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Integration and Segregation of Inputs to Higher-Order Neuropils of the Crayfish Brain

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ABSTRACT

Information about the input and output pathways of higher-order brain neuropils is essential for gaining an understanding of their functions. The present study examines the connectivity of two higher-order neuropils in the central olfactory pathway of the crayfish: the accessory lobe and its target neuropil, the hemiellipsoid body. It is known that the two subregions of the accessory lobe, the cortex and medulla, receive different inputs; the medulla receives visual and tactile inputs, whereas the cortex receives neither (Sandeman et al. [1995] J Comp Neurol 352:263–279). By using dye injections into the olfactory lobe, we demonstrate that the accessory lobe cortex and medulla also have differing connections with the olfactory lobe. These injections show that local interneurons joining the olfactory and accessory lobes branch primarily within the cortex with only limited branching within the medulla. Injections of different dyes into the two subregions of the hemiellipsoid body, HBI and HBII, show that the accessory lobe cortex and medulla also have separate output pathways. HBI is innervated by the output pathway from the cortex while HBII is innervated by the output pathway from the medulla. These injections also show that HBI and HBII are innervated by separate populations of local interneurons with differing connections to higher-order neuropils in the olfactory and visual pathways. These results suggest a segregation of olfactory and multimodal (including olfactory) inputs within both the accessory lobe and the hemiellipsoid body and provide evidence of important functional subdivisions within both neuropils. J. Comp. Neurol. 481:118–126, 2005.

Indexing terms: accessory lobe; olfaction; olfactory projection neuron; protocerebrum; vision

The processing of sensory inputs within the brain involves two main principles of structural and functional organization: segregation and integration (Tononi et al., 1994; Kandel et al., 2000). The segregation of modalities within the brain enables the extraction of important features from sensory inputs, often with separate pathways processing different features of the same stimulus. This subdivision is particularly evident within the mammalian cerebral cortex, which is made up of numerous anatomically and functionally segregated regions composed of specialized groups of neurons. Perception and the generation of behavior require the integration of the activity within these segregated regions of the brain (Tononi et al., 1998; Sporns et al., 2000, 2002). This integration is mediated by connections within and between sensory systems, particularly within higher-order associative areas, that enable the coordination of activity among different neuronal groups and the formation of dynamic links between brain regions (Engel et al., 2001; Varela et al., 2001).

Decapod crustaceans are valuable models for studying the processing of olfactory inputs within the brain both because of the important roles that olfactory cues play in crustacean behavior and because of the accessibility of their brains for neurobiological analyses. Studies of decapod crustaceans, particularly spiny lobsters and crayfish, have elucidated important features of the structural and functional organization of the crustacean olfactory organ and the primary olfactory brain neuropil, the olfactory lobe. Very little is known, however, about how olfactory inputs are processed within higher-order brain neuropils in these animals.

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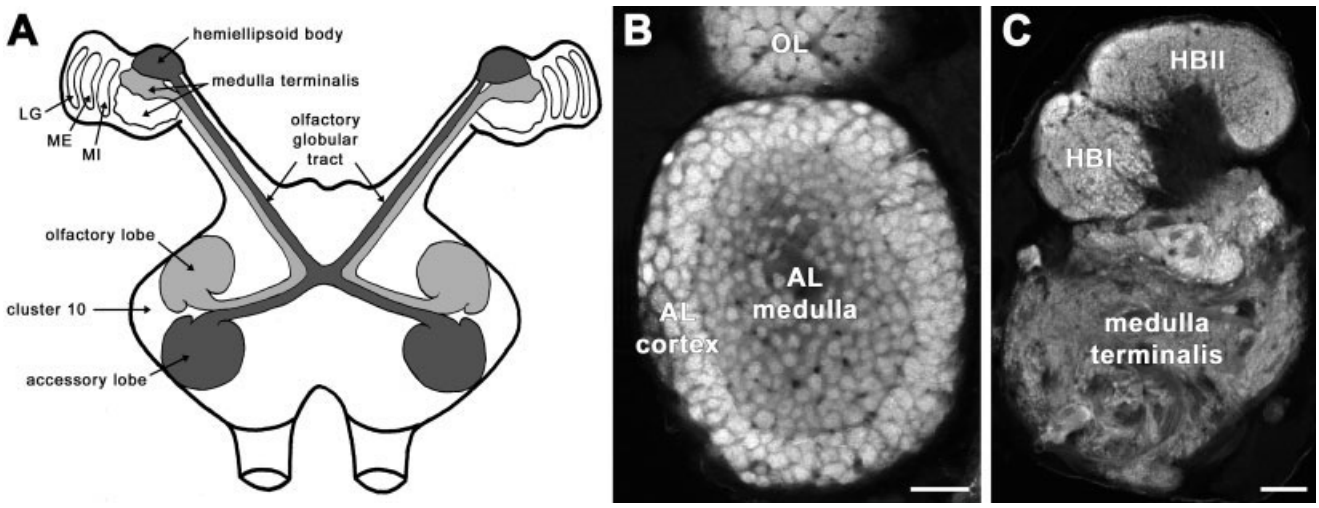


Fig. 1. Morphology of the brain of freshwater crayfish. **A:** Schematic diagram outlining the central olfactory pathway. The olfactory and accessory lobes are innervated by projection neurons whose cell bodies are located lateral to the neuropils in a dense cluster, known as cluster 10. The axons of these projection neurons form the olfactory globular tract, which bifurcates in the center of the brain and projects bilaterally to both hemiellipsoid body and medulla terminalis. Dye injections into the olfactory and accessory lobes of the crayfish *Procambarus clarkii* and *Orconectes rusticus* have demonstrated that projection neurons innervating the olfactory lobe target the medulla terminalis, whereas those innervating the accessory lobe terminate within the hemiellipsoid body (Sullivan and Beltz, 2001). **B:** Horizontal section through the olfactory and accessory lobes of *Cherax destructor* labeled with an antibody against *Drosophila* synapsin. **C:** Cross-section through the lateral protocerebrum of *C. destructor* showing synapsin labeling of the hemiellipsoid body and medulla terminalis. AL, accessory lobe; HBI, hemiellipsoid body neuropil I; HBII, hemiellipsoid body neuropil II; LG, lamina ganglionaris; ME, medulla externa; MI, medulla interna; OL, olfactory lobe. Scale bars = 100 μm in B,C.

One brain region that has been identified as an important higher-order neuropil in the olfactory pathways of crayfish and lobsters is the accessory lobe (Sandeman et al., 1993; Schmidt and Ache, 1996). The accessory lobe lies adjacent to the olfactory lobe (Fig. 1) and receives olfactory inputs from local interneurons that also innervate the ipsilateral olfactory lobe (OL-AL interneurons: Arbas et al., 1988; Mellon and Alones, 1994; Wachowiak et al., 1996). The accessory lobe also receives inputs from a large population of bilateral interneurons, known as deutocerebral commissure (DC) interneurons, which provide the lobe with higher-order multimodal inputs (Sandeman D et al., 1995; Sandeman R et al., 1995; Wachowiak et al., 1996).

Both the olfactory and accessory lobes of crayfish are glomerular in structure (Fig. 1). The olfactory lobe is comprised of radially arranged columnar glomeruli (Sandeman and Luff, 1973). In contrast, the accessory lobe has an inner region (medulla) composed of small, spherical glomeruli and an outer layer (cortex) containing larger, spherical glomeruli (Fig. 1B; Blaustein et al., 1988; Sandeman D et al., 1993, 1995). Anatomical studies of individual DC interneurons in the crayfish *Cherax destructor* have shown that each neuron has outputs within either the accessory lobe cortex or medulla (Sandeman D et al., 1995; Sandeman R et al., 1995). DC interneurons innervating these two regions also differ in the brain neuropils in which they receive their inputs (Fig. 2). Although the functional nature of the inputs to DC interneurons innervating the accessory lobe cortex remains unknown, DC interneurons innervating the medulla respond to both visual and tactile stimuli (Sandeman D et al., 1995).

The main output pathway from the accessory lobe is provided by the axons of a large population of projection

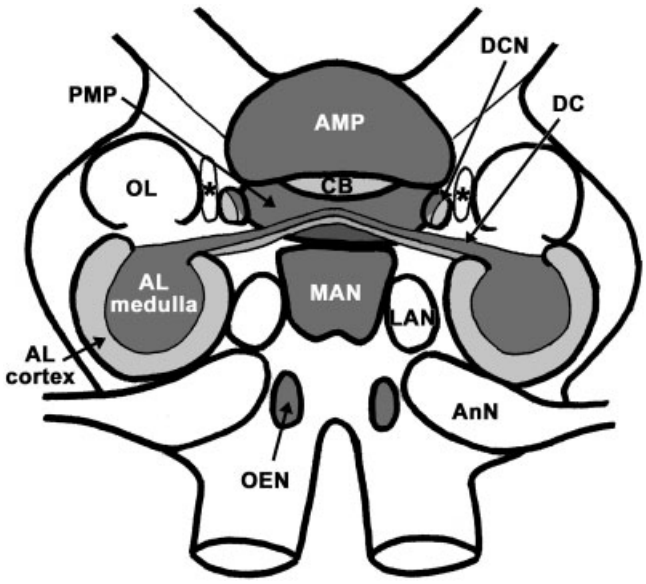


Fig. 2. Schematic diagram illustrating the brain regions innervated by DC interneurons terminating in the accessory lobe medulla (dark gray) and cortex (light gray). Whereas DC interneurons innervating the medulla have connections with several brain regions, those innervating the cortex have connections with only the DCN and CB, based on the results of Sandeman D et al. (1995). The black asterisks indicate the locations of the cell bodies of the DC interneurons. AL, accessory lobe; AMP, anterior medial protocerebral neuropil; AnN, antenna II neuropil; CB, central body; DC, deutocerebral commissure; DCN, deutocerebral commissure neuropil; LAN, lateral antennular neuropil; MAN, median antennular neuropil; OL, olfactory lobe; OEN, esophageal neuropil; PMP, posterior medial protocerebral neuropil.

neurons, whose somata are located lateral to the lobe in a densely packed cluster, known as cluster 10 (Fig. 1A). Projection neurons originating in this cluster also provide the main output pathway from the olfactory lobe (Mellon et al., 1992a,b; Wachowiak and Ache, 1994; Sullivan and Beltz, 2001). Intracellular dye fills of individual accessory lobe projection neurons in spiny lobsters (Wachowiak et al., 1996) and clawed lobsters (Sullivan et al., 2000) have revealed different morphological classes of neurons that selectively innervate different subregions of the lobe. These observations suggest that, in addition to having different input pathways, the accessory lobe subregions may also have separate output pathways.

The axons of the accessory lobe projection neurons join with those of the olfactory lobe projection neurons to form the olfactory globular tract, which bifurcates in the center of the brain before projecting bilaterally to the lateral protocerebrum (Fig. 1A). The lateral protocerebrum is located proximal to the optic neuropils (lamina ganglionaris, medulla externa, and medulla interna) and is composed of two main neuropil regions: the medulla terminalis and the hemiellipsoid body (Fig. 1A,C; Sandeman et al., 1992, 1993). The medulla terminalis is a complex of several interconnected glomerular and unstructured neuropils (Hanström, 1925, 1931, 1947; Blaustein et al., 1988; Sullivan and Beltz, 2001). The hemiellipsoid bodies of crayfish are composed of two lobes, known as neuropils I and II, containing numerous, spherical microglomeruli (Fig. 1C; Blaustein et al., 1988). Anatomical studies in the crayfish *Procambarus clarkii* and *Orconectes rusticus* have shown that projection neurons innervating the accessory lobe project exclusively to the hemiellipsoid body, whereas those innervating the olfactory lobe terminate within the medulla terminalis (Fig. 1A; Sullivan and Beltz, 2001). It is not known whether projection neurons innervating different regions of the accessory lobe have different projections within the hemiellipsoid body.

In the present study, we used focal dye injections into both the olfactory lobe and the hemiellipsoid body of *Cherax destructor* to examine and compare the patterns of connectivity of the accessory lobe cortex and medulla. These studies provide evidence of important functional divisions in both the accessory lobe and the hemiellipsoid body and suggest a segregation of olfactory and multimodal (including olfactory) inputs within both regions.

MATERIALS AND METHODS

Animals

Male and female juvenile Australian freshwater crayfish, *C. destructor* (Malacostraca, Decapoda, Parastacidae), were reared in the laboratory in aquaria with artificial freshwater and a light/dark cycle of 12:12 hours. These animals were the offspring of adult crayfish collected from dams near Sydney, Australia.

Labeling of interneuronal and projection neuron pathways

Brains were dissected from the crayfish in cold crayfish saline (mmol l^{-1}: 205 NaCl, 5.4 KCl, 10.2 $CaCl_2$, 1.2 $MgCl_2$, 2.4 $NaHCO_3$, pH 7.4) and desheathed in the regions surrounding the olfactory lobe, accessory lobe, and hemiellipsoid body. Isolated brains were placed in a well of cold saline on a Sylgard (Dow Corning; Midland, MI) -coated slide and viewed using a fixed-stage Nikon compound microscope equipped with Nomarski optics. Microelectrodes were backfilled with saturated solutions of DiA, DiD, or DiI (Molecular Probes; Eugene, OR) in 100% ethanol, which were then pressure injected into specific brain neuropil regions or nerves. Dye injections were generally made at several locations within the region being examined. After the dye injections, brains were fixed in the dark for 24 hours in 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) at room temperature. The preparations were then placed in fresh fixative and incubated in the dark at 37°C for 10 to 30 days to allow the dyes to travel along the lengths of the labeled neurons. Subsequently, the brains were rinsed for 4 hours in 0.1 M PB, suspended in 6% Noble agar, and sectioned at 100 μm on a Vibratome. Sections were then mounted in Gel Mount (Biomedia Corp., Foster City, CA) and viewed by using laser-scanning confocal microscopy.

Intracellular staining of lateral protocerebral interneurons

Brains were dissected free in cold crayfish saline and desheathed in the regions surrounding the lateral protocerebrum. Preparations were then viewed by using a fixed-stage Nikon compound microscope equipped with Nomarski optics. The morphology of individual 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 pressure injection of Lucifer yellow, by using 0.5 kPa pulses of pressure (500 msec in duration, 0.5 Hz in frequency), for 60 minutes. After the injection of Lucifer yellow, preparations were fixed overnight in 4% paraformaldehyde and then sectioned, mounted, and viewed as detailed above.

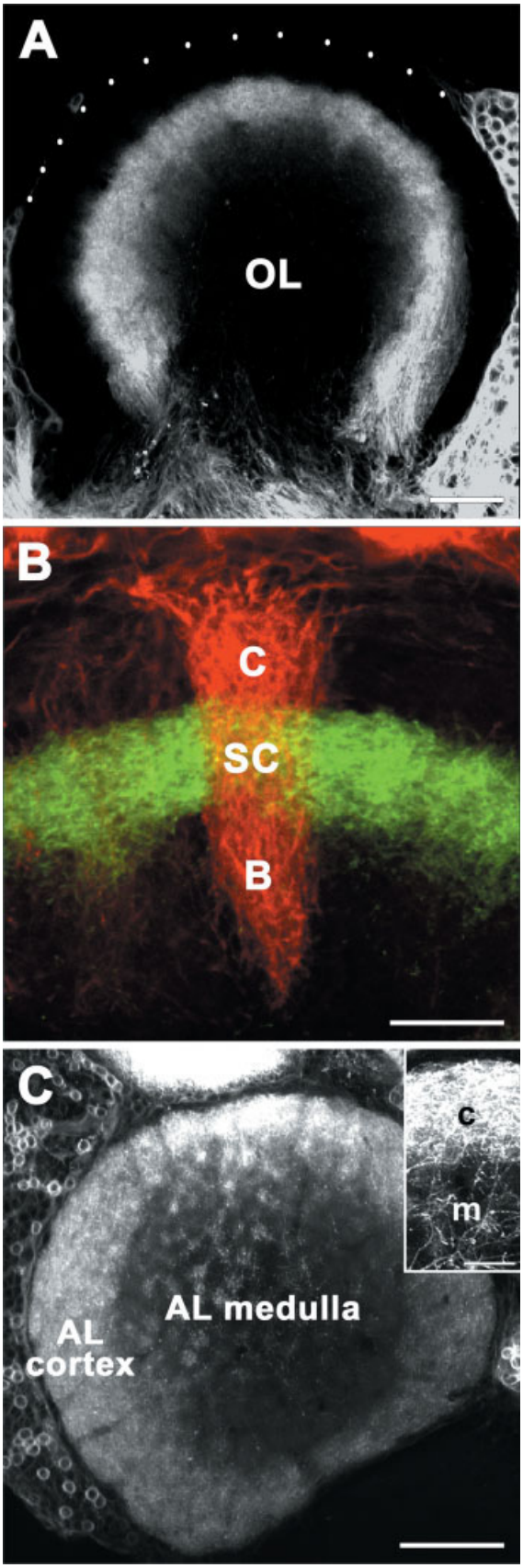
Confocal microscopy and image processing

Specimens were viewed with a Leica TCS SP laser-scanning confocal microscope equipped with argon, krypton, and helium neon lasers. Serial optical sections were taken at intervals of 1.25 μm and were saved as both three-dimensional stacks and two-dimensional projections. Images were processed to adjust brightness and contrast by using Adobe Photoshop 5.0 (Adobe Systems).

RESULTS

Projections of local interneurons innervating both the olfactory lobe and the ipsilateral accessory lobe (OL-AL interneurons)

Focal dye injections into the olfactory and accessory lobes of *C. destructor* show that these two neuropils are connected by a population of local interneurons (OL-AL interneurons) that selectively innervate specific subregions within each lobe. Dye injections into the accessory lobe show that these interneurons specifically innervate a central region of each olfactory lobe glomerulus (Fig. 3A). Olfactory lobe glomeruli are composed of three distinct subregions: the cap, subcap, and base (Sandeman and Luff, 1973). To examine the innervation of the glomeruli in more detail, we injected the olfactory nerve with DiI (red) to label the projections of olfactory receptor neurons within the olfactory lobe glomeruli and then injected the



accessory lobe with DiA (green) to label the OL-AL interneurons. These studies showed that the interneurons branch primarily within the subcap region of the olfactory lobe glomeruli (Fig. 3B).

In most preparations, dye injections into the olfactory nerve brightly labeled individual, scattered glomeruli within the olfactory lobe rather than evenly labeling all of the glomeruli. Some sorting of the afferent axons within the olfactory nerve must, therefore, occur at a site distant to the olfactory lobe. Dye injections into the olfactory lobe show that the OL-AL interneurons branch primarily within the accessory lobe cortex, with only restricted innervation of the medulla (Fig. 3C). The innervation of both the cortex and medulla was more extensive in the rostral than the caudal half of the accessory lobe.

Previous electrophysiological studies of OL-AL interneurons in spiny lobsters have shown that these neurons receive inputs in the olfactory lobe and have their axon terminals within the accessory lobe (Wachowiak et al., 1996). Because of the high degree of structural and functional homology between the olfactory pathways of lobsters and crayfish, it is likely that this is also the case for the OL-AL interneurons of *C. destructor*. The results of the present study suggest, therefore, that the accessory lobe cortex is the principal target of second-order olfactory inputs to the accessory lobe.

Neuroanatomy of the projection neuron pathways from the olfactory and accessory lobes

Dye injections into the olfactory and accessory lobes of *C. destructor* showed that the projection neuron (output) pathways from both lobes have comparable neuroanatomies to those previously described in *P. clarkii* and *O. rusticus* (Sullivan and Beltz, 2001). Both pathways, however, also exhibit several unique anatomical features. The projection neuron pathway from the olfactory lobe of *C. destructor* projects bilaterally to the medulla terminalis (Fig. 4), as in *P. clarkii* and *O. rusticus*, but also innervates basal regions of hemiellipsoid body neuropil I (HBI; Fig. 4A). The projection neuron pathway from the accessory lobe of *C. destructor* extensively innervates both hemiellipsoid body neuropils (Fig. 4), as in *P. clarkii* and *O. rusticus*, but also innervates a small region of the medulla terminalis adjacent to the base of hemiellipsoid body neuropil II (HBII; Fig. 4B). This region of the medulla termi-

Fig. 3. Branching patterns of OL-AL interneurons revealed by focal dye injections into the olfactory and accessory lobe. **A:** Dye injections into the accessory lobe show that the OL-AL interneurons specifically innervate a central region of the olfactory lobe. **B:** The olfactory lobe of a preparation in which olfactory receptor neurons were labeled by the injection of DiI (red) into the olfactory nerve and the OL-AL interneurons were labeled by injection of DiA (green) into the accessory lobe. These preparations demonstrate that the OL-AL interneurons branch primarily within the subcap region of the olfactory lobe glomeruli. **C:** Dye injections into the olfactory lobe show that the OL-AL interneurons branch primarily within the accessory lobe cortex, with only restricted innervation of the medulla. AL, accessory lobe; B, base region of olfactory lobe glomerulus; c, accessory lobe cortex; C, cap region of olfactory lobe glomerulus; m, accessory lobe medulla; OL, olfactory lobe; SC, subcap region of olfactory lobe glomerulus. Scale bars = 50 μm in A; 25 μm in B; 100 μm in C; 25 μm in inset in C.

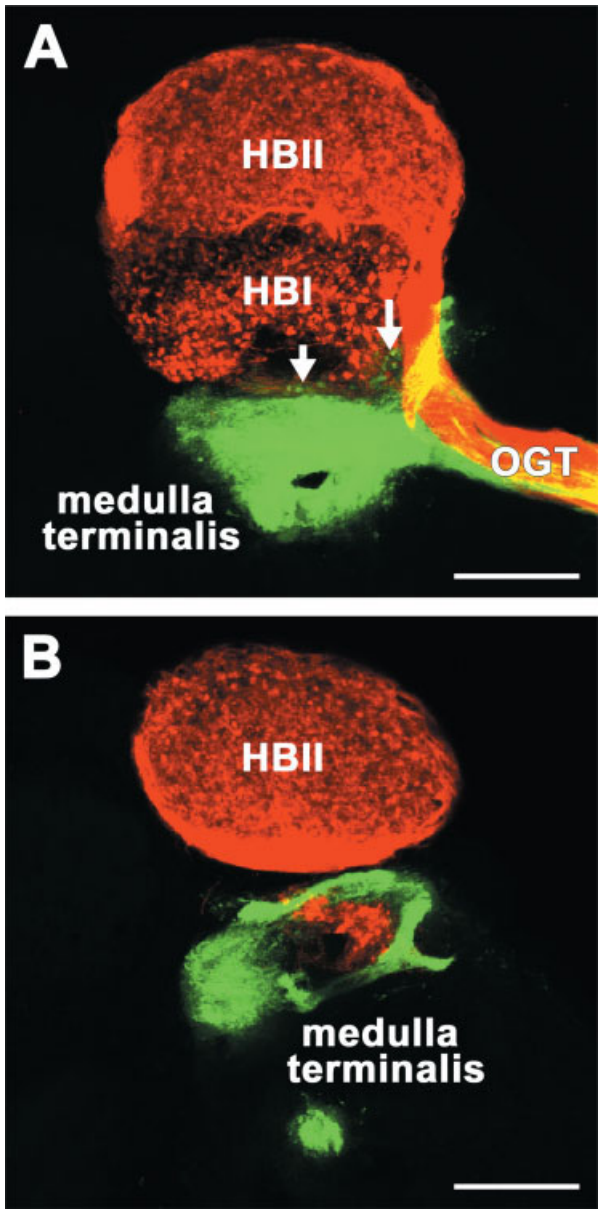


Fig. 4. Neuroanatomy of the terminal arbors of the projection neuron pathways from the olfactory and accessory lobes of *Cherax destructor*. **A,B:** Stacked confocal images of horizontal sections of the lateral protocerebrum of *C. destructor* in a brain in which the ipsilateral olfactory lobe was injected with DiA (green) and the ipsilateral accessory lobe was injected with DiI (red). The white arrows in A indicate branching of the olfactory lobe projection neuron pathway within HBI. The labeled arbors shown in A are ventral to those shown in B. The bilateral projections of both projection neuron pathways were found to be symmetrical. HBI, hemiellipsoid body neuropil I; HBII, hemiellipsoid body neuropil II; OGT, olfactory globular tract. Scale bars = 100 μm in A,B.

nalis is not innervated by the projection neuron pathway from the olfactory lobe (Fig. 4B). Unlike the olfactory and accessory lobe projection neuron pathways of *P. clarkii* and *O. rusticus*, which show pronounced bilateral asymmetries, both pathways in *C. destructor* were bilaterally symmetrical.

Connectivity of the accessory lobe cortex and medulla

To determine whether the accessory lobe cortex and medulla of *C. destructor* have separate output pathways to the hemiellipsoid body, we injected HBI with DiA (green) and HBII with DiI (red). An examination of the labeled projections within the accessory lobe revealed the presence of two distinct projection neuron pathways between the accessory lobe and the hemiellipsoid body (Fig. 5A). The first pathway is composed of projection neurons that branch primarily within the accessory lobe cortex and project to HBI, whereas the projection neurons that comprise the second pathway branch primarily within the accessory lobe medulla and project to HBII.

Connectivity of the hemiellipsoid body and the medulla terminalis

Focal dye injections into HBI and HBII also revealed the anatomies of populations of local interneurons connecting the hemiellipsoid body neuropils with several neuropil regions within the medulla terminalis (Fig. 5B–F). These studies showed that HBI and HBII are innervated by largely separate populations of local interneurons that connect these neuropils with distinct regions of the medulla terminalis. Focal dye injections into HBI showed that this neuropil is innervated by a population of local interneurons that also branch extensively within central regions of the medulla terminalis (Fig. 5B–F). Preparations in which focal dye injections were made into both HBI (green) and the olfactory lobe (blue) showed that the projections of these local interneurons overlap with the terminal arbors of the olfactory lobe output pathway within several regions of the medulla terminalis (Fig. 5E,F). Dye injections into HBII showed that the local interneurons innervating this neuropil branch extensively within the diamedullary neuropil (Fig. 5B,F), a region of the medulla terminalis adjacent to the optic neuropils, as well as a small circular neuropil located at the dorsal margin of the medulla terminalis (Fig. 5D).

While most of the labeled projections from HBI and HBII innervate separate regions of the medulla terminalis, some overlap of these arbors does occur adjacent to the base of the hemiellipsoid body (Fig. 5B), suggesting that some interneurons may innervate both hemiellipsoid body neuropils. An examination of the labeled cell bodies showed that only one soma, located adjacent to the diamedullary neuropil at the lateral margin of the medulla terminalis, was consistently labeled by injections into HBI or HBII. Intracellular labeling of this neuron with Lucifer yellow showed that it possesses a large primary neurite that courses first caudally and then medially to extensively innervate regions of the medulla terminalis adjacent to the base of the hemiellipsoid body, as well as sparsely innervate both HBI and HBII (Fig. 6). These results suggest that neuropil regions adjacent to the base of the hemiellipsoid body may be involved in the integration of the differing inputs to HBI and HBII.

DISCUSSION

The accessory lobe cortex and medulla of *C. destructor* have different input pathways (summarized in Fig. 7). Dye injections into the olfactory lobe show that OL-AL interneurons branch extensively in the accessory lobe cor-

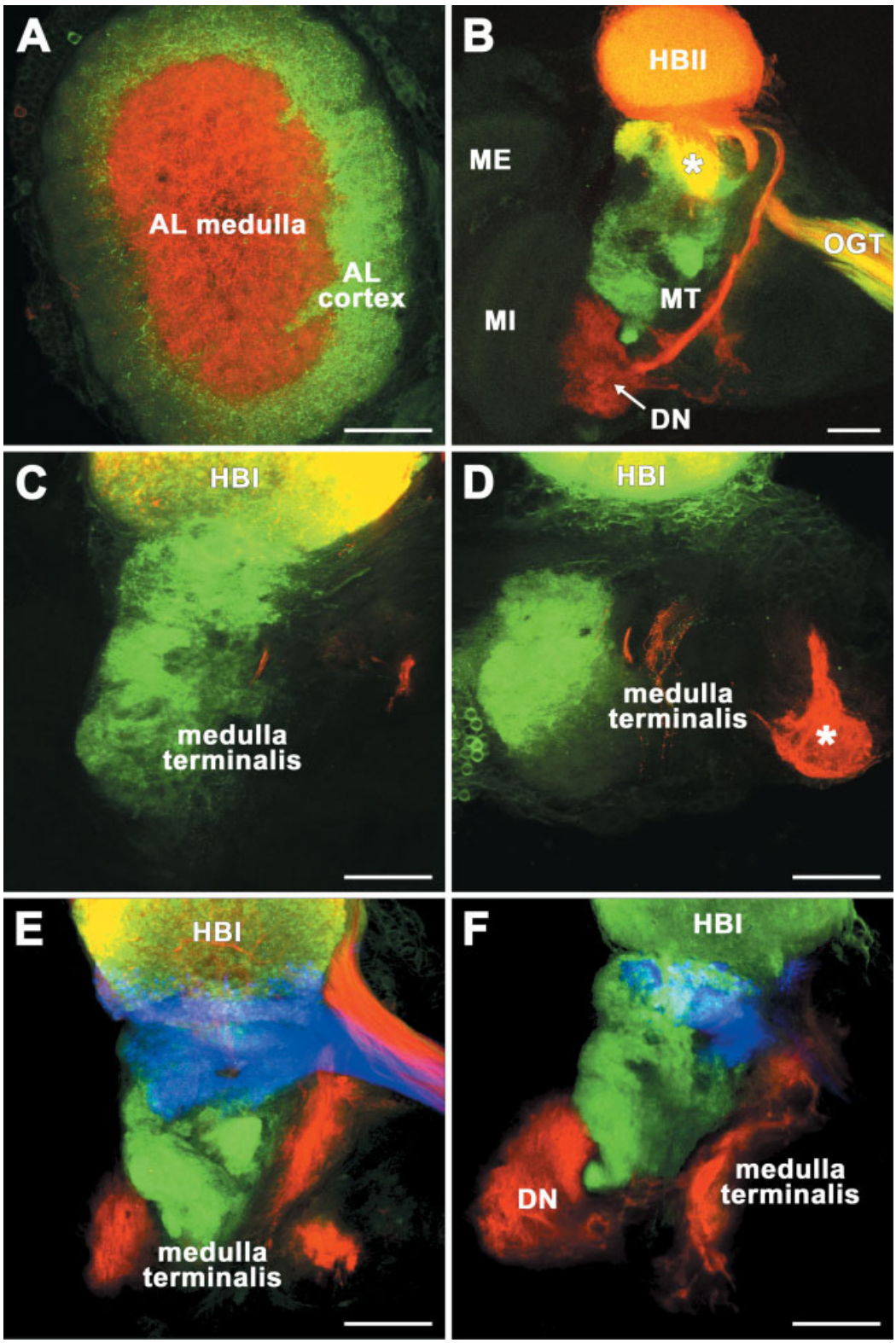
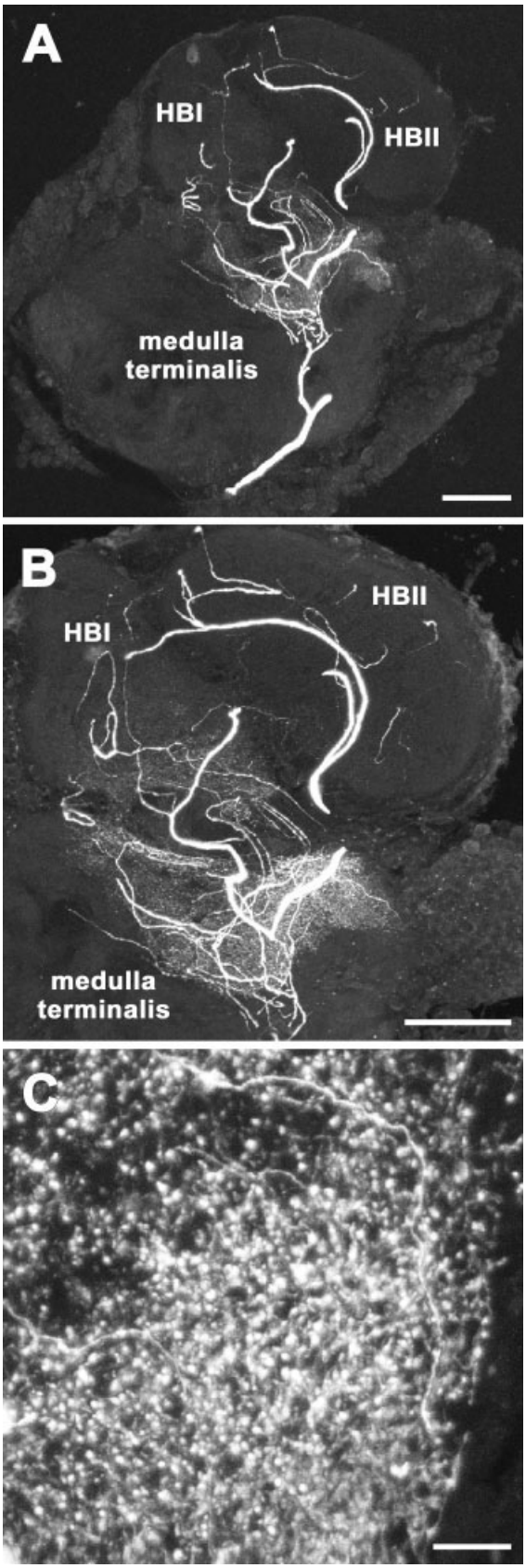


Fig. 5. Connectivity of the hemiellipsoid body neuropils of *Cherax destructor*. **A:** Horizontal section through the accessory lobe of a brain in which the ipsilateral HBI was injected with DiA (green) and the ipsilateral HBII was injected with DiI (red), demonstrating the presence of two projection neuron pathways from the accessory lobe to the hemiellipsoid body. **B–D:** Horizontal sections through the lateral protocerebrum and optic neuropils of a brain in which HBI was injected with DiA (green) and HBII was injected with DiI (red). These preparations demonstrate that HBI and HBII are innervated by largely separate populations of local interneurons. B: The asterisk shows a region of the medulla terminalis in which the arbors of interneurons innervating HBI and HBII overlap. C: The labeled arbors are dorsal to those shown in B and ventral to those shown in D. D: The asterisk indicates an undescribed, circular neuropil region of the medulla terminalis that is extensively innervated by interneurons that also branch within HBII. **E,F:** Horizontal section through the lateral protocerebrum of a brain in which HBI was injected with DiA (green), HBII was injected with DiI (red), and the ipsilateral olfactory lobe was injected with DiD (blue). These preparations show that the arbors of the local interneurons innervating HBI overlap those of the olfactory lobe projection neurons. Regions of overlap are cyan in colour. The labeled arbors shown in E are ventral to those shown in F. AL, accessory lobe; DN, diamedullary neuropil; HBI, hemiellipsoid body neuropil I; HBII, hemiellipsoid body neuropil II; ME, medulla externa; MI, medulla interna; MT, medulla terminalis; OGT, olfactory globular tract. Scale bars = 100 μm in A–F.



tex, with only minimal innervation of the medulla. Second-order olfactory inputs to the accessory lobe, therefore, appear to be processed primarily within the cortex, providing the first insight into the nature of the inputs to this region. It remains unclear what inputs the accessory lobe cortex receives from the DC interneurons that end within it. Whereas DC interneurons innervating the accessory lobe medulla respond to visual and tactile stimuli, those innervating the cortex do not respond to either (Sandeman D et al., 1995). It is possible that the accessory lobe cortex could receive higher-order olfactory inputs from the DC interneurons, as DC interneurons responding to stimulation of the olfactory nerve have been identified in the spiny lobster *Panulirus argus* (Wachowiak et al., 1996). The responses of the DC interneurons of *C. destructor* to olfactory stimuli, however, have yet to be examined.

Our current knowledge of the inputs to the accessory lobe cortex and medulla indicates that, whereas the cortex is involved in the processing of olfactory inputs, the medulla is involved in the integration of visual, tactile, and olfactory inputs (D. Sandeman et al., 1995; present study). This finding suggests that separate unimodal (olfactory) and multimodal pathways exist within the accessory lobe and that the cortex and medulla have distinct functions. It remains unclear whether the cortex and medulla are interconnected by interneurons intrinsic to the accessory lobe and, therefore, receive inputs from one another. Intrinsic interneurons have been identified in the accessory lobe of *P. argus* and, if present in *C. destructor*, could be involved in the integration of the differing inputs to the cortex and medulla. That the cortex and medulla have separate output pathways as well as separate input pathways suggests, however, that the outputs from these two regions are distinct and that the differences in their inputs are reflected in their outputs. This idea is further strengthened by an examination of the connectivity of the target neuropils of the output pathways from the two regions.

Dye injections into the hemiellipsoid body of *C. destructor* demonstrate that the projection neuron (output) pathway from the accessory lobe cortex targets HBI, whereas that from the medulla targets HBII (Fig. 7). These injections also label the populations of local interneurons that innervate the two hemiellipsoid body neuropils, confirming the observation of McKinzie et al. (2003) in *P. clarkii* that HBI and HBII are innervated by largely separate populations of local interneurons. We have found only one interneuron that innervates both neuropils. The hemiellipsoid body, therefore, is not the site at which substantial integration of the information in the output pathways from the accessory lobe cortex and medulla occurs. The functional differences between the two accessory lobe sub-

Fig. 6. Stacked confocal images of transverse Vibratome sections of the lateral protocerebrum of *Cherax destructor* in which a large interneuron innervating both HBI and HBII was labeled intracellularly with Lucifer yellow. **A,B:** Low-power images showing that this neuron branches extensively within regions of the medulla terminalis adjacent to the base of the hemiellipsoid body as well as more sparsely within HBI and HBII. **C:** Higher-magnification image showing the extensive branching of this neuron within the medulla terminalis. HBI, hemiellipsoid body neuropil I; HBII, hemiellipsoid body neuropil II. Scale bars = 100 μm in A,B; 10 μm in C.

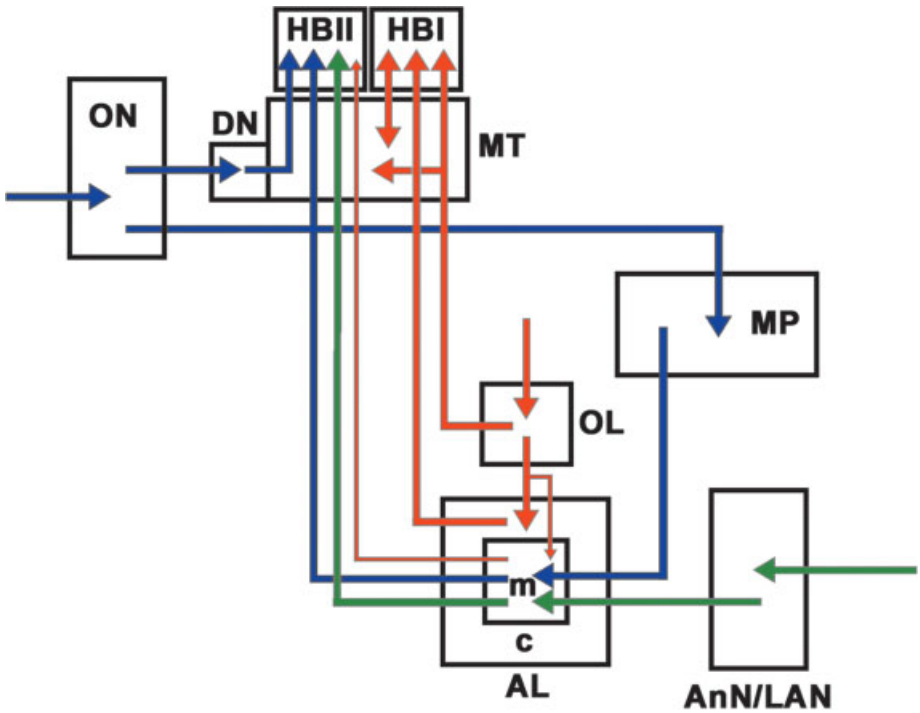


Fig. 7. Schematic representation of known pathways to and between the deutocerebral and lateral protocerebral neuropils of crayfish. Arrows indicate the flow of information. Olfactory pathways are shown in red, visual pathways are shown in blue, and tactile pathways are shown in green, based on the results of the present study and those of Blaustein et al., 1988; Derby and Blaustein, 1988; D. Sandeman et al., 1995; R. Sandeman et al., 1995; Mellon and Alones, 1997; Utting et al., 2000; Sullivan and Beltz, 2001; McKinzie et al., 2003. AL, accessory lobe; AnN, antenna II neuropil; c, accessory lobe cortex; DN, diamedullary neuropil; HBI, hemiellipsoid body neuropil I; HBII, hemiellipsoid body neuropil II; LAN, lateral antennular neuropil; m, accessory lobe medulla; MP, median protocerebrum; MT, medulla terminalis; OL, olfactory lobe; ON, optic neuropils.

regions, therefore, are likely to be reflected in the two hemiellipsoid body neuropils.

The anatomy and physiology of one group of local interneurons innervating HBII in *P. clarkii* have been examined in detail by Mellon and colleagues (Mellon et al., 1992a,b; Mellon and Alones, 1993, 1997; Mellon and Wheeler, 1999; Mellon, 2000; McKinzie et al., 2003). These interneurons, termed parasol cells, form an output pathway from HBII to unidentified targets in the medulla terminalis (Mellon et al., 1992a). Individual parasol cells respond strongly to visual and tactile inputs and more weakly to olfactory stimuli (Mellon, 2000). This finding is consistent with the known input pathways to the accessory lobe medulla, whose output pathway targets HBII (Fig. 7). Parasol cells also respond to visual stimuli after the isolation of the lateral protocerebrum and the optic neuropils from the remainder of the brain (Mellon, 2000), indicating that HBII also receives visual inputs from neurons other than the accessory lobe projection neurons. The population of interneurons innervating both HBII and the diamedullary neuropil may represent one source of visual inputs to HBII as the diamedullary neuropil connects the medulla interna (the third optic neuropil) and the medulla terminalis (Blaustein et al., 1988) and has been identified as an optic focus within the medulla terminalis (Strausfeld and Nässel, 1980). Like the accessory lobe medulla, therefore, HBII is a site of integration of higher-order multimodal inputs.

Unlike HBII, little is known about the anatomy or physiology of interneurons innervating HBI (McKinzie et al., 2003). Focal dye injections in *C. destructor* show that HBI is innervated by the output pathways from both the olfactory lobe and the accessory lobe cortex (Fig. 7). HBI appears to receive, therefore, second-order olfactory inputs from the olfactory lobe projection neurons and third-order olfactory inputs from the accessory lobe cortex projection neurons. HBI is also innervated by local interneurons that branch extensively within central neuropil regions of the medulla terminalis, some of which are also targeted by the output pathway from the olfactory lobe. Together, these observations suggest that HBI is involved primarily in the processing of olfactory inputs. The segregation of olfactory and multimodal pathways apparent within the accessory lobe, therefore, also appears to be maintained in the hemiellipsoid body.

The bilateral symmetry of the terminal arbors of the accessory lobe projection neuron pathway of *C. destructor* differs substantially from the asymmetric projections of this pathway in *P. clarkii* and *O. rusticus*. In the latter two species, the output pathway from the accessory lobe innervates both HBI and HBII ipsilaterally, but only HBII contralaterally (Sullivan and Beltz, 2001). It has been proposed that this asymmetry may enable the hemiellipsoid body to compare olfactory inputs to each of the olfactory lobes with one another, in the context of concurrent visual and tactile inputs (Sullivan and Beltz, 2001; McK-

inzie et al., 2003). The absence of this asymmetry in *C. destructor* suggests, therefore, that important functional differences may exist among the olfactory pathways of these species. Because *C. destructor* belongs to a different superfamily (Parastacoidea) than *P. clarkii* and *O. rusticus* (Astacoidea), these results suggest an evolutionary divergence in the olfactory pathways of these animals.

In summary, this study provides evidence of important functional subdivisions within both the accessory lobe and the hemiellipsoid body. These subdivisions are related anatomically, with the accessory lobe cortex having outputs to HBI and the medulla to HBII. The inputs to these neuropils defined in the present study and by D. Sandeman et al. (1995) suggest that the accessory lobe cortex and HBI are involved primarily in the processing of olfactory inputs, whereas the accessory lobe medulla and HBII are involved in the processing of multimodal (including olfactory) inputs. Separate olfactory and multimodal pathways appear to exist, therefore, within both the accessory lobe and the hemiellipsoid body. One focus of future studies will be to determine the sites within the brain in which significant integration of olfactory inputs with other sensory modalities occurs. HBI, which appears to be an important higher-order neuropil in the olfactory pathway, has extensive connections with both the medulla terminalis (Fig. 5) and the median protocerebrum (Jeremy Sullivan, unpublished observations). The specific cellular targets of these projections, however, are presently unknown.

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