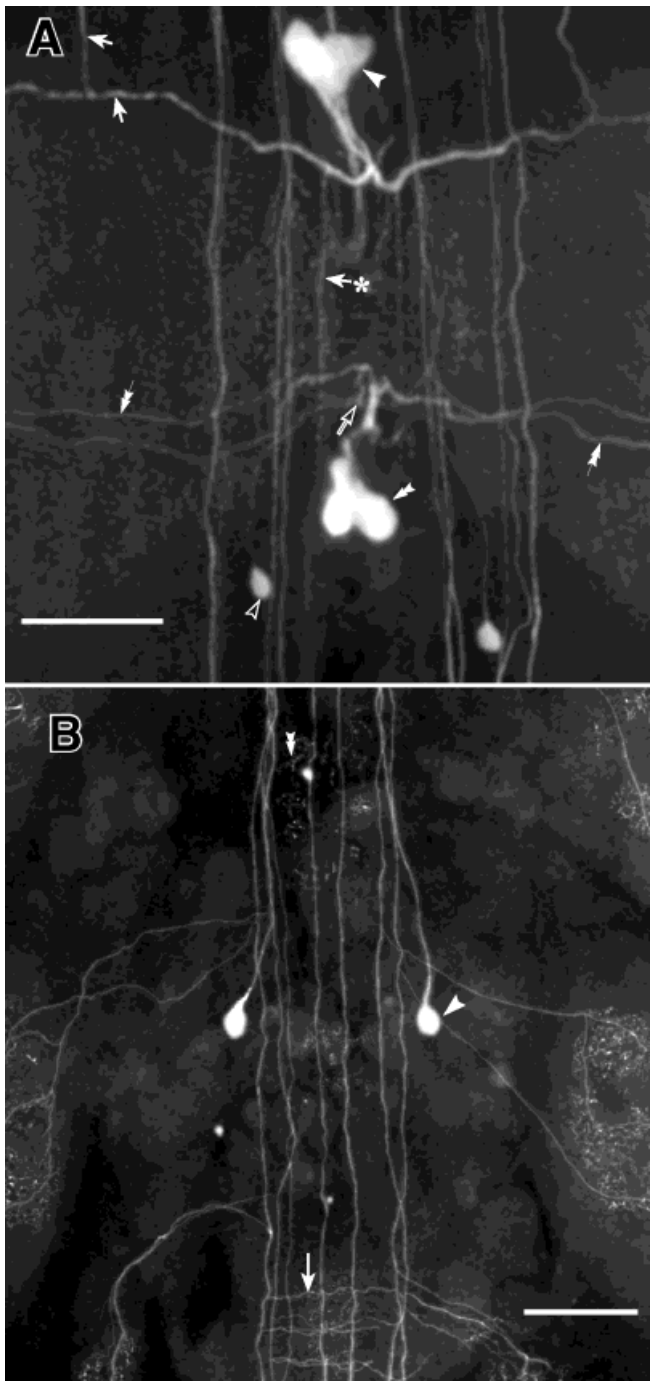

Fig. 14. Confocal photomicrographs of OA-immunoreactive neurons in the thoracic ganglia. **A:** The middle part of T1. The solid arrowhead indicates the rostral crotch cells, and the solid arrows point to the processes of one of these cells. The asterisk indicates one of the fine beaded processes that project from these cells near the midline. The double arrowhead indicates the caudal crotch cells that send processes out nerve 2 (double headed arrows). The more lateral caudal crotch cells (open arrowhead) send processes across the midline (open arrow), then rostral. **B:** The immunoreactive cells in T5 (arrowhead). These cells project rostrally and send branches out nerve 2 of at least T2 through T5. Note the weakly immunoreactive processes along the midline of T5 (double arrowhead) and the contralaterally projecting branches of the descending interneurons in A1 (arrow). Scale bars = 100 μm in A,B.

ing feature of the abdominal ganglia is the large number of contralateral branches of the descending OCT-immunoreactive fibers. In many nerve cords, contralateral branches of every descending fiber were seen in A1 through A5 and in some ganglia two or three contralateral branches of a single fiber were identified. Some additional decussating fibers passed between lateral neuropils that could not be traced to their fiber of origin. Although branches of each of the descending interneurons were followed to the ipsilateral and contralateral neuropil, the dense, weakly immunoreactive neuropil along the midline could not be associated with any particular descending fiber. In lobsters, the descending fibers from the third and fourth SEG neuromere contribute to the medial neuropil (Schneider et al., 1993). In A6, usually only two or three pairs of contralaterally projecting fibers were labeled. The obvious swellings in A6 of the descending interneurons from the SEG described in lobsters (Schneider et al., 1993) were not seen in *M. quadrispina*.

DISCUSSION

Antibodies directed against 5-HT and OCT generated reproducible patterns of immunolabeling in the central nervous system of *M. quadrispina*. The commercially available 5-HT antibody has been fully characterized, and specificity tests and controls indicate that it performs reliably under the conditions used in this study. Specificity of the OCT antibody used in this study has been shown (Schneider et al., 1993; Spörhase-Eichmann et al., 1998). Additionally, in lobsters, this antibody produced patterns of immunoreactivity similar to those produced by a polyclonal OCT antibody whose specificity in lobsters has been characterized (Schneider et al., 1993, 1996). Tests using 5-HT or OCT as blocking reagents indicated that each labeled structures in the nerve cord of *M. quadrispina* with high specificity.

Comparisons between the serotonergic and octopaminergic systems in *M. quadrispina*

Serotonergic- and octopaminergic-like immunolabeling was widespread in the nerve cord of *M. quadrispina*. Extensive lateral and medial immunoreactive neuropils are present in the brain and all ganglia and labeling by both antibodies was pervasive through most of the ganglionic neuropils, indicating considerable overlap in the distribution of 5-HT and OCT immunoreactivity throughout the central nervous system. Similarity of distribution at the light microscope level is not, of course, conclusive evidence of similarity in targets. However, the wide spread immunoreactivities and the extensive overlap of their distributions suggest that both amines have wide-ranging effects in the nervous system and that at least some of their targets may be the same.

There are far fewer OCT-immunoreactive than 5-HT–immunoreactive cells in *M. quadrispina*, even though the overall distributions of 5-HT and OCT immunoreactivities are quite similar. The large number of 5-HT–immunoreactive cell bodies in the anterior brain accounts for most of this difference, but there are also fewer segmentally repeated OCT- than 5-HT–immunoreactive somata in the ventral nerve cord. Segmentally repeated neurons are an obvious, but not the only, means to differentially control nervous output of different ganglia. For

example, segmentally disparate presynaptic inputs to a multisegmental neuron or postsynaptic inputs to its targets could allow one cell with multisegmental outputs to differentially influence targets in different neuromeres. Therefore, the smaller number of OCT cells than of 5-HT cells in *M. quadrispina*, or of OCT cells in *M. quadrispina* than in lobsters (Schneider et al., 1993), does not in itself suggest simplification or reduction in function of the central octopaminergic system as a whole. Rather, it raises interesting behavioral, physiological, and evolutionary questions regarding the aminergic systems in these animals. Some differences in aminergic modulation of behavior may turn out to be related to species differences in organization of homologous motor centers, or in receptors of individual neurons, independent or in the absence of change in the modulation/modulatory network per se (Katz and Harris-Warrick, 1999; Paul et al., 2001).

Peripheral projections of 5-HT–immunoreactive cells are also far more extensive than those of OCT-immunoreactive cells. 5-HT–immunoreactive fibers are present in every segmental nerve of the thoracic ganglia, except for nerve 1 of T5, in most of the nerves leaving the SEG, and in nerves 2 of the first five abdominal ganglia. By contrast, peripheral OCT-immunoreactive fibers are present only in nerve 2 of the eight thoracic neuromeres: the posterior three neuromeres of the SEG and T1–5. This finding indicates that 5-HT could potentially be released directly onto many more peripheral muscles, sensory neurons, and other tissues throughout the body than could OCT. In lobsters and crayfish, OCT has actions on peripheral systems not directly innervated by the nerves containing OCT-immunoreactive fibers in *M. quadrispina*, such as the abdominal musculature (Batelle and Kravitz, 1978; Kravitz et al., 1980; Pasztor and Bush, 1989; Pasztor and MacMillan, 1990). In American lobsters, concentrations of OCT sufficient to activate most of the known peripheral targets are found in the systemic circulation (Livingstone et al., 1980). If peripheral actions of OCT are similar in *M. quadrispina* to those in lobsters, OCT release into the systemic circulation could be the mechanism by which OCT reaches these targets. However, direct release onto peripheral targets allows for both higher local concentrations and differential influences on targets in close apposition to release sites. Direct release onto targets also provides better control over the time course of peripheral actions than transport by the hemolymph (Southard et al., 2000). Therefore, serotonergic modulation may be targeted differentially to specific sites in the periphery, whereas OCT seems only able to act as a systemic modulator on most peripheral OCT-sensitive tissues.

A feature common to both the serotonergic and octopaminergic systems in *M. quadrispina* is extensive bilateral projections from individual neurons in the abdominal ganglia, in contrast to the majority of solely contralateral (to the soma) projections in the thoracic and subesophageal ganglia. Bilateral projections are one way for unilateral inputs to exert bilateral influence, but bilateral effects can also be achieved by activation of systems of unilaterally projecting neurons. The aminergic innervation patterns are correlated with differences in the way the abdominal and thoracic motor systems are most often used. In the abdomen, swimmeret beating and tailflipping involve bilateral synchrony. Both systems are known to receive aminergic modulation, i.e., both amines influence axial extensor and flexor circuits (Harris-Warrick and Kravitz, 1984), and OCT inhibits fictive swimmeret motor activity and may be released by certain swimmeret command neurons (Mulloney et al., 1987). Bilateral projections in the abdomen are one way in which unilateral input to aminergic systems, such as occur with sensory stimulation (Edwards and Drummond, 1999), could produce bilateral modulation. Bilateral coordination of most motor systems in the more anterior segments, on the other hand, is much more variable and any occurrence of bilateral synchrony usually transient. Octopamine modulates a fictive walking motor pattern generated in vitro by single thoracic ganglia of crayfish (Gill and Skorupski, 1999). The anatomy of the OCT system in the thorax suggests that octopaminergic modulation in vivo could be unilateral or even directed specifically to individual ganglia. These combined observations suggest that amine distributions may give clues about their actions in at least some respects.

In general, the strength of the 5-HT immunolabel in neuropil areas was much stronger than that of the OCT immunolabel. Such differences may reflect true biological differences in endogenous concentrations of the two amines, but it is difficult to draw inferences from differences in relative intensities of labeling by different antibodies, and there are several technical issues that, although they may be inconsequential, should be considered. That somata and large immunoreactive processes within neuropil areas consistently labeled very intensely with the OCT antibody suggests that limited penetration of the antibodies was not the reason for the weak OCT label in the neuropil. However, the different fixation procedures or other inherent disparities in the antibodies used could have produced this difference in a way that we could not predict. Alternatively, the differences we observed could reflect different responses of 5-HT and OCT neurons to the dissection procedures. We have noticed that extensive handling of *M. quadrispina* before cooling and dissection can, in some instances, almost eliminate immunoreactivity for both 5-HT and OCT (our unpublished observations). Furthermore, in crabs, hemolymph levels of both 5-HT and OCT change as a result of activity (Sneddon et al., 2000). It is conceivable that differences in responses in the two aminergic systems to experimental handling could influence our results. Interestingly, nevertheless, neuropil areas in American lobsters are quite strongly OCT immunoreactive (Schneider et al., 1993, 1996), possibly suggesting another difference between amine systems in *M. quadrispina* and macrurans exhibiting fighting behavior and dominance hierarchies (Antonsen and Paul, 1997).

Interspecies comparisons

Generally, conservation of structure suggests conservation of function. However, particular care must be taken when applying this concept to modulatory systems because their overt effects will depend on the species-specific peculiarities of the motor and sensory mechanisms they influence (Katz and Harris-Warrick, 1999). For example, the walking legs of diverse decapods share a fundamentally similar shape and are, in general, used for similar purposes, such as stance, locomotion, and feeding. However, the ways different taxa have evolved to use their appendages for these behaviors vary tremendously (Paul et al., 2001). Therefore, the potential functional consequences of conserved anatomy of aminergic neurons or,

conversely, the potential consequences of anatomic differences, will depend on the degree their targets have been conserved, and the latter information is often incomplete. For example, incomplete information on the anatomy and physiology of aminergic systems and their targets in the southern hemisphere crayfish *Cherax destructor* limits interpretation of this species' unexpectedly different postural responses to injected 5-HT and OCT compared with those of northern hemisphere species (Livingstone et al., 1980; McRae, 1996). Nevertheless, the comparisons between features of the aminergic systems in *M. quadrispina* and other species summarized below suggest hypotheses concerning evolutionary change and functional adaptation that may be tested in future studies.

Fusion of the thoracic and first abdominal ganglia in *M. quadrispina* has resulted in changes in the organization of these ganglia which appear to be reflected in the morphology of some of the aminergic neurons. This finding is particularly evident in T4, T5, and A1, where the cytoarchitecture of the individual neuromeres has become rather distorted. A1 is insinuated along the midline at the posterior edge of this fused ganglionic mass and, as a result, the medial parts of the more posterior thoracic ganglia are pushed laterally. The ganglionic arrangement and anatomy of individual neurons in *M. quadrispina* are much more similar to those in crabs, which have undergone a similar fusion of thoracic and first abdominal ganglia, than to those in lobsters and crayfish (Harzsch and Dawirs, 1995; Antonsen and Paul, 2001). One result of the ganglionic fusion in *M. quadrispina* is the apparent medial position within the fused thoracic mass of the 5-HT lateral fiber bundle, which nevertheless retains its lateral position within the tracts of ascending and descending axons coursing through the ganglia and intervening remnants of connective.

In general, *M. quadrispina* has fewer aminergic neurons than other decapods studied to date. We do not know whether this is peculiar to this species or a feature of anomurans in general. Preliminary studies of another anomuran *Emerita analoga*, a hippid crab quite distantly related to *M. quadrispina*, indicate that this species also has a reduced number of serotonergic neurons (our unpublished observations). Data from more anomuran species are needed, however, to know whether reduction in number of aminergic neurons is characteristic of this infraorder, in which case it might be correlated with the reduction in the fifth leg, loss of fighting behaviors, and/or loss of the medial (except in hermit crabs) and lateral giant interneurons. In any case, loss of cell bodies does not necessarily suggest loss of function, as differential control can occur at many levels (see above).

The striking similarity among crustacean species, including *M. quadrispina*, in positions and, often, relative sizes of many 5-HT–immunoreactive somata, as well as their primary projections and the distributions of 5-HT–immunoreactive neuropils, suggests a highly conserved serotonergic system. There are similar large immunoreactive neurons in the pereiopod ganglia in lobsters (Beltz and Kravitz, 1983), crayfish (Real and Czternasty, 1990), and crabs, although the caudal lateral cells of T3 may be missing in the latter group (Harzsch and Dawirs, 1995). These cells also occur in the syncarid *Anaspides tasmaniae* (Harrison et al., 1995), which displays many primitive malacostracan features. The LPLCs may be present in isopods, although in these animals, the rostral projections of the cells are ipsilateral (Thompson et al., 1994). The large cells of the pereiopod and first abdominal ganglia in these diverse crustaceans are almost certainly homologues, and the remarkable conservation of morphology suggests functional conservation of modulation of their targets. The behavioral consequences of the modulation may differ among species, however, because of alterations in target circuitry. Of more immediate interest are specific differences between aminergic systems which, when viewed in the context of other neural behavioral differences, can help focus questions for future research.

For example, 5-HT influences the lateral giant escape circuit in crayfish, and serotonergic nerve endings come into close apposition with the lateral giant neurons at several locations in the abdominal ganglia (Glanzman and Krasne, 1983; Yeh et al., 1996, 1997). Although the two serotonergic fibers (one per side) that give rise to these nerve endings have been traced into the thorax, their cell bodies of origin are unknown (Yeh et al., 1997). *M. quadrispina* has lost both sets of giant escape interneurons (Paul, 1989, 1991; Wilson and Paul, 1987) and also has some distinct rearrangements of 5-HT immunoreactivity in the abdominal ganglia. Most provocative is the absence of the medial unpaired serotonergic neurons that are present in some abdominal ganglia in crayfish and lobsters (Beltz and Kravitz, 1983; Real and Czternasty, 1990). Associating these two differences would be completely speculative at this point, but such comparative observations suggest places to look for physiological interactions between motor circuits and aminergic networks.

The fifth leg in *M. quadrispina* is specialized for grooming, whereas in crayfish and in most other nonanomuran decapods, the fifth leg's principal use is in locomotion (Schram, 1986). In *M. quadrispina*, branches of the A1 LAMCs project into all of the first segmental nerves entering legs 1 through 4, as do their A1 homologues in crayfish (Real and Czternasty, 1990). However, *M. quadrispina* has no 5-HT–immunoreactive fibers in nerve 1 of leg 5, unlike crayfish, in which the fifth leg nerve contains serotonergic fibers that are branches of the T5 LAMCs (Real and Czternasty, 1990). 5-HT generally increases tonicity of muscle in crustaceans (Livingstone et al., 1980); this role may not be necessary in non–load-bearing legs such as the fifth pereiopods of *M. quadrispina* and most other anomurans (Paul et al., 2001). We conjecture that hermit crabs have retained 5-HT fibers in their fifth as well as fourth legs, because they use both pairs to support their heavy gastropod shells (Herreid and Full, 1986). In crayfish, the role of leg 5 in walking is quite different from the roles of the other pereiopods (Jamon and Clarac, 1995); this is paralleled by the different sources of 5-HT fibers (Real and Czernasty, 1990). These data suggest that the release of 5-HT may be controlled at least in part independently in the fifth and the more anterior legs. Furthermore, the loss of serotonergic fibers in *M. quadrispina*'s fifth leg nerve may, therefore, not directly affect serotonergic modulation of legs 1 through 4, leaving open the possibility that 5-HT's influence on walking may be similar between these groups.

The putative neurosecretory structures formed by the LPLCs in *M. quadrispina* have no anatomic equivalent described (as yet) in any other species. The only neurosecretory structure formed by any of the LPLCs described in any other species is in nerve 1 of T5 in crayfish, which is formed by the cells of T4 (Real and Czternasty, 1990). At

this point, it is impossible to make even a reasonably informed guess as to the function of these unusual structures in *M. quadrispina*. However, most of the putative peripheral 5-HT neurosecretory system is conserved between *M. quadrispina* and other species, suggesting that many of the peripheral targets of 5-HT may remain the same.

Many of the OCT-immunoreactive cells described in American lobsters (Schneider et al., 1993, 1996) are apparently absent from *M. quadrispina,* but whether this finding reflects a change in pharmacologic profile of the neurons, as has been shown to occur in crustaceans (e.g., see review in Katz and Tazaki, 1992), or a loss of the neurons themselves remains to be determined. The absence of segmentally repeated OCT-immunoreactive neurons in *M. quadrispina* could reflect loss of function or consolidation of function into the remaining neurons or a combination. Present evidence suggests that both have occurred.

With respect to the loss of OCT-immunoreactive cells in the third and fourth pereiopod ganglia of *M. quadrispina*, it is provocative that among these cells in American lobsters are the six claw octopamine cells (CLOCs) (Bräunig et al., 1994). Projections of these cells enter nerve 1 of the first pereiopod ganglia. American lobsters show subordinate behavior if they lose one of their claws (Lang et al., 1977), and Kravitz and coworkers are investigating the potential roles of the CLOCs in this behavior (Beltz, 1999). *M. quadrispina* do not become submissive after losing a claw (our unpublished observations); perhaps loss of these neurons was associated with loss of this behavior.

In lobsters, a total of 28 segmentally repeated OCT-immunoreactive crotch cells in the pereiopod ganglia and the caudal two neuromeres of the subesophageal ganglion contribute to the peripheral neurosecretory system in the second segmental nerves. At least two different neurons project into each nerve 2, except for the fifth pereiopod ganglion, which does not contain any immunoreactive fibers. In *M. quadrispina*, only 12 of these cells are present, but the morphologies of most of them are identical to their homologues in lobsters. In particular, there are no OCT-immunoreactive crotch cells in the third and fourth pereiopod ganglia. However, a pair of caudal cells in the fifth pereiopod ganglion sends branches out the second segmental nerves of at least the next three rostral pereiopod ganglia. A similar pair of cells in the American lobster's fifth pereiopod ganglion sends processes to nerve 2 of pereiopod ganglion 4, but apparently to no further rostral ganglia (Schneider et al., 1993). The sources of peripheral OCT neurosecretion in the second segmental nerves of pereiopod ganglia 3 and 4 have been changed from segmentally repeated neurons in the lobster to a single pair of cells in *M. quadrispina*, suggesting that consolidation of function has occurred. It is not known whether the different neurons projecting into the second nerves of lobsters serve different functions; it is possible that some change in function has also occurred in association with the loss of segmentally repeated OCT-immunoreactive neurons in *M. quadrispina*.

SUMMARY

Despite overall similarity in the serotonergic and octopaminergic systems of decapods, several significant differences exist that are likely to have functional consequences at some level. Although gain or loss of aminergic structures itself does not necessarily suggest change of function at a behavioral level, it does suggest some sort of change in functional control of the system at a cellular or command level, which may be equally interesting. Very few functional roles have been assigned to any individual aminergic neurons. We have shown that comparative studies between species with different behaviors, anatomies, and neuromuscular circuits provide hints as to where to look for some of the interactions between aminergic neurons and other systems.

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LITERATURE CITED

Antonsen BL, Paul DH. 1997. Serotonin and octopamine elicit stereotypical agonistic behaviors in the squat lobster *Munida quadrispina* (Anomura, Galatheidae). J Comp Physiol A 181:501–510.

Antonsen BL, Paul DH. 2000. The leg depressor and levator muscles in the squat lobster *Munida quadrispina* (Galatheidae) and the crayfish *Procambarus clarkii* (Astacidae) have multiple heads with potentially different functions. Brain Behav Evol 56:63–85.

Antonsen BL, Paul DH. 2001. Aminergic systems in the squat lobster *Munida quadrispina* (Anomura, Galatheidae): a case made for comparative neurobiology. In: K. Wiese, editor. The crustacean nervous system. New York: Springer Verlag (in press).

Battelle BA, Kravitz EA. 1978. Targets of octopamine action in the lobster: cyclic nucleotide changes and physiological effects in haemolymph, heart, and exoskeletal muscle. J Pharmacol Exp Ther 205:438–448.

Beltz BS. 1999. Distribution and functional anatomy of amine-containing neurons in decapod crustaceans. Microsc Res Tech 44:105–120.

Beltz BS, Kravitz EA. 1983. Mapping of serotonin-like immunoreactivity in the lobster nervous system. J Neurosci 3:585–602.

Beltz BS, Kravitz EA. 1987. Physiological identification, morphological analysis, and development of identified serotonin-proctolin containing neurons in the lobster ventral nerve cord. J Neurosci 7:533–546.

Bräunig P, Walter I, Schneider H, Kravitz EA. 1994. A subdivision of octopamine neurosecretory cells in the lobster. Soc Neurosci Abstr 20:1789.

Doernberg SB, Cromarty SI, Heinrich R, Beltz BS, Kravitz EA. 2001. Agonistic behavior in naïve juvenile lobsters depleted of serotonin by 5,7-dihydroxytryptamine. J Comp Physiol A 187:91–103.

Edwards DH, Drummond JM. 1999. Effect of social experience on reflex responses of serotonergic neurons in crayfish. Soc Neurosci Abstr 25: 905.

Elofsson R. 1983. 5-HT-like immunoreactivity in the central nervous system of the crayfish, *Pacifasticus leniusculus*. Cell Tissue Res 232:221–236.

Elson RC. 1996. Neuroanatomy of a crayfish thoracic ganglion: sensory and motor roots of the walking nerves and possible homologies with insects. J Comp Neurol 365:1–17.

Evans PD, Kravitz EA, Talamo BR. 1975. Octopamine neurons: morphology, release of octopamine and possible physiological role. Brain Res 90:340–347.

Evans PD, Kravitz EA, Talamo BR. 1976a. Octopamine release at two points along lobster nerve trunks. J Physiol (Lond) 262:71–89.

Evans PD, Kravitz EA, Talamo BR, Wallace BG. 1976b. The association of octopamine with specific neurones along lobster nerve trunks. J Physiol (Lond) 262:51–70.

Flamm RE, Harris-Warrick RM. 1986. Aminergic modulation in the lobster stomatogastric ganglion. I. Effects on motor pattern and activity of neurons within the pyloric circuit. J Neurophysiol 55:847–865.

Florey E, Rathmayer M. 1978. The effects of octopamine and other amines on the heart and on neuromuscular transmission in decapod crustaceans: further evidence for role as a neurohormone. Comp Biochem Physiol 61C:229–237.

Gill MD, Skorupski P. 1999. Antagonistic effects of phentolamine and octopamine on rhythmic motor output of crayfish thoracic ganglia. J Neurophysiol 82:35–89.

Glanzman DL, Krasne FB. 1983. Serotonin and octopamine have opposite modulatory effects on the crayfish lateral giant escape reaction. J Neurosci 3:2263–2269.

Harris-Warrick RM, Kravitz EA. 1984. Cellular mechanisms for modulation of posture by octopamine and serotonin in the lobster. J Neurosci 4:1976–1993.

Harrison PJ, MacMillan DL, Young HM. 1995. Serotonin immunoreactivity in the ventral nerve cord of the primitive crustacean *Anaspides tasmaniae* closely resembles that of crayfish. J Exp Biol 198:531–535.

Harzsch S, Dawirs RR. 1995. A developmental study of serotonin-immunoreactive neurons in the larval central nervous system of the spider crab *Hyas araneus* (Decapoda, Brachyura). Invert Neurosci 1:53–65.

Herreid CF, Full RJ. 1986. Locomotion of hermit crabs (*Coenobita compressus*) on beach and treadmill. J Exp Biol 120:283–296.

Hörner M, Weigner W, Edwards DH, Kravitz EA. 1997. Excitation of identified serotonergic neurons by escape command neurons in lobsters. J Exp Biol 200:2017–2033.

Huber R, Orzeszyna M, Pokorny N, Kravitz EA. 1997a. Biogenic amines and aggression: experimental approaches in crustaceans. Brain Behav Evol 50(Suppl 1):60–68.

Huber R, Smith K, Delago A, Isaksson K, Kravitz EA. 1997b. Serotonin and aggressive motivation in crustaceans: altering the decision to retreat. Proc Natl Acad Sci USA 94:5939–5942.

Jamon M, Clarac F. 1995. Locomotor patterns in freely moving crayfish (*Procambarus clarkii*). J Exp Biol 198:683–700.

Johansson KUI. 1991. Identification of different types of serotonin-like immunoreactive olfactory interneurons in four orders of decapod crustacea. Cell Tissue Res 264:357–362.

Katz PS, Harris-Warrick RM. 1999. The evolution of neuronal circuits underlying species-specific behavior. Curr Opin Neurobiol 9:628–633.

Katz PS, Tazaki K. 1992. Comparative and evolutionary aspects of the crustacean stomatogastric system. In: Harris-Warrick RM, Marder E, Selverston AI, Moulins M, editors. Dynamic biological networks: the stomatogastric nervous system. Cambridge: MIT Press. p 221–261.

Kravitz EA, Glusman S, Harris-Warrick RM, Livingstone MS, Schwarz T, Goy MF. 1980. Amines and a peptide as neurohormones in lobsters: actions of neuromuscular preparations and preliminary behavioral studies. J Exp Biol 89:159–175.

Lang F, Govind CK, Costello WJ, Greene SI. 1977. Developmental neuroethology: changes in escape and defensive behavior during growth in the lobster. Science 197:682–685.

Langworthy K, Helluy S, Benton J, Beltz B. 1997. Amines and peptides in the brain of the American lobster: immunocytochemical localization patterns and implications for brain function. Cell Tissue Res 288:191–206.

Livingstone MS, Harris-Warrick RM, Kravitz EA. 1980. Serotonin and octopamine produce opposite postures in lobsters. Science 208:76–79.

Livingstone M, Schaeffer SF, Kravitz EA. 1981. Biochemistry and ultrastructure of serotonergic nerve endings in the lobster: serotonin and octopamine are contained in different nerve endings. J Neurobiol 12: 27–54.

McRae T. 1996. On the postural effects induced in female *Cherax destructor* (Clark) by serotonin and octopamine. Freshwater Crayfish 11:293–298.

Mulloney B, Acevedo L, Bradbury, AG. 1987. Modulation of crayfish swimmeret rhythm by octopamine and the neuropeptide proctolin. J Neurophysiol 58:584–597.

Musolf BE, Spörhase-Eichmann U, Edwards DH. 1997. Sensory stimulation enhanced 5-HT-IR of motor-neurons to the crayfish hindgut. Neurosci Abstr 23:1788.

Pasztor VM, Bush BM. 1989. Primary afferent responses of a crustacean mechanoreceptor are modulated by proctolin, octopamine, and serotonin. J Neurobiol 20:234–254.

Pasztor VM, MacMillan DL. 1990. The actions of proctolin, octopamine and serotonin on crustacean proprioceptors show species and neurone specificity. J Exp Biol 152:485–504.

Paul DH. 1989. A neurophylogenist's view of decapod crustacea. Bull Mar Sci 45:487–504.

Paul DH. 1991. Pedigrees of neurobehavioral circuits: tracing the evolution of novel behavior by comparing motor patterns, muscles, and neurons in members of related taxa. Brain Behav Evol 38:226–239.

Paul DH, Then MA, Magnuson DS. 1985. Evolution of the telson neuromusculature in decapod crustacea. Biol Bull 168:106–124.

Paul DH, Antonsen BL, Faulkes Z. 2001. Synergies between disparate motor systems: loci for behavioral evolution. In: Wiese K, editor. The crustacean nervous system. New York: Springer Verlag (in press).

Real D, Czternasty G. 1990. Mapping of serotonin-like immunoreactivity in the ventral nerve cord of crayfish. Brain Res 521:203–212.

Rossi-Durand C. 1993. Peripheral proprioceptive modulation in crayfish walking leg by serotonin. Brain Res 632:1–15.

Sandeman RE, Sandeman DC. 1987. Serotonin-like immunoreactivity of giant olfactory interneurons in the crayfish brain. Brain Res 403:371–374.

Sandemen DC, Sandeman RE, Aitken AR. 1988. Atlas of serotonin-containing neurons in the optic lobes of the crayfish, *Cherax destructor*. J Comp Neurol 269:465–478.

Sandeman D, Sandeman R, Derby C, Schmidt M. 1992. Morphology of the brain of crayfish, crabs, and spiny lobsters: a common nomenclature for homologous structures. Biol Bull 183:304–326.

Schneider H, Trimmer BA, Rapus J, Eckert M, Valentine DE, Kravitz EA. 1993. Mapping of octopamine-immunoreactive neurons in the central nervous system of the lobster. J Comp Neurol 329:129–142.

Schneider H, Budhiraja P, Walter I, Beltz BS, Peckol E, Kravitz EA. 1996. Developmental expression of the octopamine phenotype in lobsters, *Homarus americanus*. J Comp Neurol 371:3–14.

Schram FR. 1986. Crustacea. Oxford: Oxford University Press. p 190.

Sillar KT, Heitler WJ. 1985. The neural basis of escape swimming behaviour in the squat lobster *Galathea strigosa* I. Absence of cord giant axons and anatomy of motor neurones involved in swimming. J Exp Biol 117:251–269.

Sneddon U, Taylor AC, Huntingform FA, Watson DG. 2000. Agonistic behaviour and biogenic amines in shore crabs *Carcinus maenus*. J Exp Biol 203:537–545.

Spörhase-Eichmann U, Musolf BE, Edwards DH. 1998. Dopamine- and octopamine-immunoreactive neurons in the central nervous system of the crayfish. Soc Neurosci Abstr 24:1841

Southard RC, Haggard J, Crider ME, Whiteheart SW, Cooper RL. 2000. Influence of serotonin on the kinetics of vesicular release. Brain Res 871:16–28.

Thompson KSJ, Zeidler MP, Bacon JP. 1994. Comparative anatomy of serotonin-like immunoreactive neurons in isopods: putative homologues in several species. J Comp Neurol 347:553–569.

Wilson LJ, Paul DH. 1987. Tailflipping of *Munida quadrispina* (Galatheidae): conservation of behavior and underlying musculature with loss of anterior contralateral flexor motor neurons and motor giant. J Comp Physiol A 161:881–890.

Wine JJ, Krasne FB. 1982. The cellular organization of crayfish escape behavior. In: Bliss DH Sandeman DC, Atwood HL, editors. The biology of crustacea, Vol IV: neural integration and behavior. New York: Academic Press. p 241–292.

Yeh S-R, Fricke RA, Edwards DH. 1996. The effect of social experience on serotonergic modulation of the escape circuit of crayfish. Science 271: 366–369.

Yeh S-R, Musolf BE, Edwards DH. 1997. Neuronal adaptations to changes in the social dominance status of crayfish. J Neurosci 17:697–708.