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Development and Connectivity of Olfactory Pathways in the Brain of the Lobster *Homarus americanus*

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ABSTRACT

The main output pathways from the olfactory lobes (primary olfactory centers) and accessory lobes (higher-order integrative areas) of decapod crustaceans terminate within both of the main neuropil regions of the lateral protocerebrum: the medulla terminalis and the hemiellipsoid body. The present study examines the morphogenesis of the lateral protocerebral neuropils of the lobster, *Homarus americanus*, and the development of their neuronal connections with the paired olfactory and accessory lobes. The medulla terminalis was found to emerge during the initial stages of embryogenesis and to be the target neuropil of the output pathway from the olfactory lobe. In contrast, the hemiellipsoid body is first apparent during mid-embryonic development and is innervated by the output pathway from the accessory lobe. The dye injections used to elucidate these pathways also resulted in the labeling of a previously undescribed pathway linking the olfactory lobe and the ventral nerve cord. To increase our understanding of the morphology of the olfactory pathways in *H. americanus* we also examined the connectivity of the lateral protocerebral neuropils of embryonic lobsters. These studies identified several interneuronal populations that may be involved in the higher-order processing of olfactory inputs. In addition, we examined the neuroanatomy of ascending pathways from the antenna II and lateral antenna I neuropils (neuropils involved in the processing of chemosensory and tactile inputs). These studies showed that the ascending pathways from these neuropils innervate the same regions of the medulla terminalis and that these regions are different from those innervated by the olfactory lobe output pathway. J. Comp. Neurol. 441:23–43, 2001.

Indexing terms: crustacean; decapod; deutocerebrum; olfaction; projection neuron

The lateral protocerebra of decapod crustaceans are paired brain neuropils located proximal to the three optic ganglia (lamina ganglionaris, medulla externa, and medulla interna). In addition to having connections to the optic ganglia (Hanström, 1925; Strausfeld and Nässel, 1980; Blaustein et al., 1988), the lateral protocerebrum has also long been recognized as an important center in the olfactory pathway as it is the target region of output neurons from olfactory neuropils in the deutocerebrum (Hanström, 1924, 1925; Blaustein et al., 1988; Sandeman et al., 1993). The lateral protocerebrum, therefore, is thought to play an important role in the higher-order integration of multimodal sensory inputs.

The lateral protocerebrum is composed of two main regions: the medulla terminalis and the hemiellipsoid body (Fig. 1; Sandeman et al., 1992, 1993; Sandeman and Scholtz, 1995). The medulla terminalis is a complex of several interconnected neuropil regions, many of which are glomerular in structure (Hanström, 1925, 1931, 1947; Blaustein et al., 1988; Sullivan and Beltz, 2001). The hemiellipsoid body varies markedly between species in both its size and anatomy (Bellonci, 1882; Hanström, 1924, 1925, 1931, 1947; Blaustein et al., 1988; Sandeman et al., 1993; Strausfeld, 1998). In the lobster, *Homarus americanus*, the hemiellipsoid body is composed of an outer (cap) neuropil that surrounds an inner (core) neuropil region (Sullivan and Beltz, 2001). Anatomic studies of the brains of lobsters and other decapod crustaceans indicate that the medulla terminalis and the hemiellipsoid body are innervated by the output pathways from the olfactory and accessory lobes, which are located in the

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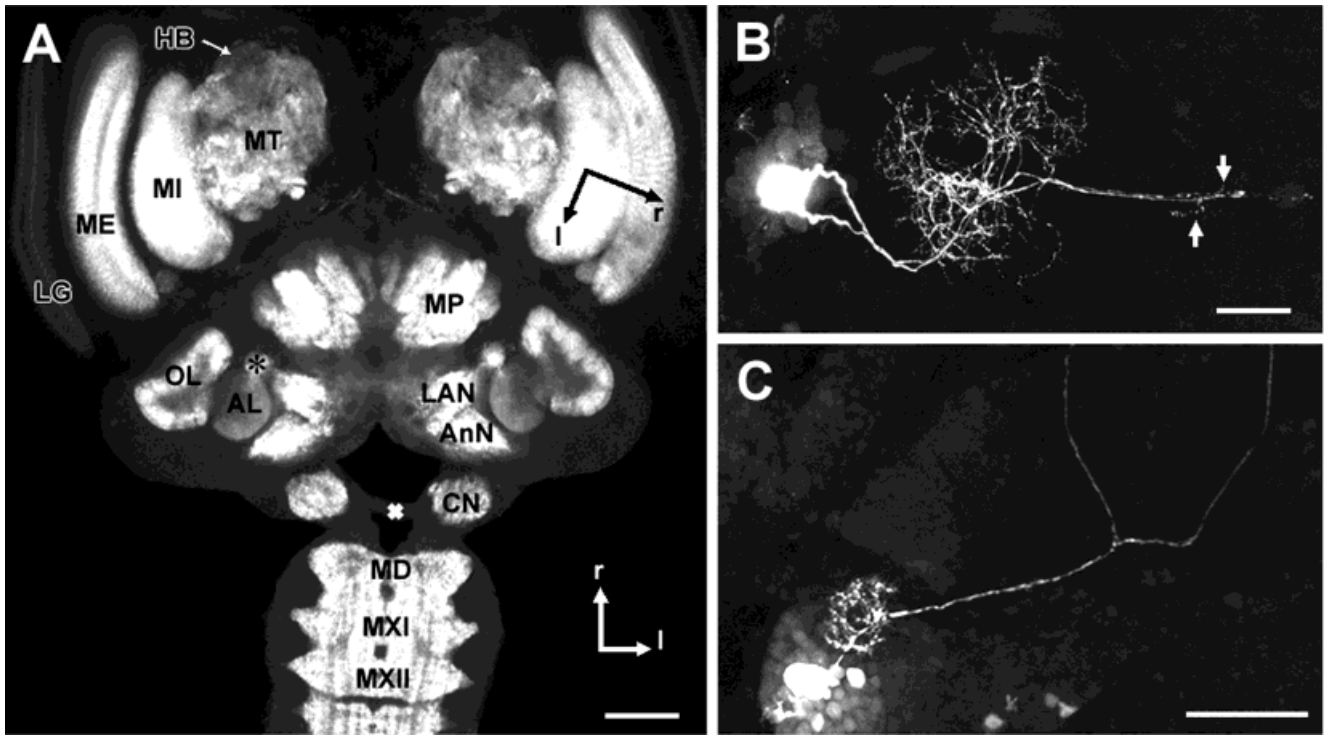


Fig. 1. **A:** Montage of confocal images of the brain and ventral nerve cord of an embryonic lobster (E85%) stained with an antibody against *Drosophila* synapsin. AL, accessory lobe; AnN, antenna II neuropil; CN, commissural neuropil; HB, hemiellipsoid body; l, lateral; LAN, lateral antenna I neuropil; LG, lamina ganglionaris; MD, mandibular neuromere; ME, medulla externa; MI, medulla interna; MP, median protocerebrum; MT, medulla terminalis; MXI, first maxillary neuromere; MXII, second maxillary neuromere; OL, olfactory lobe; r, rostral. The olfactory globular tract neuropil is identified by the black asterisk and the commissural nerve by the white cross. The orientation of the lateral protocerebral and optic neuropils differs from that of the rest of the central nervous system as the eyestalks are rotated relative to the axis of the body. **B,C:** Stacked confocal images illustrating the morphologies of olfactory lobe projection neurons in the deutocerebra of embryonic lobsters. The neurons were filled intracellularly with Lucifer yellow. Previous studies of olfactory projection neurons in embryonic lobsters have shown that each neuron extensively innervates either the ipsilateral olfactory lobe or the ipsilateral accessory lobe. B: Two olfactory lobe projection neurons labeled in an embryo at E38%. The axons of both neurons branch in the olfactory globular tract neuropil (arrows) before projecting to the lateral protocerebrum. C: Projection neuron innervating the olfactory lobe of an embryo at E28%. The axon of the neuron bifurcates in the center of the brain before projecting bilaterally to the lateral protocerebrum. Scale bars = 100 μm in A, 50 μm in B,C.

deutocerebrum (Hanström, 1925, 1931, 1947; Blaustein et al., 1988; Sullivan and Beltz, 2001).

In decapod crustaceans, olfactory stimuli are perceived by receptor neurons innervating specialized sensilla, known as aesthetascs, located along the lateral flagellum of the first antennae (antennae I). The axons of these olfactory receptor neurons project ipsilaterally to the deutocerebrum where they terminate in the glomerular neuropil of the paired olfactory lobes. The olfactory lobe glomeruli are sites of synaptic contact between the receptor neurons, projection neurons and several classes of local interneurons. In lobsters and crayfish, the latter two classes of neuron also innervate an additional glomerular neuropil, the accessory lobe, which lies adjacent to the olfactory lobe (Fig. 1; Arbas et al., 1988; Mellon and Alones, 1994; Sandeman and Sandeman, 1994; Wachowiak et al., 1996; Sullivan et al., 2000). In contrast to the olfactory lobe, which receives only primary olfactory inputs, the accessory lobe receives higher-order olfactory, visual, and mechanosensory inputs (Sandeman et al., 1995).

The main output pathways from both the olfactory and the accessory lobes are provided by the axons of a large population of projection neurons whose somata lie adjacent to the lobes in a densely packed cluster (Mellon et al., 1992a,b; Wachowiak and Ache, 1994; Wachowiak et al., 1996; Mellon, 2000; Sullivan and Beltz, 2001). On leaving the lobes, the axons of the projection neurons form a large tract, known as the olfactory globular tract (OGT; Hanström, 1925), which bifurcates in the center of the brain before projecting bilaterally to the lateral protocerebrum, via the protocerebral tracts (Fig 1; Hanström, 1925, 1931, 1947; Tsvileneva and Titova, 1985; Blaustein et al., 1988; Sandeman et al., 1992). Recent anatomic studies of the brains of lobsters (*Homarus americanus*) and crayfish (*Procambarus clarkii* and *Orconectes rusticus*) have shown that the olfactory and accessory lobes have separate projection neuron pathways that target different regions of the lateral protocerebrum (Sullivan and Beltz, 2001). In all three species, projection neurons innervating the accessory lobe (accessory lobe projection neurons) terminate bilaterally within the hemiellipsoid bodies. In contrast, projection neurons innervating the olfactory lobe (olfactory lobe projection neurons) primarily target neuropil regions of the medulla terminalis adjacent to the hemiellipsoid body. The olfactory lobe projection neuron tract of *H.*

americanus also has an additional branch that projects into the hemiellipsoid body (Sullivan and Beltz, 2001).

In addition to the olfactory and accessory lobe projection neurons, a variety of other neurons that respond to chemical stimulation of the antennae I also possess axons that ascend the protocerebral tract, although not within the OGT. These neurons are functionally diverse with many also responding to visual and/or tactile inputs (Tautz et al., 1986; Tautz, 1987; Derby and Blaustein, 1988; Schmidt and Ache, 1996a). Morphologic analyses indicate that these neurons do not have dendritic branches within the olfactory and accessory lobes but collectively innervate several other regions of the brain, including the median protocerebrum, medial antenna I neuropil, lateral antenna I neuropil, and antenna II neuropil (Derby and Blaustein, 1988; Schmidt and Ache, 1996a). It is not known, however, which of the lateral protocerebral or optic neuropils these neurons innervate on leaving the protocerebral tract.

Studies of the development of the brain of *H. americanus* have shown that the neuropils in the deutocerebrum arise at quite different stages of embryogenesis. The anlagen of the olfactory lobes are first apparent in histologic sections during the initial stages of embryonic development (Helluy et al., 1993, 1995). Similarly, the medulla terminalis, the principal target of the olfactory lobe projection neuron pathway, also appears early in embryogenesis (Harzsch et al., 1999a). In contrast, the anlagen of the accessory lobes do not emerge until mid-embryonic development (Helluy et al., 1993, 1995). The emergence and development of the hemiellipsoid bodies, which in adult lobsters receive projections from both the olfactory and accessory lobe projection neuron pathways, however, have yet to be examined.

In the present study, we used histologic and immunocytochemical techniques to examine the appearance and maturation of both the medulla terminalis and the hemiellipsoid body of *H. americanus*. In addition, we used focal dye injections into the olfactory and accessory lobes at successive stages of embryogenesis to follow the maturation of their projection neuron pathways to the lateral protocerebrum. To further our understanding of the functional roles of the lateral protocerebral neuropils, we also examined their anatomic connections with the antenna II and lateral antenna I neuropils and with other regions of the central nervous system.

These studies demonstrate that the medulla terminalis and hemiellipsoid body differ markedly in both their ontogeny and connectivity. Although the medulla terminalis arises during the initial stages of embryogenesis and is innervated by the olfactory lobe projection neuron pathway, the hemiellipsoid body emerges during mid-embryonic development and is innervated by the projection neuron pathway from the accessory lobe. The accessory and olfactory lobe projection neuron pathways, therefore, innervate separate, nonoverlapping regions of the lateral protocerebrum during the embryonic development of the lobster. Dye injections into the olfactory lobes of embryonic and larval lobsters also resulted in the labeling of a previously unidentified interneuronal pathway connecting the olfactory lobe and the mandibular and maxillary neuromeres of the ventral nerve cord. Examination of the connectivity of the antenna II and lateral antenna I neuropils showed that the ascending pathways from these two neuropils innervate the same regions of the medulla terminalis. The regions innervated by these ascending pathways, however, differ from those innervated by the olfactory lobe projection neuron pathway.

MATERIALS AND METHODS

Animals

Embryonic, larval, and juvenile lobsters, *Homarus americanus* (Malacostraca, Decapoda, Homarida), were obtained from the New England Aquarium (Boston, MA) and maintained at 14°C in aquaria with circulating artificial sea water and a light/dark cycle of 12:12 hours. The developmental stages of embryos were determined by using the percentage staging system of Helluy and Beltz (1991) in which egg extrusion is defined as E0% and hatching as E100%. *H. americanus* hatches as a prelarva, which moults within hours to the first of three pelagic larval stages (Herrick, 1895; Helluy and Beltz, 1991; Talbot and Helluy, 1995). The third larval stage (Stage III) metamorphoses into the first postlarval stage (Stage IV), which settles to the benthos and establishes the lifestyle typical of juvenile and adult lobsters (Herrick, 1895; Phillips and Sastry, 1980; Charmantier, 1987; Charmantier et al., 1991). Subsequently, lobsters continue to moult and grow throughout their lifetimes (Wolff, 1978; Cooper and Uzmann, 1980).

Immunocytochemistry

To examine the development of the neuropils of the deutocerebrum and lateral protocerebrum, embryonic and larval brains were labeled immunocytochemically by using an antibody against *Drosophila* synapsin. The *Drosophila* synapsin antibody (SYNORF1) intensely labels neuropil regions in decapod crustaceans and has been used in several studies of the anatomy and development of crustacean central nervous systems (Harzsch et al., 1997, 1998, Harzsch et al., 1999a,b; Benton and Beltz, 2001; Sullivan and Beltz, 2001).

Brains and optic ganglia were removed from animals immersed in cold lobster saline (mmol l^{-1}: 462 NaCl, 16 KCl, 34.4 $CaCl_2$, 17.1 $MgCl_2$, 11.1 glucose, 10 HEPES, pH 7.4) and fixed for 24 hours in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C. Subsequently, the preparations were rinsed for 4 hours in 0.1 M phosphate buffer containing 0.3% Triton X-100 (PBTx) then incubated in the anti-synapsin SYNORF1 antibody (1:50 in PBTx; Klagges et al., 1996; antibody provided by E. Buchner, Universität Würzburg, Germany) for 24 hours at 4°C. After incubation in the primary antibody, tissues were rinsed over several hours in PBTx and then incubated overnight at 4°C in an Alexa 488–conjugated goat anti-mouse antibody (Molecular Probes; Eugene, OR) diluted 1:50 in PBTx. Subsequently, the preparations were rinsed for 3 hours in PB, mounted in Gelmount (Biomedia; Foster City, CA), and viewed by using a laser-scanning confocal microscope.

Histology

Toluidine blue-stained serial sections (3–7 μm) of plastic (JB-4)-embedded embryonic brains were also examined to follow the development of the lateral protocerebral neuropils. This material was prepared by Helluy et al. (1995) and was made available for the present study.

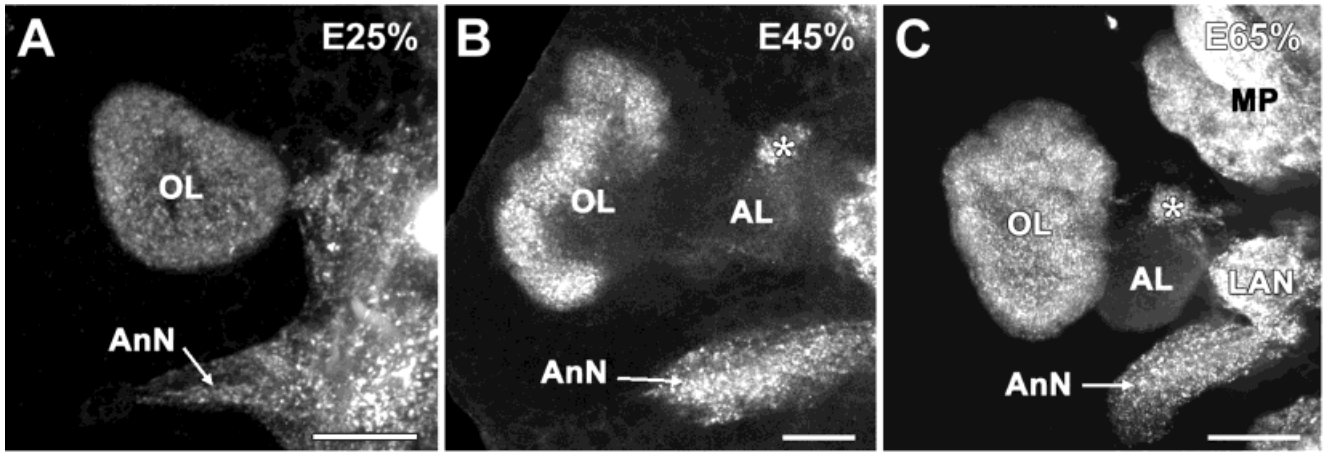


Fig. 2. Confocal images of the brain of *Homarus americanus* at E25% (**A**), E45% (**B**), and E65% (**C**), showing synapsin immunoreactivity in the developing olfactory and accessory lobes. Whole-mount preparations, anterior at top. AL, accessory lobe; AnN, antenna II neuropil; LAN, lateral antenna I neuropil; MP, median protocerebrum; OL, olfactory lobe. B,C: The olfactory globular tract neuropil is identified by the white asterisks. Scale bars = 25 μm in A,B, 50 μm in C.

Labeling of projection neuron pathways from the olfactory lobe, accessory lobe, lateral antenna I neuropil, and antenna II neuropil

The brain and ventral nerve cord of embryonic and larval lobsters were dissected free, placed in a well of cold saline on a poly-L-lysine (0.01% in ddH_2O; Sigma) -coated slide, and then viewed by using a Nikon compound microscope equipped with Nomarski optics. The morphology of the projection neuron pathways to the lateral protocerebrum were examined by focal injections of the lipophilic tracers DiI, DiA, or DiD (Molecular Probes) into the olfactory lobe, accessory lobe, lateral antenna I neuropil, and antenna II neuropil. Small crystals of DiI were deposited within the brain regions by iontophoretic injection of saturated solutions of DiI in 100% ethanol by using depolarizing current (85 nA for ~1 hour; method modified from Whitington et al., 1993). Saturated solutions of DiA or DiD in 100% ethanol were pressure injected into the neuropils, as these dyes could not be successfully iontophoresed.

After the dye injections, brains were fixed in 4% paraformaldehyde in 0.1 M PB (pH 7.4) at room temperature and left in the dark for 24–72 hours to allow the dyes to travel along the lengths of the projection neuron pathways. Subsequently, the brains were cleared and mounted in Hypaque meglumine (Nycomed, Inc.; Princeton, NJ) and viewed by using laser-scanning confocal microscopy. Although the size of the injection site varied between preparations, the staining patterns did not differ between animals or with the use of different dyes.

Labeling of neurons innervating the lateral protocerebral neuropils

The connectivity of the hemiellipsoid body and the neuropils of the medulla terminalis that are innervated by the olfactory lobe projection neuron pathway were examined by iontophoretic injections of DiI into these regions of the lateral protocerebrum. The hemiellipsoid bodies of older embryos (E80%–E100%) can be visualized with the use of Nomarski optics. Therefore, DiI-filled electrodes, could be guided into the hemiellipsoid bodies of these embryos visually. To accurately locate the neuropil regions innervated by the olfactory lobe projection neuron tract, this tract was first labeled by pressure injections of DiA into the olfactory lobe. The brains were then fixed at room temperature in 4% paraformaldehyde in 0.1 M PB (pH 7.4) and left in the dark for 48 hours. Subsequently, the preparations were rinsed in 0.1 M PB for 2 hours then placed in a well of 0.1 M PB on a poly-L-lysine (0.01% in ddH_2O) -coated slide. The terminal arbors of the projection neuron pathway were visualized by using a Nikon compound fluorescence microscope equipped with a FITC filter. A small crystal of DiI was then deposited iontophoretically amongst the DiA-labeled arbors to visualize other neurons branching in these neuropil regions. Preparations were then processed and viewed as detailed above.

Intracellular staining of individual neurons

The brain and ventral nerve cord of embryonic lobsters were dissected free and placed in a well of cold lobster saline on a poly-L-lysine (0.01% in ddH_2O; Sigma) -coated slide. Preparations were then viewed by using a fixed-stage Nikon compound microscope equipped with Nomarski optics. The morphology of individual projection neurons and lateral protocerebral interneurons was examined by intracellular staining of the cells with Lucifer yellow CH (Sigma). Neurons were penetrated in the soma and stained by iontophoretic injection of Lucifer yellow, by using hyperpolarizing current pulses of up to 6 nA (500 msec in duration, 1 Hz in frequency), for 4–7 minutes. After the injection of Lucifer Yellow, preparations were fixed in 4% paraformaldehyde for 4 hours, dehydrated in an ethanol series (8 minutes in each of 50, 70, 80, 90, 95, 100%), and cleared in methyl salicylate. Subsequently, the brains were mounted in DPX (Fluka; Buchs, Switzerland) and viewed by using a laser-scanning confocal microscope.

Confocal microscopy and image processing

Specimens were viewed with a Leica TCS SP laser-scanning confocal microscope equipped with argon, krypton, and helium neon lasers. Serial optical sections were

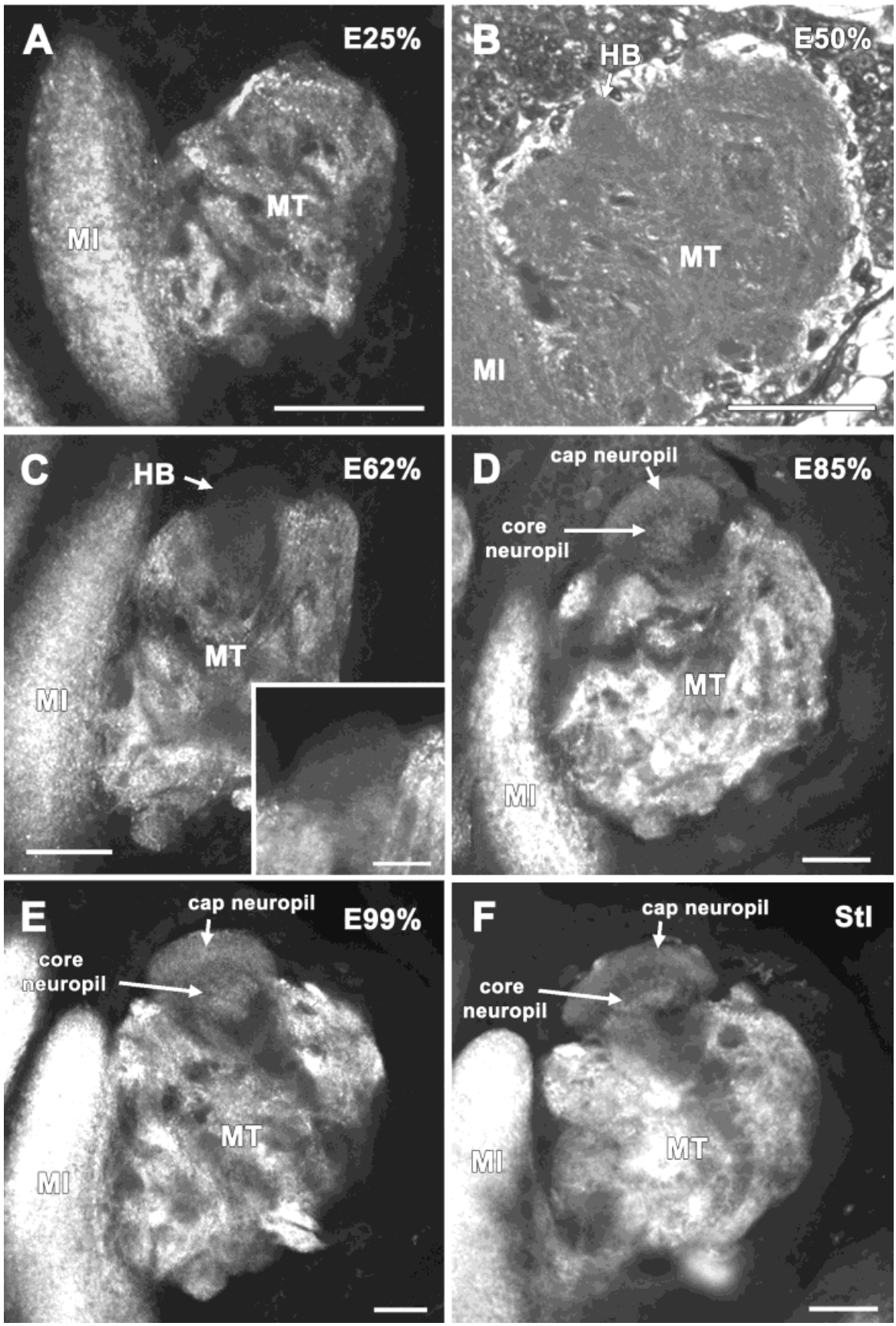


Fig. 3. Development of the lateral protocerebrum. HB, hemiellipsoid body; MI, medulla interna; MT, medulla terminalis; StI, first larval stage. **A:** Confocal image of the MT and MI of an embryo at E25% labeled with an antibody against *Drosophila* synapsin. **B:** Photomicrograph of a toluidine blue-stained 5 μm horizontal section through the lateral protocerebrum of an embryo at E50%. **C–F:** Synapsin immunoreactivity in the developing lateral protocerebrum at E62% (C), E85% (D), E99% (E), and the first larval stage (F). The inset in C is a higher magnification image of the hemiellipsoid body at E62%. Scale bars = 50 μm in A–F, 25 μm in inset in C.

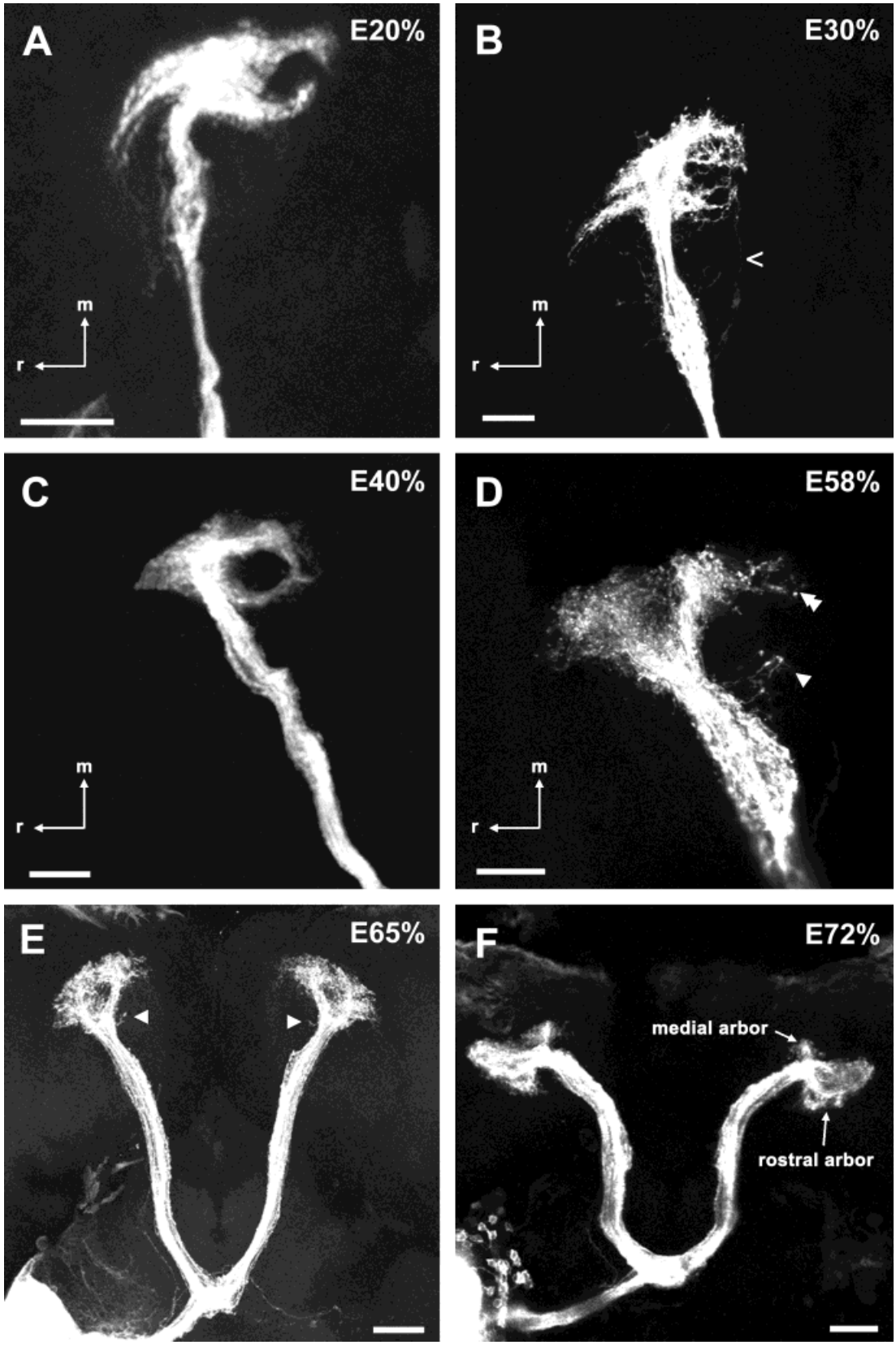


Fig. 4. Development of the projection neuron pathways from the olfactory lobe of *Homarus americanus*. The projection neuron pathway from the olfactory lobe projects bilaterally to neuropil regions within the medulla terminalis. The bilateral arbors of the tract are symmetrical throughout embryogenesis. **A–D:** Stacked confocal images of the projection neuron pathway to the ipsilateral medulla terminalis labeled by dye injections into the olfactory lobe at E20% (A), E30% (B), E40% (C), and E58% (D). **E,F:** Stacked confocal images of the bilateral projection neuron pathways labeled by dye injections into the olfactory lobe at E65% (E) and E72% (F). The olfactory lobe projection neuron pathway initially has three main arbors within the medulla terminalis: one projecting rostrally and two projecting caudally. The two caudally directed branches are gradually withdrawn during mid-embryonic development. The more medial of the two caudally directed branches is identified by a double arrowhead in D and the more lateral branch by a single arrowhead in D and E. The open arrowhead in B identifies a fine tract of fibers projecting to the ipsilateral medulla terminalis that are also labeled by the dye injections into the olfactory lobe. Whole-mount preparations. m, medial; r, rostral. Scale bars = 25 μm in A–D, 50 μm in E,F.

taken at intervals of 1–1.5 μm and were saved as both three-dimensional stacks and as two-dimensional projections. Images were processed to adjust brightness and contrast by using Paint Shop Pro 4.12 (JASC, Inc.) and Adobe Photoshop 5.0 (Adobe Systems).

RESULTS

Development of neuropils in the olfactory pathway

Deutocerebral neuropils. The primordia of the olfactory lobes are first apparent in the developing brain of *H. americanus* at ~E10% (Helluy et al., 1993, 1995). During their initial development, the olfactory lobes appear as rounded regions of synapsin-immunoreactive neuropil (Fig. 2A). The glomerular structure characteristic of the olfactory lobes of adult lobsters is first distinguishable by E45% (Helluy et al., 1993). By contrast, the accessory lobes of *H. americanus* do not emerge until ~E45% (Fig. 2B; Helluy et al., 1993, 1995) and are only weakly synapsin-immunoreactive (Fig. 2B,C). Synapsin-immunoreactive glomeruli are first apparent within the developing accessory lobes during the first larval stage (J. Benton, personal communication). These glomeruli become more clearly defined during the subsequent larval stages (Helluy et al., 1995).

Lateral protocerebral neuropils. In most decapod crustaceans the lateral protocerebrum and the optic neuropils are located within the eyestalks and are, consequently, referred to collectively as the eyestalk neuropils. The medulla terminalis and the medulla interna are the first of the five eyestalk neuropils to form during embryogenesis in *H. americanus* (Harzsch et al., 1999a). As in other decapod crustaceans, the medulla terminalis and medulla interna of *H. americanus* arise from a common anlage but separate during subsequent development (Elofsson, 1969; Harzsch et al., 1997, 1999a). The medulla externa is the next of the eyestalk neuropils to form and is followed, thereafter, by the lamina ganglionaris (Harzsch et al., 1999a).

By E20%, the earliest stage of development examined in the present study, the medulla terminalis, medulla interna, medulla externa, and lamina ganglionaris are all apparent in synapsin-labeled preparations of the brain of *H. americanus*. The developing medulla terminalis is composed of several contiguous neuropil regions (Fig. 3), as it is in adult lobsters (Sullivan and Beltz, 2001). The hemiellipsoid body is first apparent in toluidine-blue stained brains at ~E50% as a semicircular region of neuropil on the medial margin of the medulla terminalis (Fig. 3B). The hemiellipsoid body, therefore, emerges at a slightly later stage of development than the accessory lobe. The hemiellipsoid body is first distinguishable in synapsin-labeled preparations at E55–60% but, like the accessory lobe, is only weakly synapsin immunoreactive (Fig. 3C). During subsequent development the hemiellipsoid body becomes progressively larger and more ovoid in shape (Fig. 3D–F). The cap and core neuropils characteristic of the hemiellipsoid bodies of adult lobsters are first clearly distinguishable from one another at ~E80% (Fig. 3D). The developing core neuropil initially lies within the medulla terminalis but gradually moves medially until it is located adjacent to the medial edge of the medulla terminalis (Fig. 3D–F).

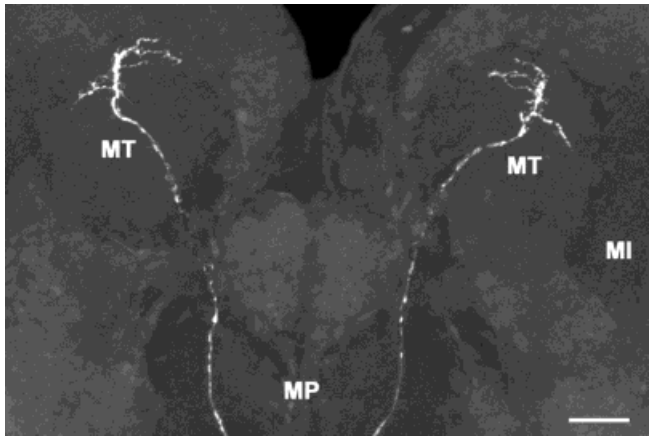


Fig. 5. Stacked confocal image of a Lucifer yellow-stained olfactory lobe projection neuron in an embryo at E30%. Image shows the bilateral projections of the neuron within the medulla terminalis (MT). Dorsal view of whole-mount preparation. MI, medulla interna; MP, median protocerebrum. Scale bar = 25μm.

Development of the projection neuron pathways from the deutocerebral lobes

Olfactory lobe projection neuron pathway. Focal dye injections into the developing olfactory lobes of embryonic lobsters showed that the projection neuron pathway from this lobe bifurcates in the center of the brain before projecting bilaterally to the medulla terminalis (Fig. 4). The bilateral arbors of the projection neuron tract were found to be symmetrical throughout embryogenesis. Figure 4A shows the terminal arbors of the projection neuron pathway from the ipsilateral olfactory lobe at E20%. The projection neuron tract courses to the medial half of the medulla terminalis where it divides into three main branches. Two of these branches arc outward caudally toward one another, whereas the third branch projects rostrally. This pattern of arborization within the medulla terminalis was also observed at E30% (Fig. 4B) and E40% (Fig. 4C). Intracellular dye fills of individual projection neurons innervating the olfactory lobe between E20% and E40% showed that the bilateral projections of these neurons are symmetrical and have dendrites within two or more of the three main branches in the medulla terminalis of the olfactory lobe projection neuron tract (Fig. 5).

The morphology of the terminal arbors of the olfactory lobe projection neuron tract changes markedly during mid-embryonic development (Fig. 4). Between E50% and E60% the two caudal branches of the projection neuron tract are gradually withdrawn (Fig. 4D,E). Concomitant with the loss of these branches, the rostrally directed branch of the projection neuron tract becomes more expansive and an additional, medially directed branch arises in the region between the rostral and caudal branches. By E60% the projection neuron tract labeled by dye injections into the olfactory lobe has only two major arbors within the medulla terminalis (Fig. 4E,F). These arbors arise in the medial half of the medulla terminalis, after a bifurcation in the projection neuron tract, with one branch projecting medially and the other rostrally. This basic pattern of arborization is maintained throughout the subsequent stages of embryogenesis. However, the rostral

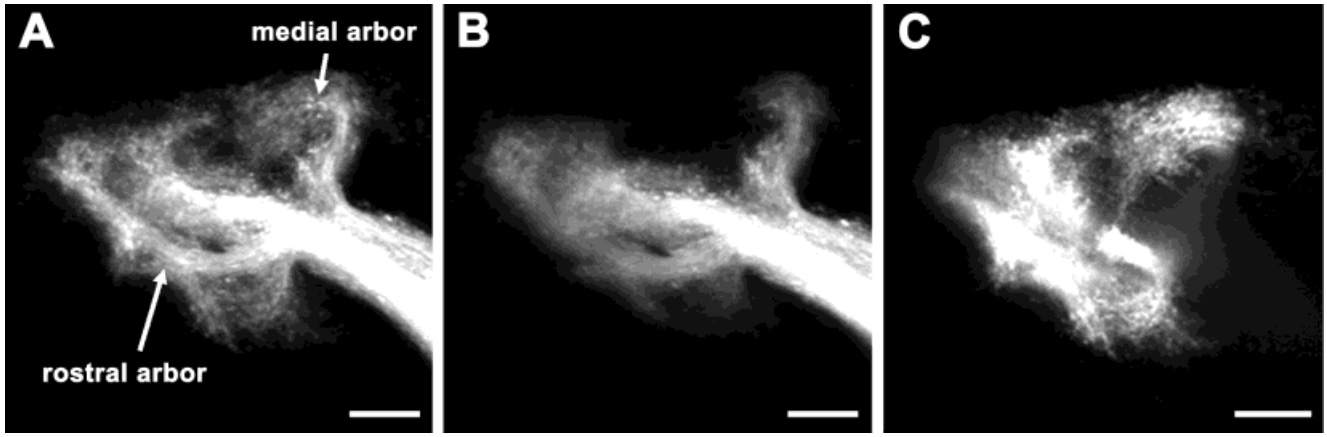


Fig. 6. Stacked confocal images of the terminal arbors of the olfactory lobe projection neuron pathway in an embryo at E80%. The projection neurons were labeled by injecting DiD into the contralateral olfactory lobe. The entire stack of confocal images is shown in **A**, whereas the dorsal half of the terminal arbors of the projection neuron pathway are shown in **B** and the ventral half in **C**. Scale bars = 25 μm in A–C.

arbors of the projection neuron pathway become increasingly larger than the medial arbors (Fig. 4E,F).

Although the rostral and medial arbors project away from one another at the bifurcation point of the projection neuron tract (Fig. 6A,B), they both then course ventrally and turn toward one another (Fig. 6A,C). The terminal arbors of the two branches meet each other in ventral neuropil regions of the medulla terminalis where they overlap slightly (Fig. 6C).

In addition to the projection neuron pathways, dye injections into the olfactory lobes of embryonic lobsters also labeled two other neuronal populations. The first group of neurons possessed axons that formed a fine tract that branched in the median protocerebrum before projecting to the ipsilateral medulla terminalis (Fig. 7). Within the medulla terminalis the labeled tract possessed two main branches that arborized adjacent to the caudal margins of the terminal arbors of the olfactory lobe projection neuron tract. The locations of the cell bodies of these neurons could not be determined but were not located in either the eyestalk or the ventral nerve cord.

The second group of neurons labeled by dye injections into the olfactory lobe was a population of eight neurons whose cell bodies were located in the first two neuromeres of the ventral nerve cord (Fig. 8A–D). Labeling of these cells was observed throughout the period of embryonic development examined in this study (E20%–E100%) and in each of the four larval stages. The cell bodies of the eight neurons occurred as two bilateral pairs. The first two pairs of cell bodies were located anterolaterally in the mandibular neuromere, whereas the second two pairs occurred posterolaterally in the first maxillary neuromere. The axon of each neuron bifurcated within the neuropil region ipsilateral to its cell body. One axonal branch then projected rostrally toward the brain, whereas the other crossed the midline to the contralateral neuropil where it coursed rostrally and fasciculated with the ipsilateral branches of the opposite pair of neurons. The axons of the four neurons from the first maxillary neuropil fasciculated with those of the neurons in the mandibular neuromere forming bilateral tracts, which projected to the olfactory lobes (Fig. 8A–C). The projections of these neurons within

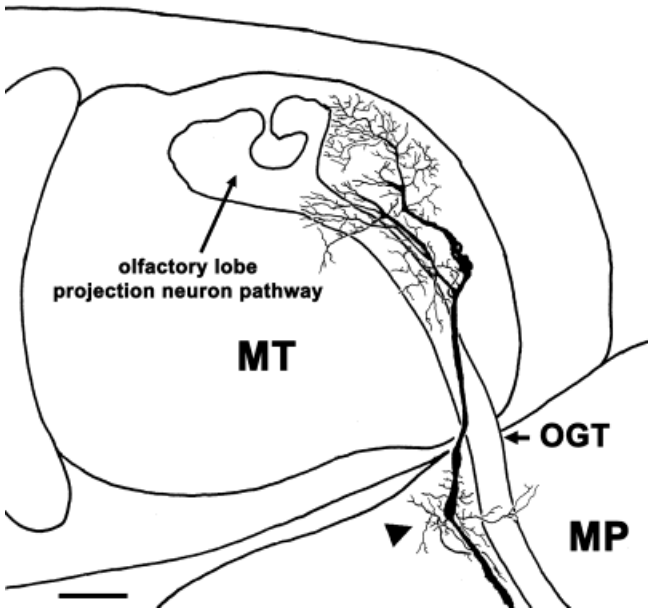


Fig. 7. In addition to labeling the axons of the olfactory lobe projection neurons, dye injections into the developing olfactory lobe also resulted in the labeling of a fine tract of fibers projecting to the ipsilateral medulla terminalis. These neurons possess dendritic branches within the median protocerebrum (arrowhead) and within regions of the medulla terminalis caudal to the terminal arbors of the olfactory lobe projection neuron pathway. The drawing is a reconstruction from a series of confocal images from an embryo at E80%. OGT, olfactory globular tract; MP, median protocerebrum, MT, medulla terminalis. Scale bar = 50 μm.

the olfactory lobes were examined in more detail by injecting DiA into the left half of the mandibular neuromere. These experiments demonstrated that the neurons arborize extensively within both the ipsilateral and contralateral olfactory lobes (Fig. 8E,F). In addition to these four pairs of neurons, a pair of neurons with cell bodies on the midline of the first maxillary neuromere was also

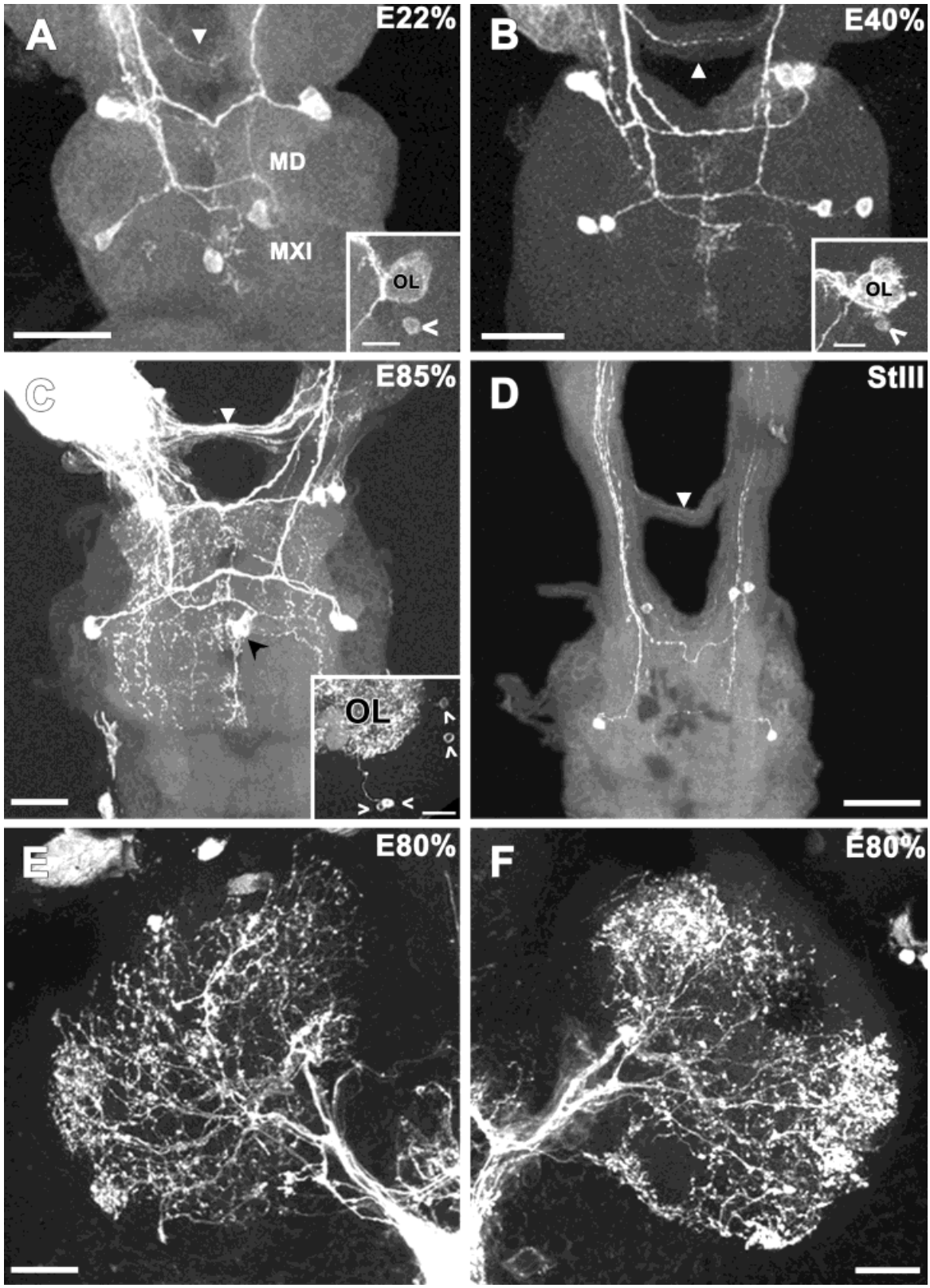


Fig. 8. The olfactory lobe of embryonic lobsters is innervated by a group of neurons whose somata lie within the first two neuromeres of the ventral nerve cord. OL, olfactory lobe; MD, mandibular neuromere; MXI, first maxillary neuromere; StIII, third larval stage. **A–D:** Stacked confocal images of neurons labeled by dye injections into the left olfactory lobe of embryos at E22% (A), E40% (B), E85% (C), and the third larval stage (D). The white arrowheads in A–C show the location of the commissural nerve, whereas the black arrowhead in C shows the cell bodies of a pair of neurons that were only labeled by dye injections in the olfactory lobes of older embryos (E75%–E100%). The insets in A–C show details of the olfactory lobe contralateral to that into which the dye was injected. Labeled projection neuron cell bodies (open arrowheads) occur next to the contralateral olfactory lobe, indicating that some projection neurons in embryonic lobsters branch within both olfactory lobes. The contralateral olfactory lobe is also innervated by the neurons ascending from the ventral nerve cord. **E,F:** Stacked confocal images of preparations in which DiA was injected into the left half of the mandibular neuromere of embryos at E80%. The projections of the labeled neurons to the ipsilateral olfactory lobe are shown in E, whereas those to the contralateral olfactory lobe are shown in F. Scale bars = 50 μm in A–C, 100 μm in D, 25 μm in E,F, 25 μm in insets in A–C.

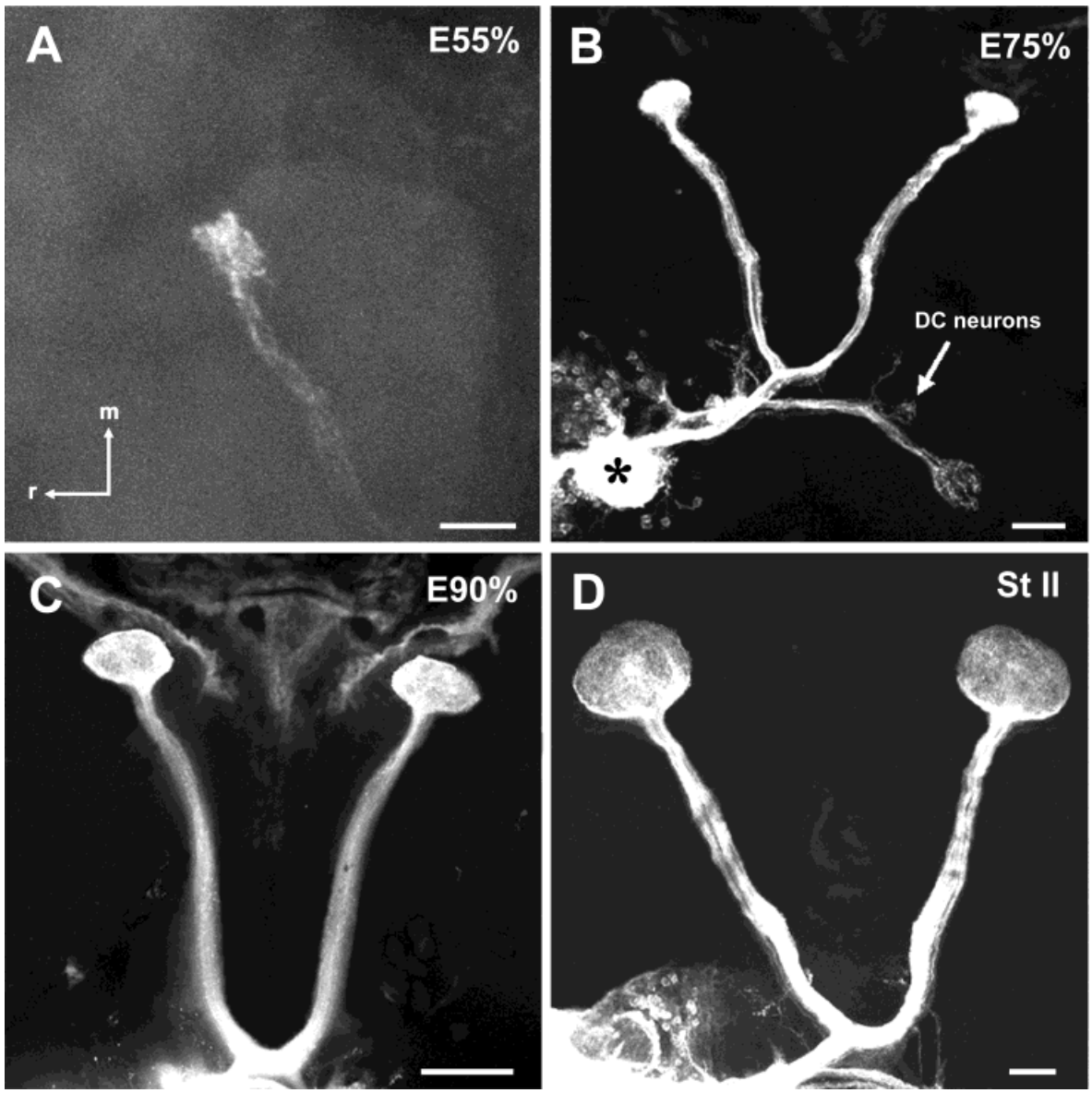


Fig. 9. Stacked confocal images of the projection neuron pathways from the developing accessory lobe. The accessory lobe projection neuron pathway projects bilaterally to the hemiellipsoid body. The bilateral projections are symmetrical. **A:** Projection neuron pathway to the ipsilateral hemiellipsoid body of an embryo at E55%. m, medial; r, rostral. **B–D:** Bilateral accessory lobe projection neuron pathways in embryos at E75% (B) and E90% (C) and in the second larval stage (StII; D). The black asterisk in B shows the site of the dye injection. In addition to labeling the olfactory projection neurons, dye injections into the accessory lobe also label the axons of deutocerebral commissure (DC) neurons (B). Scale bars = 20 μm in A, 50 μm in B, 100 μm in C, 50 μm in D.

labeled by dye injections into the olfactory lobes of older embryos (~E75%–E100%; Fig. 8C). Labeling of this additional pair of neurons was not observed in any of the larval stages.

Dye injections in the developing olfactory lobe also resulted in the labeling of projection neurons whose somata were located in the contralateral cluster 10 (Fig. 8A–C, insets). These results suggest that a small number of projection neurons in embryonic lobsters may innervate both olfactory lobes.

Accessory lobe projection neuron pathway. By using Nomarski optics, the developing accessory lobe is first apparent at ~E55%. Therefore, this stage of development was the first at which it was possible to inject dye into the lobe. Dye injections into the accessory lobes of embryos at E55% showed that the projection neuron pathway from this lobe terminates bilaterally within the hemiellipsoid body in small, knot-like arbors (Fig. 9A). During subsequent development, the terminal arbors of the projection neuron tract become progressively larger and more hemielliptical (Fig. 9).

Double labeling of the projection neuron tracts from the olfactory and accessory lobes showed that the terminal arbors of the two pathways do not overlap during embryogen-

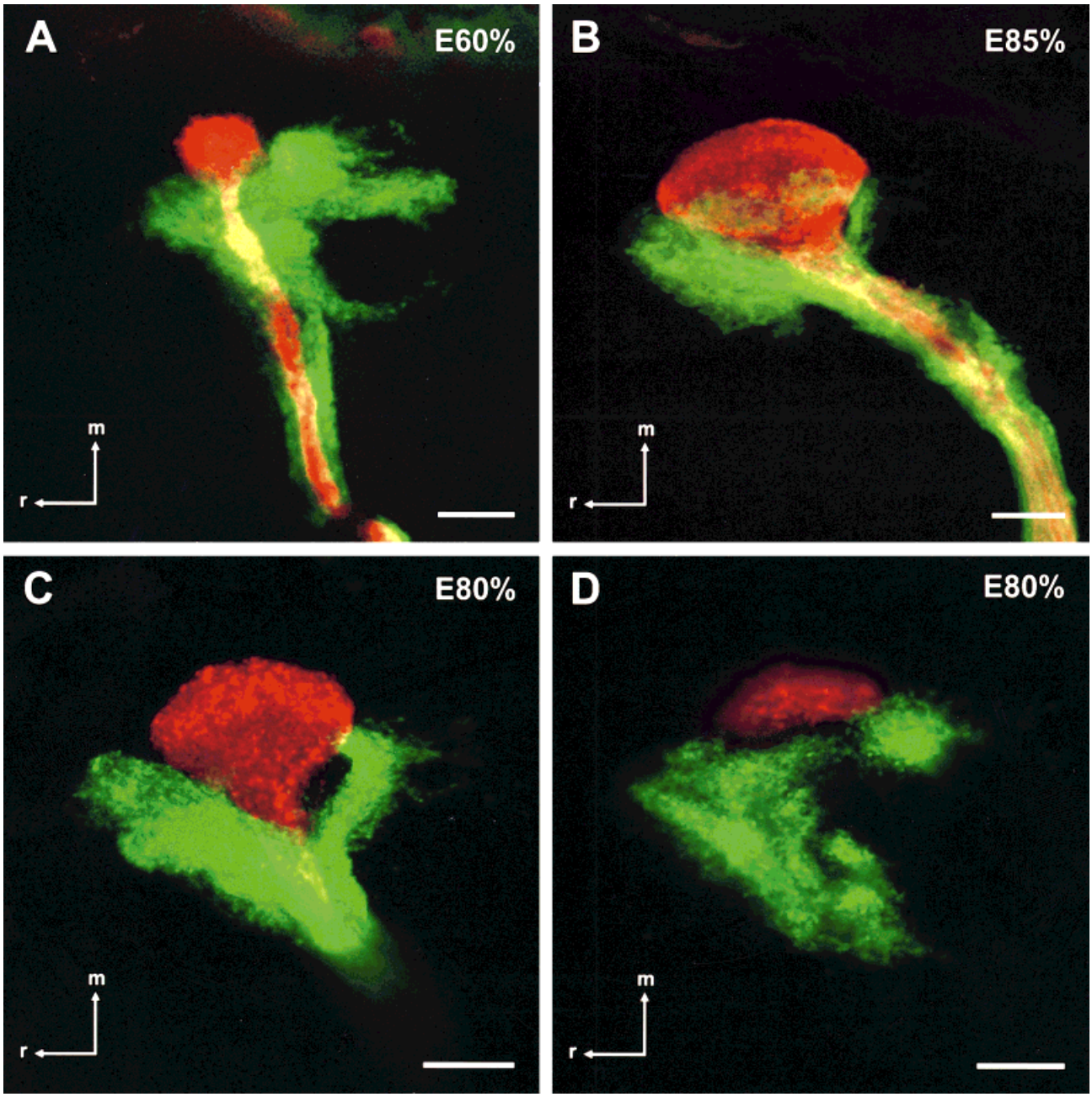


Fig. 10. Stacked confocal images showing the relative positions of the projection neuron pathways from the olfactory lobe (green) and the accessory lobe (red) of embryonic lobsters. The terminal arbors of the projection neuron pathways from the olfactory and accessory lobes do not overlap one another during embryogenesis. **A,B:** Projection neuron pathways to the ipsilateral lateral protocerebra of embryos at E60% (A) and E85% (B). **C,D:** Stacked confocal images of the dorsal (C) and ventral (D) margins of the olfactory and accessory lobe pathways of an embryo at E80%. m, medial; r, rostral. Scale bars = 25 μm in A–D.

esis (Fig. 10). As the hemiellipsoid body first emerges, it is located adjacent to the medial margins of the olfactory lobe projection neuron pathway (Fig. 10A). After the morphogenesis of the olfactory lobe projection neuron pathway between E55% and E60%, the accessory lobe projection neuron tract sits within the region bordered by the medial and rostral branches of the olfactory lobe projection neuron tract (Fig. 10C). The two branches of the olfactory lobe projection neuron pathway then course ventrally around the outside of the arbors of the accessory lobe projection tract. The ventral margins of the arbors of the accessory lobe projection neuron pathway lie adjacent to the region where the medial and rostral arbors of the olfactory lobe projection neuron tract turn and meet one another (Fig. 10D).

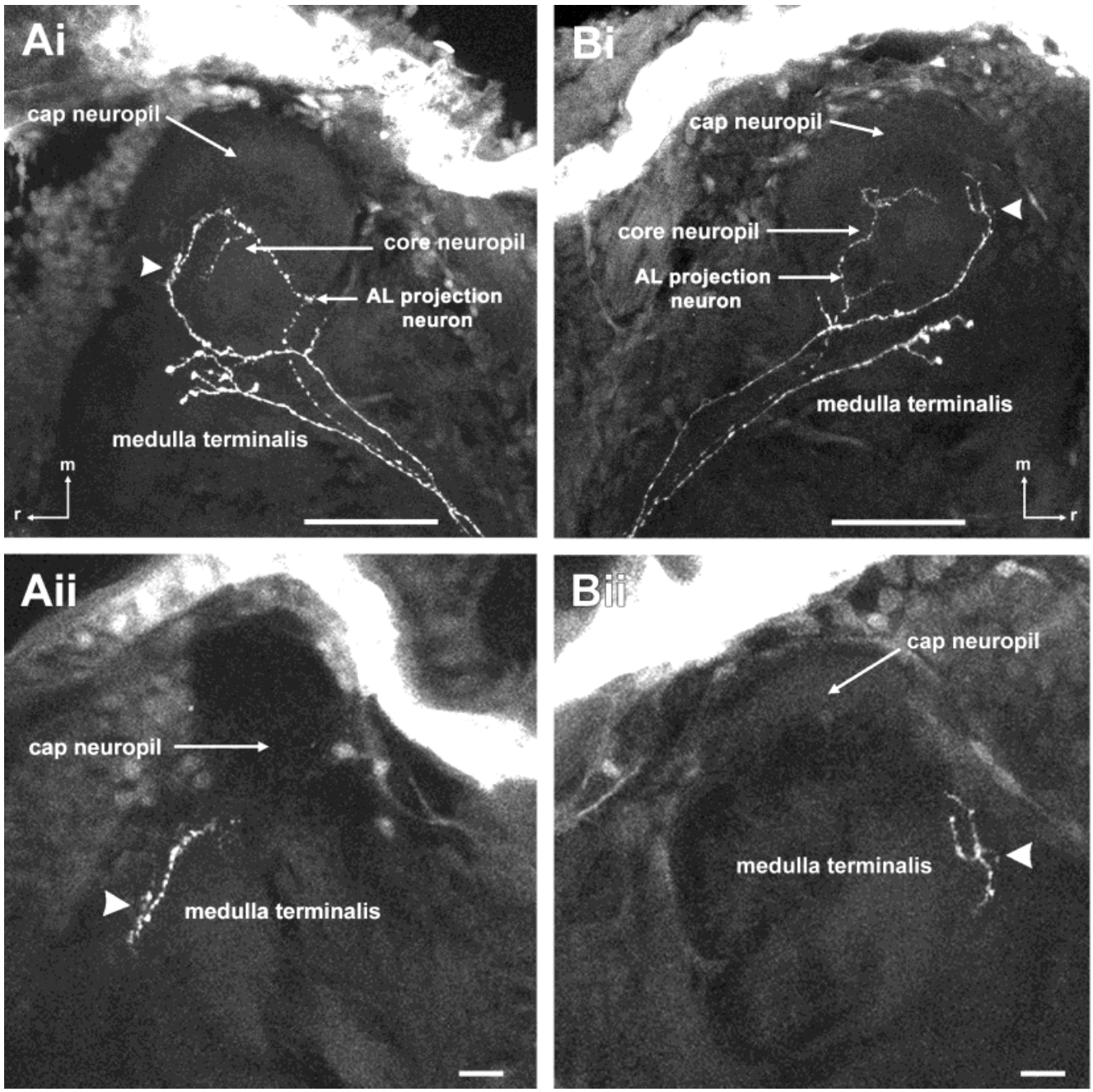


Fig. 11. Stacked confocal images of the bilateral projections within the lateral protocerebrum of three projection neurons stained in the brain of an embryo at E98%. The neurons were stained intracellularly with Lucifer yellow. Two of the projection neurons innervated the olfactory lobe, whereas the third was an accessory lobe (AL) projection neuron. The fluorescent strip of tissue along the top of the preparation is the cuticle of the lobster, which in embryos is autofluorescent. Dorsal views of whole-mount preparations. **Ai,Bi:** Stacked confocal images of the projections of the three neurons within the lateral protocerebrum ipsilateral (Ai) and contralateral (Bi) to their cell bodies. The white arrowheads in Ai and Bi indicate the region of the olfactory lobe projection neuron shown at higher magnification in **Aii** and **Bii**. The axon of this neuron courses around the outside of the hemiellipsoid body and then branches within neuropil regions of the medulla terminalis lateral to the ventral base of the cap neuropil of the hemiellipsoid body. m, medial; r, rostral. Scale bars = 50 μm in Ai,Bi, 10 μm in Aii,Bii.

Intracellular dye labeling of individual projection neurons provided further insights into the organization of the projection neuron pathways from the deutocerebral lobes. Figure 11Ai, Bi shows stacked confocal images of the bilateral arbors of two olfactory lobe projection neurons and a third accessory lobe projection neuron, labeled in an embryo at E98%. The arbors of all three neurons were bilaterally symmetrical. The more lateral of the two olfac-

tory lobe projection neurons arborized within neuropil regions adjacent to the hemiellipsoid body (those innervated by the rostral arbor of the olfactory lobe projection neuron pathway) and possessed dendritic branches that terminated in small, knot-like endings (Fig. 11Ai,Bi), similar to those of olfactory lobe projection neurons in juvenile lobsters (Sullivan and Beltz, 2001). The second olfactory lobe projection neuron possessed branches within both the medial and rostral arbors of the projection neuron tract. The rostrally projecting axon of this neuron arborized finely within neuropil regions adjacent to the other olfactory lobe projection neuron before coursing medially and ventrally around the hemiellipsoid body. The axon then arborized again within neuropil regions of the medulla terminalis medial to the ventral margins of the hemiellipsoid body (Fig. 11Aii,Bii). The third labeled projection neuron was a Type I accessory lobe projection neuron (terminology from Sullivan et al., 2000). These neurons innervate only the outer margins of the accessory lobe. The axons of the labeled neuron projected bilaterally to the core neuropil of the hemiellipsoid body where they branched sparsely (Fig. 11Ai,Bi). We were unable to obtain enough complete projection neuron fills to determine whether there is a consistent difference in the projection patterns of Type I and Type II accessory lobe projection neurons.

Connectivity of the lateral protocerebrum in embryonic lobsters

Connectivity of the hemiellipsoid body. Focal dye injections into the hemiellipsoid bodies of embryos (E80%–E100%) showed that, in addition to being innervated by accessory lobe projection neurons, this neuropil is also innervated by two groups of interneurons whose cell bodies are located adjacent to the medulla terminalis (Fig. 12A). These interneurons connect the hemiellipsoid body to several regions of the brain (Fig. 12A,B). Within the eyestalk neuropils, the labeled interneurons innervated several neuropil regions of the ipsilateral medulla terminalis as well as the caudal margins of the medulla interna (Fig. 12A). Neuronal connections between the hemiellipsoid body and the medulla interna were also observed after dye injections into the hemiellipsoid bodies of adult lobsters (Fig. 12C,D). In both embryonic and adult lobsters, the labeled interneurons that innervated the medulla interna terminated in clusters of fine branches (Fig. 12A,C,D).

In addition to the branches within the eyestalk neuropils, several of the neurons labeled by dye injections into the hemiellipsoid bodies of embryonic lobsters possessed axons that projected to the median protocerebrum (Fig. 12A,B). The axons of these neurons formed a fine tract that ran parallel to the OGT before coursing laterally to innervate lateral regions of the median protocerebrum (Fig. 12B). A small number of axons then coursed further caudally before crossing the midline to innervate both anterior and posterior regions of the median protocerebrum on the contralateral side of the brain (Fig. 12B).

Connectivity of the regions of the medulla terminalis innervated by the olfactory lobe projection neuron pathway. To examine the connectivity of the target neuropils of the olfactory lobe output pathway, the projection neuron tract from the olfactory lobe was first visualized by injecting DiA into the lobe. Small crystals of DiI were then deposited amongst the terminal arbors of the projection neuron tract. These dye injections resulted in the labeling of the other neurons that branch within these regions of the medulla terminalis. Injections were made into the rostral arbors of the projection neuron pathway, the largest of the two main branches of the projection neuron tract, in embryos from E80% to E100%.

Dye injections into the terminal arbors of the olfactory lobe projection neuron pathway showed that the neuropils innervated by this tract also contain the branches of several additional neuronal populations (Fig. 13). The cell bodies of these neurons are located within four distinct clusters. Three of these clusters are situated next to the lateral protocerebral neuropils (Fig. 13A), whereas the fourth is located adjacent to the ipsilateral commissural neuropil (Fig. 13C). The lateral protocerebral interneurons labeled by the dye injections also innervated several neuropil regions of the ipsilateral medulla terminalis (Fig. 13A). Because of the proximity of these neuropils to the site of the dye injections, it was not possible to examine the fine details of the branching patterns of the labeled neurons. Therefore, the anatomy of these lateral protocerebral interneurons were examined by staining individual neurons intracellularly with Lucifer yellow (Fig. 14). Each of the stained lateral protocerebral neuropils innervated several regions of the medulla terminalis in addition to those innervated by the terminal arbors of the olfactory lobe projection neuron pathway. There was considerable variation between neurons, however, in the extent of their dendritic branching (Fig. 14A,B). Intracellular labeling of individual neurons indicated that some of these neurons also branch in the hemiellipsoid body (Fig. 14C).

In addition to their arbors within the lateral protocerebrum, several of the neurons labeled by the DiI injections also possessed axons that projected caudally through the protocerebral tract (Fig. 13). These axons formed two tracts that arborized within the ipsilateral median protocerebrum before turning medially and crossing the midline to innervate comparable neuropils on the contralateral side of the brain (Fig. 13B). An additional neuron possessed an axon that projected to the midline of the brain, caudal to the chiasm of the OGT, where it branched in a characteristic "H" shape (Fig. 13B,C). The two caudal branches of the "H" arose on opposite sides of the midline and projected to the ventral nerve cord by means of the esophageal connectives (Fig. 13C). Within the ventral nerve cord, the two axonal branches ran parallel to one another on either side of the midline. On reaching the second maxillary neuromere, however, both axons turned medially and crossed the midline to the contralateral half of the neuromere where they arborized within restricted neuropil regions (Fig. 13D). The third axonal branch of this neuron arose on the side of the brain contralateral to its cell body and projected rostrally to the anterior median protocerebrum (Fig. 13B). Neither this neuron or the other lateral protocerebral interneurons labeled by the DiI injections innervated the contralateral eyestalk, although this is characteristic of neurons innervating other regions of the medulla terminalis (unpublished observation; Derby and Blaustein, 1988).

The DiI injections also labeled a group of 1–3 neurons whose cell bodies were located adjacent to the ipsilateral commissural neuropil (Fig. 13C). These neurons branched extensively within the commissural neuropil before pro-

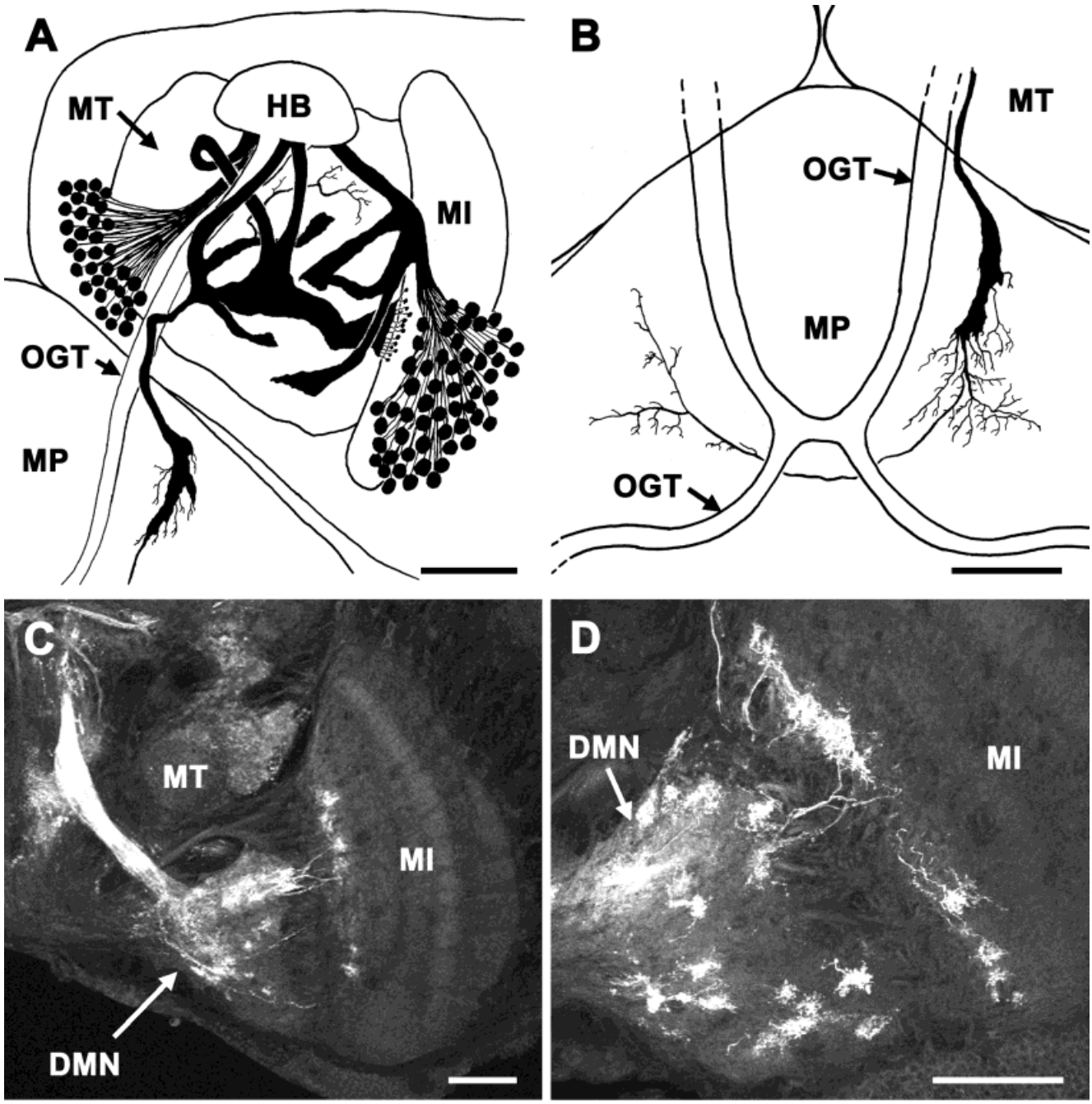


Fig. 12. Connectivity of the hemiellipsoid body of *Homarus americanus*. DMN, diamedullary neuropil; HB, hemiellipsoid body; MI, medulla interna; MP, median protocerebrum; MT, medulla terminalis; OGT, olfactory globular tract. **A,B:** Reconstructions from series of confocal images of the neurons labeled by focal injections of DiI into the hemiellipsoid bodies of embryos at E80%. In addition to labeling the accessory lobe projection neurons, the dye injections also labeled two groups of interneurons whose cell bodies lie adjacent to the medulla terminalis (A). These neurons connect the hemiellipsoid body to several regions of the medulla terminalis as well as the caudal margin of the medulla interna (A). Labeled axons also descend to the median protocerebrum (A,B) where they branch on both sides of the brain (B). **C,D:** Confocal images of 100 μm horizontal sections through the eyestalk neuropils of juvenile lobsters in which focal injections of DiA were made into the cap (C) and core (D) neuropils of the hemiellipsoid body. The labeled neurons branch within the diamedullary neuropil, a region of the medulla terminalis (Blaustein et al., 1988), and the caudal margins of the medulla interna. Scale bars = 50 μm in A, 25 μm in B, 100 μm in C,D.

jecting to the lateral protocerebrum. It was not possible to accurately determine whether these neurons also branched within the medial protocerebrum.

Ascending pathways from the lateral antenna I neuropil and antenna II neuropil. Dye injections into the lateral antenna I neuropil of late-embryonic lobsters

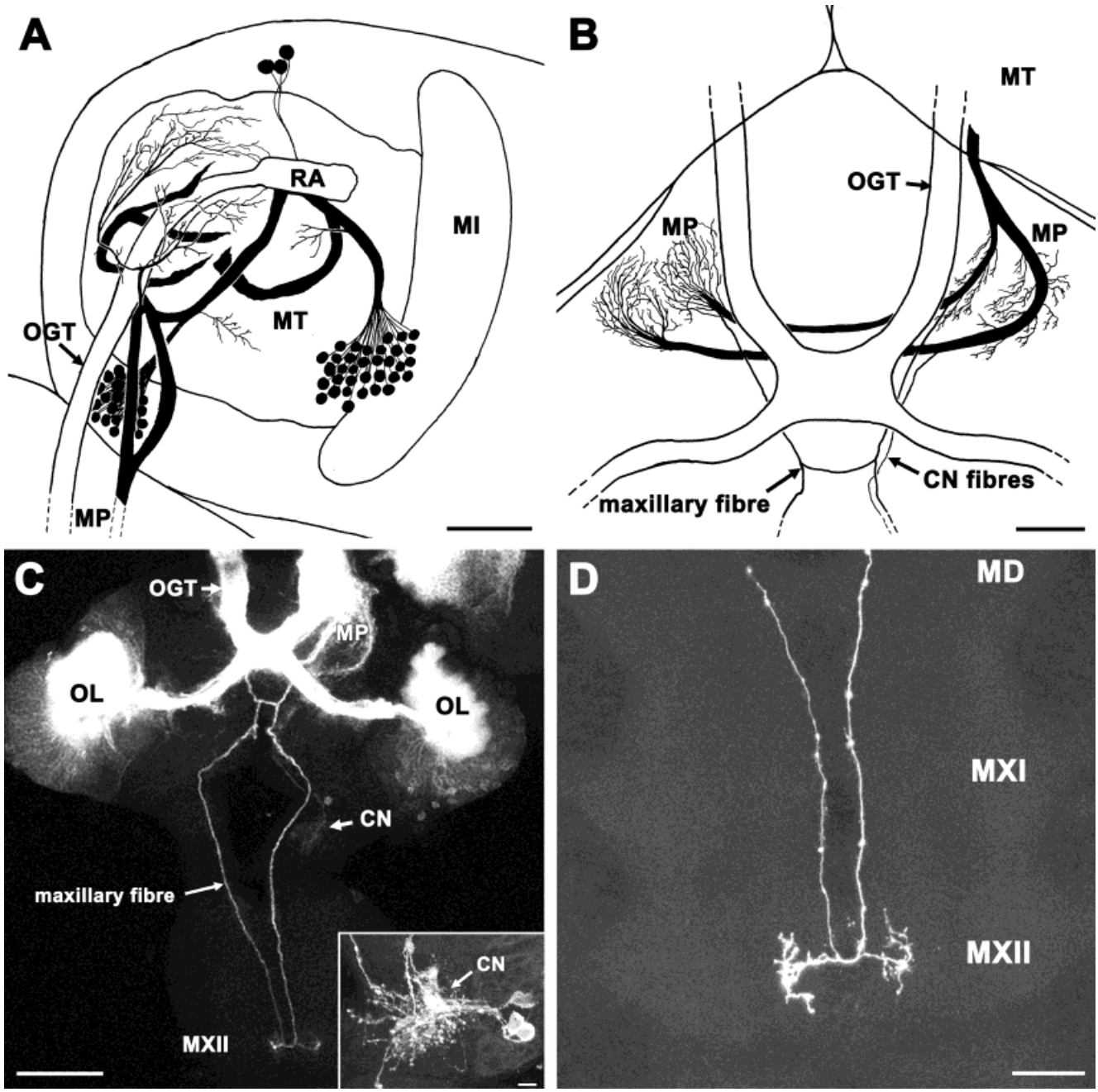
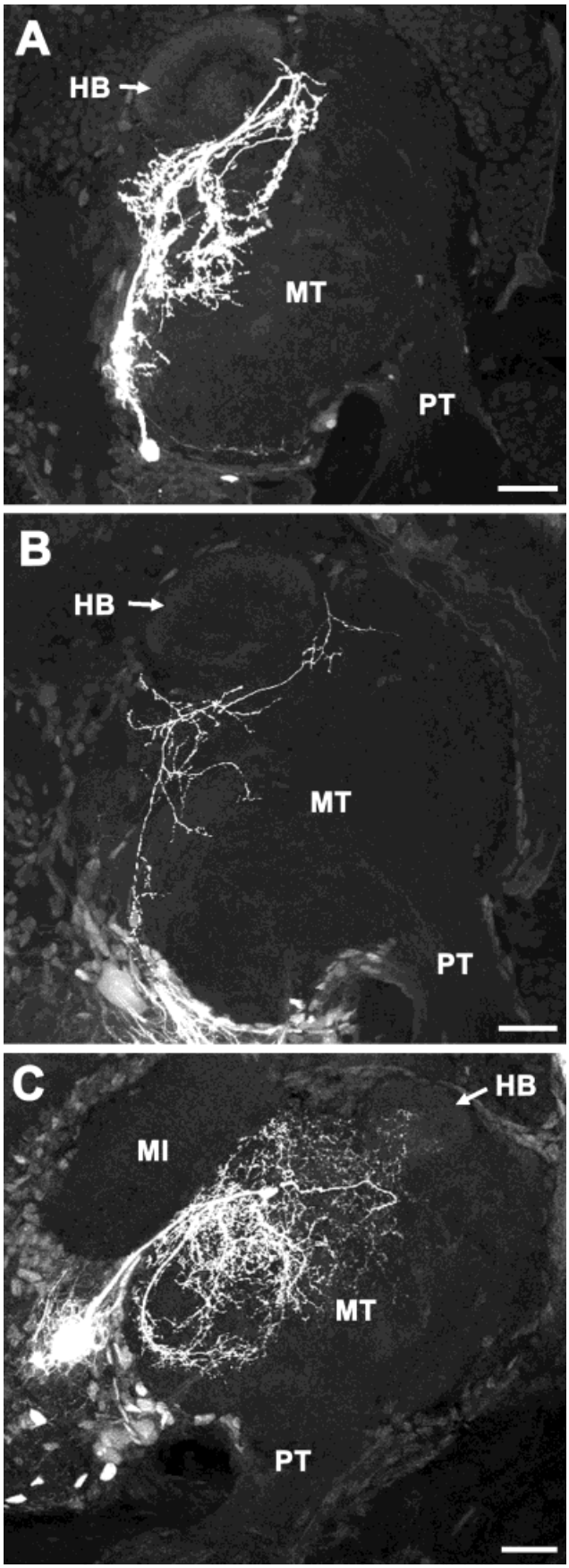


Fig. 13. Connectivity of the neuropil regions of the medulla terminalis innervated by the rostral arbor of the olfactory lobe projection neuron pathway. CN, commissural neuropil; MD, mandibular neuromere; MI, medulla interna; MP, median protocerebrum; MT, medulla terminalis; MXI, first maxillary neuromere; MXII, second maxillary neuromere; OGT, olfactory globular tract; OL, olfactory lobe; RA, rostral arbor of the olfactory lobe projection neuron pathway. **A,B:** Reconstructions from series of confocal images of neurons labeled by focal injections of DiI into the rostral arbor of the olfactory lobe projection neuron pathway of an embryo at E85%. Neurons that branch within the neuropils innervated by the rostral arbor of the projection neuron pathway also branch in several other regions of the ipsilateral medulla terminalis (A) and bilateral regions of the median protocerebrum (B). In addition, the axons of labeled neurons also projected to the commissural neuropil and the second maxillary neuromere of the ventral nerve cord. **C,D:** Stacked confocal images of embryos at E95% (C) and E80% (D) in which DiI was injected into the rostral arbor of the olfactory lobe projection neuron pathway. The **inset** in C shows three neurons labeled in an embryo at E80% that arborize within the commissural neuropil and possess axons that project to the ipsilateral lateral protocerebrum. Scale bars = 100 μm in A, 50 μm in B, 100 μm in C, 25 μm in D, 10 μm in inset in C.

(E80%–E100%) showed that this neuropil has connections to both the ipsi- and contralateral medulla terminalis (Fig. 15). Although the basic structure of the labeled arbors within the bilateral medulla terminale were largely symmetrical, the innervation of the ipsilateral medulla terminalis was considerably more extensive than that of the



contralateral medulla terminalis (Fig. 15A,B). The axons of the labeled neurons project to the medulla terminale in several tracts. The largest tract of labeled axons coursed rostrally on entering the medulla terminalis and terminated in a rounded arbor within neuropil regions of the medulla terminalis adjacent to the medulla interna. Some of the axons within this tract turned laterally before reaching these neuropil regions and projected laterally along the edge of the medulla interna. Within the ipsilateral eyestalk, several of these neurons then turned rostrally and arborized within the medulla interna (Fig. 15A). Innervation of the contralateral medulla interna, however, was not observed. The smaller tracts of labeled axons projected bilaterally to several neuropil regions of the medulla terminalis (Fig. 15A,B). In addition to neurons projecting rostrally from the lateral antenna I neuropil, a bilateral group of 1–3 neurons whose cell bodies were located medial to the hemiellipsoid body were also labeled by the dye injections (Fig. 15).

Labeling of the ascending pathways from the lateral antenna I neuropil and the projection neuron tracts from the olfactory and accessory lobes indicated that the terminal arbors of the three pathways do not overlap within the lateral protocerebrum (Fig. 15C,D). The largest of the neuronal tracts labeled by dye injections into the lateral antenna I neuropil coursed into the medulla terminalis alongside the olfactory lobe projection neuron pathway and innervated neuropil regions adjacent to the rostral arbor of the projection neuron tract. Other neurons innervating the lateral antenna I neuropil also branched within neuropil regions adjacent to those innervated by the medial arbor of the olfactory lobe projection neuron tract. Despite the close apposition of the output tracts from the olfactory lobe and the lateral antenna I neuropil, the terminal arbors of the two pathways remained separate from one another throughout the medulla terminalis.

Focal dye injections into the antenna II neuropil indicated that the ascending pathways from this neuropil to the bilateral eyestalk neuropils are essentially identical in morphology to those of the LAN. Injections of different dyes into the antenna II neuropil (DiD) and the ipsilateral lateral antenna I neuropil (DiA) resulted in the colabeling of all of the tracts and arbors connecting these neuropils to the medulla terminalis and the medulla interna (Fig. 15E,F). The ascending pathways from these neuropils, therefore, appear to be completely overlapping. It was not possible to determine the extent to which individual ascending neurons were colabeled by the two dyes. The descending neurons with cell bodies adjacent to the hemiellipsoid body were colabeled in each preparation, however, indicating that they innervate both the lateral antenna I neuropil and the antenna II neuropil.

DISCUSSION

Embryonic development of the lateral protocerebrum. Dye injections into the developing olfactory and accessory lobes of embryonic lobsters show that the pro-

Fig. 14. Stacked confocal images of local interneurons innervating the lateral protocerebra of embryos at E85% (**A**), E82% (**B**), and E85% (**C**). The neurons were stained intracellularly with Lucifer yellow. Dorsal views of whole-mount preparations. Rostral is to the right. HB, hemiellipsoid body; MI, medulla interna; MT, medulla terminalis; PT, protocerebral tract. Scale bars = 25 μm in A–C.

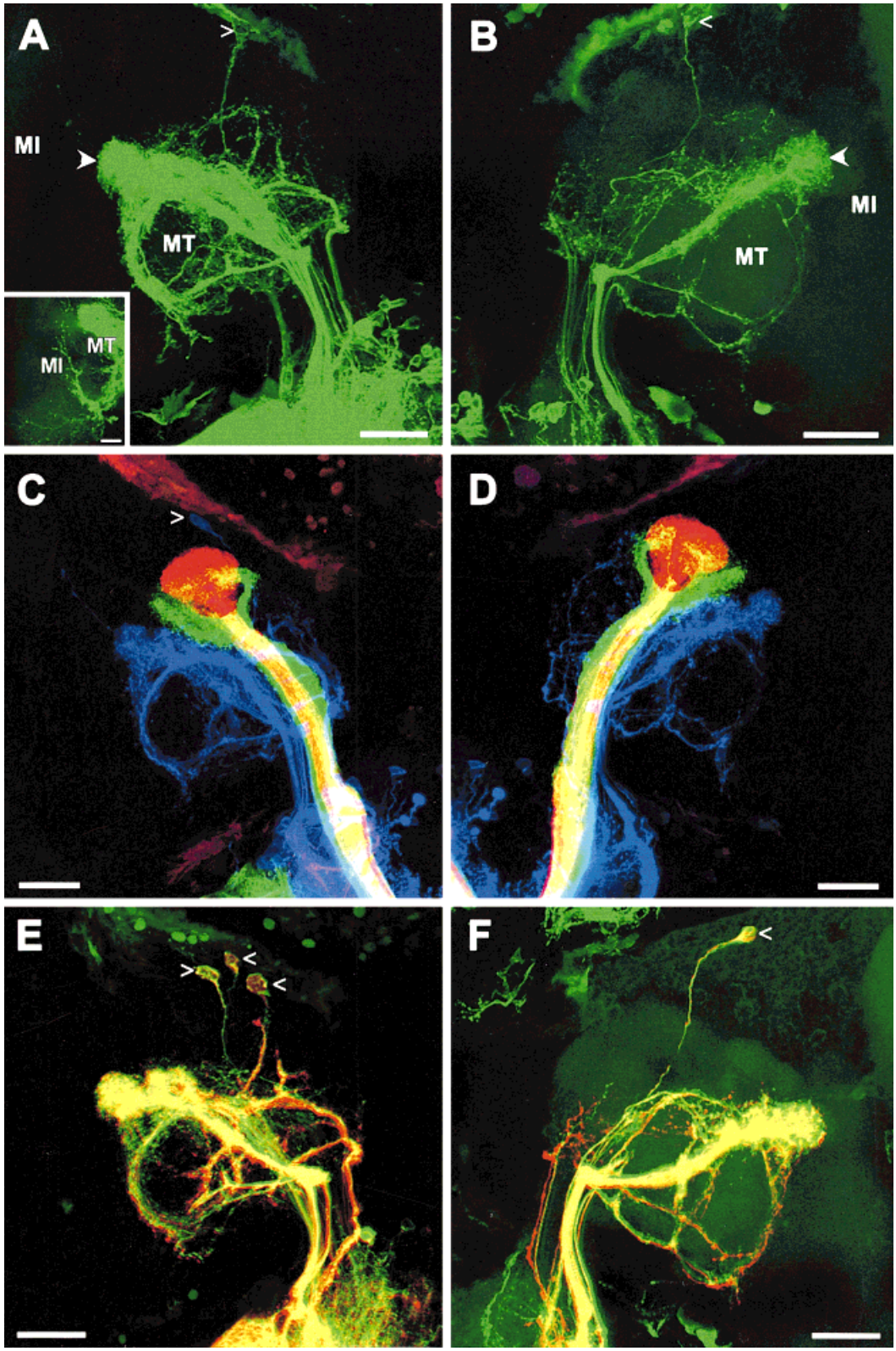


Fig. 15. Neuronal pathways connecting the eyestalk neuropils with the lateral antenna I neuropil and the antenna II neuropil. MI, medulla interna; MT, medulla terminalis. **A,B:** Stacked confocal images of the neuronal pathways labeled by focal injections of DiA into the ipsilateral (A) and contralateral (B) lateral antenna I neuropils of embryos at E80%. The largest tract of labeled neurons terminates in a rounded arbor (filled arrowheads) within rostral regions of the medulla terminalis. The **inset** in A shows the branches of labeled neurons projecting to the ipsilateral medulla interna. The open arrowheads in A–C and in E,F show neuronal cell bodies adjacent to the medulla terminalis that were labeled by the dye injections. **C,D:** Stacked confocal images of the brains of embryos at E85% in which the lateral antenna I neuropil was injected with DiD (blue), the olfactory lobe projection neuron pathway labeled with DiA (green) and the accessory lobe projection neuron pathway labeled with DiI (red). Images show the labeled neurons within the ipsilateral (C) and contralateral (D) eyestalk neuropils. **E,F:** Stacked confocal images of embryos at E82% in which the lateral antenna I neuropil was injected with DiA (green) and the antenna II neuropil injected with DiD (shown as red). Images show the labeled neurons within the ipsilateral (E) and contralateral (F) eyestalk neuropils. Scale bars = 50 μm in A–F, 25 μm in inset in A.

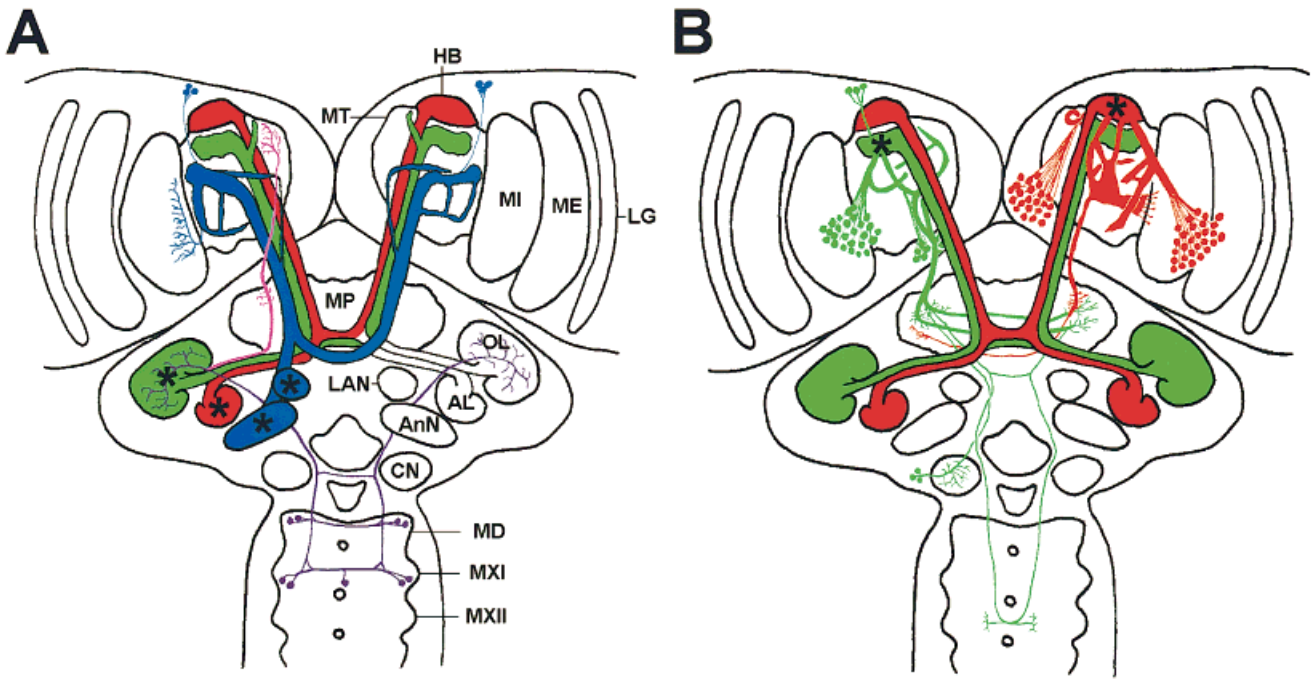


Fig. 16. Schematic diagrams summarizing the patterns of connectivity established in the present study. The black asterisks indicate the sites of dye injection. **A:** Connectivity of the olfactory lobe (OL), accessory lobe (AL), lateral antenna I neuropil (LAN), and the antenna II neuropil (AnN). **B:** Connectivity of the hemiellipsoid body (HB) and the region of the medulla terminalis (MT) innervated by the olfactory lobe projection neuron pathway. CN, commissural neuropil; LG, lamina ganglionaris; MD, mandibular neuromere; ME, medulla externa; MI, medulla interna; MP, median protocerebrum; MXI, first maxillary neuromere; MXII, second maxillary neuromere.

jection neuron pathways from the two lobes target different regions of the lateral protocerebrum. Whereas projection neurons innervating the olfactory lobe terminate within the medulla terminalis, those innervating the accessory lobe project exclusively to the hemiellipsoid body (summarized in Fig. 16A). Studies of the ontogeny of the brain of *H. americanus* also indicate that the two lateral protocerebral neuropils arise at stages of development that are similar to those of the deutocerebral lobes from which they will receive inputs. Both the medulla terminalis and the olfactory lobe emerge during the initial stages of embryogenesis (Helluy et al., 1993, 1995; present study). However, both the accessory lobe and the hemiellipsoid body first appear during mid-embryonic development.

Because of the very early stages of development (<E10%) at which the medulla terminalis and the olfactory lobe are first apparent in histologic sections it was not possible to determine which of these neuropils is the first to form. An examination of the brain at successive stages of mid-embryonic development, however, demonstrated that the hemiellipsoid body emerges at a slightly later (~5%) stage of embryogenesis than the accessory lobe. It has yet to be determined when projection neurons innervating the emerging accessory lobe first reach the lateral protocerebrum. The slight delay between the formation of the accessory lobe and the emergence of the hemiellipsoid body, however, suggests that the latter neuropil region may begin to form as projection neurons from the accessory lobe first innervate the lateral protocerebrum.

Dye labeling of olfactory lobe projection neurons showed that, in embryonic lobsters (E20%–E100%), these neurons innervate medial regions of the medulla terminalis (Fig. 16A). During the initial half of embryogenesis, the olfactory lobe projection neuron tract possesses three main arbors within the medulla terminalis. The terminal arbors of the projection neuron tract, however, are rearranged after the appearance of the hemiellipsoid body. As in adult lobsters, the olfactory lobe projection neuron tract of older embryos (E60%–E100%) bifurcates into two main branches (Sullivan and Beltz, 2001; present study). The rostral arbor of the embryonic projection neuron pathway closely resembles the branch of the adult tract that innervates the medulla terminalis. Axons in both tracts innervate neuropil regions adjacent to the lateral border of the hemiellipsoid body and terminate in small, knot-like endings (Sullivan and Beltz, 2001; present study). In adult lobsters, the second branch of the olfactory lobe projection neuron tract forms a cortex around the cap neuropil of the hemiellipsoid body (Sullivan and Beltz, 2001). In contrast, the medial branch of the olfactory lobe projection neuron tract of embryonic lobsters courses around, but does not innervate, the hemiellipsoid body. The olfactory lobe projection neuron pathway, therefore, must undergo further rearrangement during the postembryonic development of the lobster as projection neuron axons, most likely from the medial branch of the embryonic projection neuron tract, grow into the hemiellipsoid body.

Neuroanatomy of the olfactory pathway of embryonic lobsters—the olfactory lobe. Previous studies of the olfactory lobes of decapod crustaceans have shown that these neuropils are connected to several regions of the brain by projection and local interneurons (Hanström, 1925; Sandeman and Sandeman, 1987; Arbas et al., 1988;

Blaustein et al., 1988; Mellon et al., 1992a; Mellon and Alones, 1994; Schmidt and Ache, 1996b; Wachowiak et al., 1996; Sullivan and Beltz, 2001). The results of the present study indicate that, in embryonic and larval lobsters, the olfactory lobes are also innervated by a group of neurons whose somata are located in the mandibular and maxillary neuromeres of the ventral nerve cord (Fig. 16A). These neurons possess axons that project bilaterally to the paired olfactory lobes, where they arborize extensively. Dye injections into the olfactory lobes of juvenile lobsters also label axons that travel within the ipsilateral circumesophageal connective (unpublished observation). Although it was not possible to determine the complete morphologies of these neurons in juvenile lobsters because of the distances involved, these results suggest that the olfactory lobes of juvenile lobsters also have direct neuronal connections with the ventral nerve cord.

The mandibles and maxillae of lobsters and other decapods are innervated by a variety of setal types that are responsive to chemical stimuli (Shelton and Laverack, 1970; Derby, 1982; Corotto et al., 1992; Lavalli and Factor, 1992). Behavioral studies suggest that these setae, and those present on the other mouthparts (the three pairs of maxillipeds), are involved in the recognition of edible foodstuffs (Hindley, 1975; Derby et al., 1984; Steiner and Harpaz, 1987). Anatomic and physiological studies in insects have shown that some sensory neurons innervating receptors on the mouthparts project to glomeruli in the olfactory (antennal) lobes by means of the ventral nerve cord (fruit flies: Singh and Nayak, 1985; Stocker et al., 1990; Stocker, 1994; Gao et al., 2000; mosquitoes: Anton, 1996; Distler and Boeckh, 1997; moths: Bogner et al., 1986; Kent et al., 1986, 1999; Lee and Altner, 1986). The results of the present study indicate that this is not the case in embryonic and larval lobsters, as dye injections into the olfactory lobes did not label neurons with axons in the segmental nerves of the ventral nerve cord. The labeled neurons in the mandibular and maxillary neuromeres of *H. americanus* could, however, transfer chemosensory information from afferents innervating the mouthparts to the olfactory lobe glomeruli. Interneurons with cell bodies in the anterior neuromeres of the ventral nerve cord and axons that project bilaterally to the olfactory lobes have been described in *Drosophila* (Stocker et al., 1990) but have not yet been examined electrophysiologically.

Neuroanatomy of the olfactory pathway of embryonic lobsters—the lateral protocerebrum. Aesthetasc sensilla are first present on the developing antennules of *H. americanus* after the moult to the second larval stage (Herrick, 1895; Charmantier et al., 1991; Helluy and Beltz, 1991; Helluy et al., 1993). Therefore, although the olfactory system of the lobster is not functionally active until this stage of development, the olfactory and accessory lobes of late-embryonic lobsters (E80%–E100%) possess connections with other regions of the brain that are similar to those previously described in adult lobsters. Unlike the deutocerebral lobes, little is known about the connectivity of the lateral protocerebral neuropils of adult decapods. In the present study, we examined the connectivity of the lateral protocerebral neuropils of embryonic lobsters that are innervated by the terminal arbors of the projection neuron pathways from the olfactory and accessory lobes. The aim of these studies was to gain insights into the neuroanatomy of the olfactory pathways in the lobster brain that would provide directions for future studies of adult decapod crustaceans.

Investigations of neurons innervating the hemiellipsoid bodies of adult crayfish, *P. clarkii*, have led to the identification of a population of local interneurons that connect the hemiellipsoid body with ventral neuropil regions of the medulla terminalis (Mellon et al., 1992a,b; Mellon and Alones, 1997). The results of the present study indicate that the hemiellipsoid bodies of embryonic lobsters are innervated by at least two major populations of interneurons (summarized in Fig. 16B). These interneurons have dendritic branches in several regions of the medulla terminalis, as well as in the medulla interna and the median protocerebrum. Interneurons connecting the hemiellipsoid body and the medulla interna were also observed in adult lobsters and have been previously described in the brain of the mud shrimp, *Calocaris macandreae* (Hanström, 1925). However, further electrophysiological and anatomic studies will be required to determine the direction of signal transfer in these neurons.

The projection neuron pathways from the olfactory lobes of embryonic lobsters innervate neuropil regions of the medulla terminalis adjacent to the hemiellipsoid body. The neuropils innervated by the rostral branch of the projection neuron tract are also innervated by the dendrites of several other populations of interneurons (Fig. 16B). These neurons link the neuropils with other regions of the medulla terminalis as well as with the median protocerebrum, the commissural neuromere and the second maxillary neuromere of the ventral nerve cord. Unlike the hemiellipsoid body, the neuropil regions innervated by the olfactory lobe projection neuron tract do not have connections with the medulla interna.

Neuroanatomy of non-olfactory chemosensory pathways in the brains of embryonic lobsters. Although anatomic and behavioral studies have identified the aesthetascs as constituting the olfactory organs of decapod crustaceans (reviews: Ache, 1991; Atema, 1995; Derby, 2000), the lateral and medial antenna I flagella also possess additional setal types that are innervated by chemosensory afferents (lateral flagellum: Laverack, 1964; Fuzessary, 1978; Gleeson, 1982; medial flagellum: Hodgson, 1958; Ameyaw-Akumfi and Hazlett, 1975; Fuzessary and Childress, 1975; Hindley, 1975; Tierney et al., 1988). The axons of these non-aesthetasc chemosensory afferents are thought to project primarily to the lateral antenna I neuropil (Schmidt and Ache, 1992), which is also innervated by mechanosensory afferents from the antennule (Sandeman and Okajima, 1973; Yoshino et al., 1983; Roye, 1986; Schmidt and Ache, 1992). Chemosensory afferents also innervate setae on the second antennae (antennae II; Tazaki and Shigenaga, 1975; Voigt and Atema, 1992). The axons of these neurons, along with those of mechanosensory afferents, terminate in the antenna II neuropil (Taylor, 1975; Tautz and Müller-Tautz, 1983). The lateral antenna I and antenna II neuropils, therefore, seem to be involved in the processing of inputs from both non-olfactory chemosensory afferents as well as mechanosensory afferents. Both neuropils also have direct neuronal connections with the olfactory and accessory lobes (lateral antenna I neuropil: Mellon and Alones, 1994; Schmidt and Ache, 1996b; Arbas et al., 1988; antenna II neuropil: Arbas et al., 1988). Because the direction of information flow in these neurons is not known, it is unclear whether the

lateral antenna I and antenna II neuropils also receive information about olfactory stimuli.

Previous studies of individual neurons responding to chemical stimulation of the antennules have identified neurons branching within the lateral antenna I neuropil and/or the antenna II neuropil that possess axons that ascend the protocerebral tract (spiny lobster: Schmidt and Ache, 1996a; crayfish: Derby and Blaustein, 1988). These neurons are functionally diverse with many cells responding to visual as well as chemosensory and tactile stimuli (Derby and Blaustein, 1988; Schmidt and Ache, 1996a). Dye injections into the lateral antenna I and antenna II neuropils of embryonic lobsters indicate that the ascending pathways from these two neuropils target the same neuropil regions of the bilateral medulla terminale and the ipsilateral medulla interna (Fig. 16A). Therefore, it is likely that the higher-order processing of chemosensory and tactile inputs from both pairs of antennae is accomplished within the same neuropil regions of the medulla terminalis and medulla interna.

Labeling of the ascending pathways from the lateral antenna I neuropil (or the antenna II neuropil) and the olfactory lobe projection neuron pathway with different dyes indicates that the terminal arbors of these pathways innervate separate, non-overlapping regions of the medulla terminalis. These results suggest that the neuropil regions of the medulla terminalis dedicated to the higher-order processing of olfactory inputs are both functionally and anatomically distinct from those involved in the processing of non-olfactory chemosensory inputs from the two pairs of antennae. Further studies will be needed to determine the ways in which information from the olfactory and non-olfactory chemosensory pathways is processed within the medulla terminalis and the functional roles these pathways play in modulating the behavior of the lobster.

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LITERATURE CITED

Ache BW. 1991. Phylogeny of smell and taste. In: Getchell TV, Doty RL, Bartoshuk LM, Snow JB, editors. Smell and taste in health and disease. New York: Raven Press. p 3–18.

Ameyaw-Akumfi C, Hazlett B. 1975. Sex recognition in the crayfish, *Procambarus clarkii*. Science 190:1225–1226.

Anton S. 1996. Central olfactory pathways in mosquitoes and other insects. In: Proceedings of the Ciba Foundation, Symposium no. 200, Olfaction in Mosquito-Host Interactions. Chichester: Wiley. p 184–196.

Arbas EA, Humphreys CJ, Ache BW. 1988. Morphology and physiological properties of interneurons in the olfactory midbrain of the crayfish. J Comp Physiol A 164:231–241.

Atema J. 1995. Chemical signals in the marine environment: dispersal, detection, and temporal signal analysis. Proc Natl Acad Sci USA 92:62–66.

Bellonci G. 1882. Nuove ricerche sulla struttura del ganglio ottico della *Squilla mantis*. Mem. D. Accad. d. Sc. d. Ist. di Bologna Ser. 4, T. 3.

Benton JL, Beltz BS. 2001. Effects of serotonin depletion on local interneurons in the developing olfactory pathway of lobsters. J Neurobiol 15:193–205.

Blaustein DN, Derby CD, Simmons RB, Beall AC. 1988. Structure of the brain and medulla terminalis of the spiny lobster *Panulirus argus* and the crayfish *Procambarus clarkii*, with an emphasis on olfactory centers. J Crustacean Biol 8:493–519.

Bogner F, Boppré M, Ernst K-D, Boeckh J. 1986. CO_2-sensitive receptors on the labial palps of *Rhodogastria* moths (Lepidoptera: Arctiidae): physiology, fine structure and central projections. J Comp Physiol A 158:741–749.

Charmantier G. 1987. Le dévelopment larvaire et la métamorphose chez les homards (Crustacea, Decapoda). Oceanis 13:137–165.

Charmantier G, Charmantier-Daures M, Aiken DE. 1991. Metamorphosis in the lobster *Homarus* (Decapoda): a review. J Crustacean Biol 11:481–495.

Cooper RA, Uzmann JR. 1980. Ecology of juvenile and adult *Homarus*. In: Cobb JS, Phillips BJ, editors. The biology and management of lobsters, Vol. 2. New York: Academic Press. p 97–142.

Corotto F, Voigt R, Atema J. 1992. Spectral tuning of chemoreceptor cells of the third maxilliped of the lobster, *Homarus americanus*. Biol Bull 183:456–462.

Derby CD. 1982. Structure and function of cuticular sensilla of the lobster *Homarus americanus*. J Crustacean Biol 2:1–21.

Derby CD. 2000. Learning from spiny lobsters about chemosensory coding of mixtures. Physiol Behav 69:203–209.

Derby CD, Blaustein DN. 1988. Morphological and physiological characterization of individual olfactory interneurons connecting the brain and eyestalk ganglia of the crayfish. J Comp Physiol A 163:777–794.

Derby CD, Reilly PM, Atema J. 1984. Chemosensitivity of the lobster *Homarus americanus* to secondary plant compounds: unused receptor capabilities. J Chem Ecol 10:879–892.

Distler PG, Boeckh J. 1997. Central projections of the maxillary and antennal nerves in the mosquito *Aedes aegypti*. J Exp Biol 200:1873–1879.

Elofsson R. 1969. The development of the compound eyes of *Penaeus duorarum* (Crustacea: Decapoda) with remarks on the nervous system. Z Zellforsch 97:323–350.

Fuzessery ZM. 1978. Quantitative stimulation of antennular chemoreceptors of the spiny lobster, *Panulirus argus*. Comp Biochem Physiol 60:303–308.

Fuzessery ZM, Childress J. 1975. Comparative chemosensitivity to amino acids and their role in the feeding activity of bathpelagic and littoral crustaceans. Biol Bull 149:522–538.

Gao Q, Yuan B, Chess A. 2000. Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. Nat Neurosci 3:780–785.

Gleeson RA. 1982. Morphological and behavioral identification of the sensory structures mediating pheromone reception in the blue crab, *Callinectes sapidus*. Biol Bull 163:162–171.

Hanström B. 1924. Untersuchungen über das Gehirn, inbesondere die Sehganglion der Crustaceen. Ark Zool 16:1–117.

Hanström B. 1925. The olfactory centres in crustaceans. J Comp Neurol 38:221–250.

Hanström B. 1931. Neue Untersuchungen über Sinnesorgane und Nervensystem der Crustaceen. Z Morph Oekol Tiere 23:80–236.

Hanström B. 1947. The brain, the sense organs, and the incretory organs of the head in the Crustacea Malacostraca. Kungliga Fysiografiska Sallskapets Handlingar N F 58:1–44.

Harzsch S, Anger K, Dawirs RR. 1997. Immunocytochemical detection of acetylated -tubulin and *Drosophila* synapsin in the embryonic crustacean nervous system. Int J Dev Biol 41:477–484.

Harzsch S, Miller J, Benton J, Dawirs RR, Beltz B. 1998. Neurogenesis in the thoracic neuromeres of two crustaceans with different types of metamorphic development. J Exp Biol 210:2465–2479.

Harzsch S, Benton J, Dawirs RR, Beltz B. 1999a. A new look at embryonic development of the visual system in decapod crustaceans: neuropil formation, neurogenesis and apoptotic cell death. J Neurobiol 39:294–306.

Harzsch S, Miller J, Benton J, Beltz B. 1999b. From embryo to adult: persistent neurogenesis and apoptotic cell death shape the lobster deutocerebrum. J Neurosci 19:3472–3485.

Helluy SM, Beltz BS. 1991. Embryonic development of the American lobster: quantitative staging and characterization of the embryonic moult cycle. Biol Bull 180:355–371.

Helluy S, Sandeman R, Beltz B, Sandeman D. 1993. Comparative brain ontogeny of the crayfish and clawed lobster: implications of direct and larval development. J Comp Neurol 335:343–354.

Helluy S, Ruchhoeft ML, Beltz BS. 1995. Development of the olfactory and accessory lobes in the American lobster: an allometric analysis and its implications for the deutocerebral structure of decapods. J Comp Neurol 357:433–445.

Herrick FH. 1895. The American lobster: a study of its habits and development. Bull US Fish Commission 15:1–252.

Hindley J. 1975. The detection, location and recognition of food by juvenile banana prawns *Penaeus merguiensis* de Man. Mar Behav Physiol 3:193–210.

Hodgson ES. 1958. Electrophysiological studies of arthropod chemoreception. III. Chemoreceptors of terrestrial and freshwater arthropods. Biol Bull 115:114–125.

Kent KS, Harrow ID, Quartararo P, Hildebrand JG. 1986. An accessory olfactory pathway in Lepidoptera: the labial pit organ and its central projections in *Manduca sexta* and certain other sphinx moths and silk moths. Cell Tissue Res 245:237–245.

Kent KS, Oland LA, Hildebrand JG. 1999. Development of the labial pit organ glomerulus in the antennal lobe of the moth *Manduca sexta*: the role of afferent projections in the formation of identifiable olfactory glomeruli. J Neurobiol 40:28–44.

Klagges BR, Heimbeck G, Godenschwege TA, Hofbauer A, Pflugfelder GO, Reifegerste R, Reisch D, Schaupp M, Buchner S, Buchner E. 1996. Invertebrate synapsins: a single gene codes for several isoforms in *Drosophila*. J Neurosci 16: 3154–3165.

Laverack MS. 1964. The antennular sense organs of *Panulirus argus*. Comp Biochem Physiol 13:301–321.

Lavalli KL, Factor JR. 1992. Functional morphology of the mouthparts of juvenile lobsters, *Homarus americanus* (Decapoda: Nephropidae), and comparisons with the larval stages. J Crustacean Biol 12:467–510.

Lee J-K, Altner H. 1986. Primary sensory projections of the labial palp-pit organ of *Pieris rapae* L. (Lepidoptera: Pieridae). Int J Insect Morphol Embryol 15:439–448.

Mellon DeF. 2000. Convergence of multimodal sensory input onto higher-level neurons of the crayfish olfactory pathway. J Neurophysiol 84: 3043–3055.

Mellon DeF, Alones V. 1994. Identification of three classes of multiglomerular, broad-spectrum neurons in the crayfish olfactory midbrain by correlated patterns of electrical activity and dendritic arborization. J Comp Physiol A 177:55–71.

Mellon DeF, Alones VE. 1997. Response properties of higher level neurons in the central olfactory pathway of the crayfish. J Comp Physiol A 181:205–216.

Mellon DeF, Alones V, Lawrence MD. 1992a. Anatomy and fine structure of neurons in the deutocerebral projection pathway of the crayfish olfactory system. J Comp Neurol 321:93–111.

Mellon DeF, Sandeman DC, Sandeman RE. 1992b. Characterization of oscillatory olfactory interneurones in the protocerebrum of the crayfish. J Exp Biol 167:15–38.

Phillips BF, Sastry AN. 1980. Larval ecology. In: Cobb JS, Phillips BF, editors. The biology and management of lobsters, Vol. 2. New York: Academic Press. p 11–57.

Roye DB. 1986. The central distribution of movement sensitive afferent fibres from the antennular short hair sensilla of the blue crab, *Callinectes sapidus*. Mar Behav Physiol 12:181–196.

Sandeman DC, Okajima A. 1973. Statocyst-induced eye movements in the crab *Scylla serrata*. III. The anatomical projections of sensory and motor neurones and the responses of the motor neurones. J Exp Biol 59:17–38.

Sandeman DC, Sandeman RE. 1994. Electrical responses and synaptic connections of giant-immunoreactive neurons in the crayfish olfactory and accessory lobes. J Comp Neurol 341:130–144.

Sandeman D, Scholtz G. 1995. Ground plans, evolutionary changes and homologies in decapod crustacean brains. In: Breidbach O, Kutsch W, editors. The nervous systems of invertebrates: an evolutionary and comparative approach. Basel: Birkhäuser Verlag. p 329–347.

Sandeman D, Beltz B, Sandeman R, 1995. Crayfish brain interneurons that converge with serotonin giant cells in accessory lobe glomeruli. J Comp Neurol 352:263–279.

Sandeman DC, Sandeman RE, 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.

Sandeman DC, Scholtz G, Sandeman RE. 1993. Brain evolution in decapod crustacea. J Exp Zool 265:112–133.

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

Schmidt M, Ache BW. 1992. Antennular projections to the midbrain of the spiny lobster. I. Sensory innervation of the lateral and medial antennular neuropils. J Comp Neurol 318:277–290.

Schmidt M, Ache BW. 1996a. Processing of antennular input in the brain of the spiny lobster, *Panulirus argus*. I. Non-olfactory chemosensory and mechanosensory pathway of the lateral and median antennular neuropils. J Comp Physiol A 178:579–604.

Schmidt M, Ache BW. 1996b. Processing of antennular input in the brain of the spiny lobster, *Panulirus argus*. II. The olfactory pathway. J Comp Physiol A 178:605–628.

Shelton RG, Laverack MS. 1970. Receptor hair structure and function in the lobster *Homarus gammarus* (L.). J Exp Mar Biol Ecol 4:201–210.

Singh RN, Nayak S. 1985. Fine structure and primary sensory projections of sensilla on the maxillary palp of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). Int J Insect Morphol Embryol 14:291–306.

Steiner J, Harpaz S. 1987. Behavior stereotypes of food acceptance and the rejection of "bitter" food in the fresh water prawn *Macrobrachium rosenbergii*. Chem Senses 12:89–97.

Stocker RF. 1994. The organization of the chemosensory system in *Drosophila melanogaster*: a review. Cell Tissue Res 275:3–26.

Stocker RF, Lienhard MC, Borst A, Fischbach KF. 1990. Neuronal architecture of the antennal lobe in *D. melanogaster*. Cell Tissue Res 232:237–248.

Strausfeld NJ. 1998. Crustacean-insect relationships: the use of brain characters to derive phylogeny amongst segmented invertebrates. Brain Behav Evol 52:186–206.

Strausfeld NJ, Nässel DR. 1980. Neuroarchitectures serving compound eyes of Crustacea and insects. In: Autrum H, editor. Handbook of sensory physiology, Vol. VII/6, Comparative physiology and evolution of vision in invertebrates. New York: Springer-Verlag. p 1–132.

Sullivan JM, Beltz BS. 2001. Neural pathways connecting the deutocerebrum and lateral protocerebrum in the brains of decapod crustaceans. J Comp Neurol 440:9–22.

Sullivan JM, Benton JL, Beltz BS. 2000. Serotonin depletion *in vivo* inhibits the branching of olfactory projection neurons in the lobster deutocerebrum. J Neurosci 20:7716–7721.

Talbot P, Helluy S. 1995. Reproduction and embryonic development. In: Factor JR, editor. Biology of the lobster *Homarus americanus*. San Diego: Academic Press. p 177–216.

Tautz J. 1987. Interneurons in the tritocerebrum of the crayfish. Brain Res 407:230–239.

Tautz J, Müller-Tautz R. 1983. Antennal neuropile in the brain of the crayfish: morphology of neurons. J Comp Neurol 218:415–425.

Tautz J, Erber J, Ache B. 1986. Chemosensitive interneurons ascend the optic nerve in the crayfish. Naturwissenschaften 73:154–156.

Taylor RC. 1975. Integration in the crayfish antennal neuropile: Topographic representation of multiple channel coding of mechanoreceptive submodalities. J Neurobiol 6:475–499.

Tazaki I, Shigenaga Y. 1975. Chemoreception in the antenna of the lobster, *Panulirus japonicus*. Comp Biochem Physiol A 147:195–199.

Tierney AJ, Voigt R, Atema J. 1988. Response properties of chemoreceptors from the medial antennular filament of the lobster *Homarus americanus*. Biol Bull 174:364–372.

Tsvileneva VA, Titova VA. 1985. On the brain structures of decapods. Zool Jb Anat 113:217–266.

Voigt R, Atema J. 1992. Tuning of chemoreceptor cells of the second antenna of the American lobster (*Homarus americanus*) with a comparison of four of its other chemoreceptor organs. J Comp Physiol A 171:673–683.

Wachowiak M, Ache BW. 1994. Morphology and physiology of multiglomerular olfactory projection neurons in the spiny lobster. J Comp Physiol A 175:35–48.

Wachowiak M, Diebel CE, Ache BW. 1996. Functional organization of olfactory processing in the accessory lobe of the spiny lobster. J Comp Physiol A 178:211–226.

Whitington PM, Leach D, Sandeman R. 1993. Evolutionary change in neural development within the arthropods: axonogenesis in the embryos of two crustaceans. Development 118:449–461.

Wolff T. 1978. Maximum size of lobsters (*Homarus*) (Decapoda, Nephropidae). Crustaceana 34:1–14.

Yoshino M, Kondoh Y, Hisada M. 1983. Projection of statocyst sensory neurons associated with crescent hairs in the crayfish *Procambarus clarkii* Girard. Cell Tissue Res 230:37–48.