

MULTISENSORY CONVERGENCE IN THE MUSHROOM BODIES OF ANTS AND BEES*

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The mushroom bodies, central neuropils in the arthropod brain, are involved in learning and memory and in the control of complex behavior. In most insects, the mushroom bodies receive direct olfactory input in their calyx region. In Hymenoptera, olfactory input is layered in the calyx. In ants, several layers can be discriminated that correspond to different clusters of glomeruli in the antennal lobes, perhaps corresponding to different classes of odors. Only in Hymenoptera, the mushroom body calyx also receives direct visual input from the optic lobes. In bees, six calycal layers receive input from different classes of visual interneurons, probably representing different parts of the visual field and different visual properties. Taken together, the mushroom bodies receive distinct multisensory information in many segregated input layers.

Keywords: Neuroanatomy – learning – memory – insects – multimodal information

INTRODUCTION

Social Hymenoptera (social bees, social wasps and all ants) are characterized by division of labor and they feature remarkable behavioral performances. Extraordinary learning and memory abilities have been demonstrated in honeybees [6, 15], bumblebees [10] and ants [2, 17]. Behavioral abilities and plasticity are controlled by the mushroom bodies, a pair of higher brain centers [4, 18] that are particularly large in Hymenoptera.

Hymenopteran mushroom bodies are composed of many (ca. 170,000 in honey bees) intrinsic neurons (Kenyon cells) whose dendrites form a cup-like input region called the calyx. An axon extends from each dendritic tree through the peduncle to branch in two lobes (the vertical and medial lobes; [19]). Calyces receive collateral input from olfactory projection neurons originating from the antennal lobe on the same side of the brain [13]. Only in Hymenoptera, the calyces also receive substan-

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tial input from the optic lobes on both sides of the brain [3, 7, 8, 16]. In honey bees, ants and paper wasps, the calyx is concentrically subdivided into three compartments: the lip receives input from the antennal lobes; the collar receives visual input; the basal ring is divided into two layers [7, 13, 16], one receiving visual and the other one antennal input [3]. The current study describes multimodal input to the calyx.

MATERIAL AND METHODS

Carpenter ant workers (*Camponotus ocreatus*) were collected locally and kept in the laboratory. Honey bee foragers (*Apis mellifera*) were collected from flowers. Animals' head capsules were cut open and particular parts of the optic lobes or antennal lobes were filled with Dextran-coupled fluorescent dyes (Texas Red and Fluorescein, MW 3000, Molecular Probes) or with biocytin (Fig. 1b) to label sensory afferents into the mushroom bodies. Glass capillary microelectrodes with broken tips were used to inject tracers or to deposit tiny tracer crystals in the tissue. After dye diffusion (4–12 hours), brains were aldehyde-fixed, embedded and sectioned according to [3]; biocytin-stained material was reacted with avidin (Vectorstain ABC kit) after fixation and processed according to [9]. Sections were viewed and photographed using brightfield (biocytin-labeled material) or epifluorescent microscopy and the appropriate filters.

RESULTS

Antennal input to the calyx

In honey bees, antennal lobes comprise about 150 glomeruli arranged in four clusters that are defined by the tributary of the antennal nerve and by their position in the antennal lobe [5]. In carpenter ants (*Camponotus ocreatus*), we counted about 199 glomeruli distributed in five clusters: a large anterior cluster (cluster 1; Fig. 1d, f) and four smaller clusters situated more posteriorly (Fig. 1e, f), each of which receives a branch of the antennal nerve that enters the antennal lobe from the antenna. Projection neurons connect the antennal lobe and the mushroom bodies' calyx [13]. In ants and in honey bees, these projection neurons run in two major tracts: the lateral antenno-cerebral tract (Fig. 1a, f) projects laterally with respect to the mushroom body lobes. It gives off blebbed collaterals to the dorso-lateral protocerebrum, the so-called lateral horn (Fig. 1a) before continuing to the calyces. Projection neurons of the inner antenno-cerebral tract project medially with respect to the mushroom body lobes, give off collaterals to the calyces, and have a recurrent branch that terminates in the lateral horn (Fig. 1a).

When injecting tracer into many antennal lobe glomeruli simultaneously, the entire lip region is stained, although not homogeneously, and appearing striated (Fig. 1b). The staining intensity of the different layers reflects the amount of dye taken up

by individual glomeruli and projection neurons. In the lip of ants, 3–6 layers can be discriminated in different preparations (Fig. 1b); in honey bees, at least three layers are discernable (not shown).

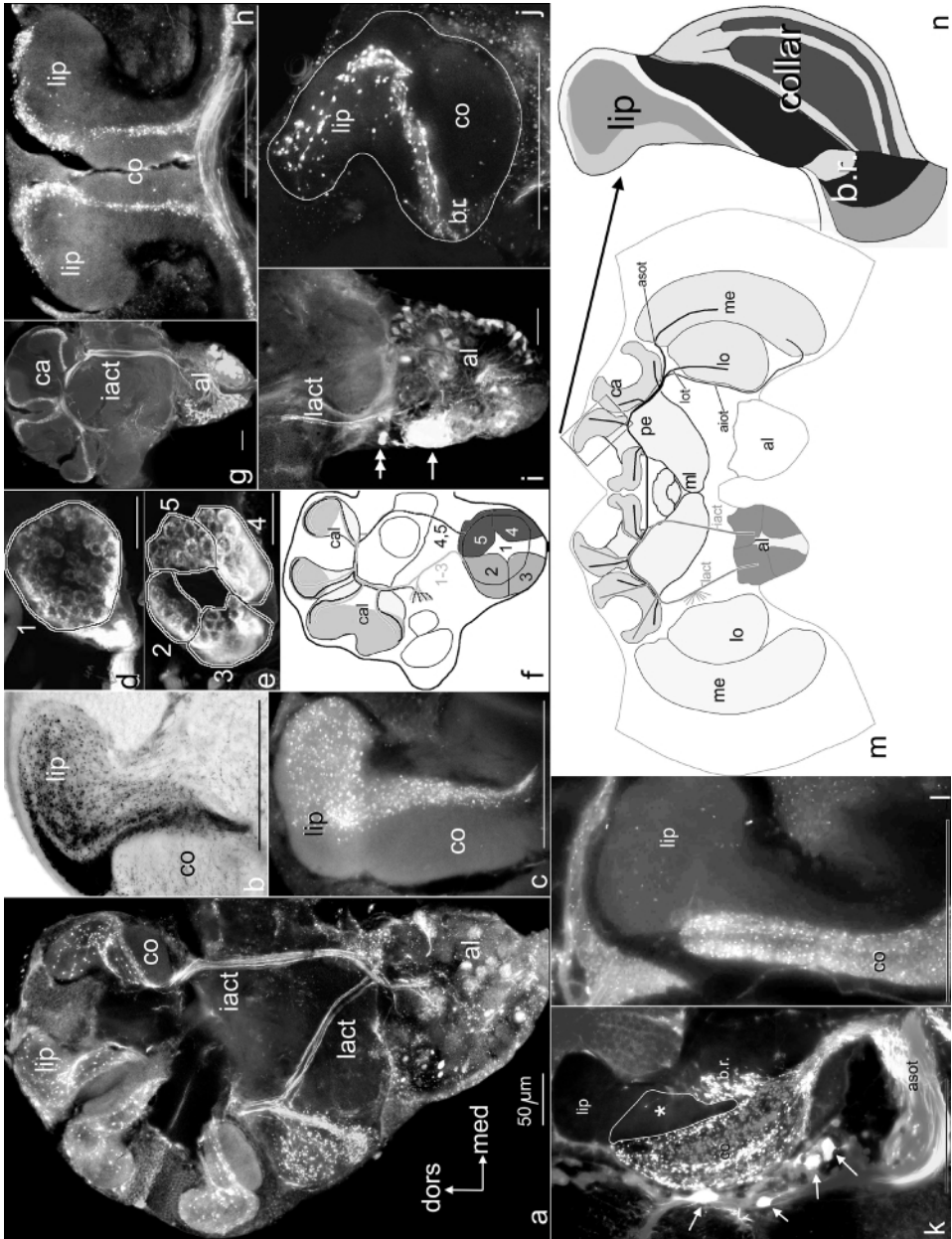
Tracing in ants a small number of projection neurons originating from only one or two clusters of glomeruli reveals that different layers in the lip region receive input from different glomeruli clusters (Fig. 1f). In specimens where projection neurons from the postero-median glomeruli cluster (cluster 4; Fig. 1e) were filled, the narrow outer layer of the lip was stained exclusively (Fig. 1g, h). In contrast, projection neurons originating from the postero-dorsal glomeruli cluster 2 (Fig. 1e) terminate in the median layers of the lip (Fig. 1i, j). Projection neurons mainly originating from the anterior-most glomeruli cluster (cluster 1; Fig. 1d) terminate in medium and inner lip layers but not in the outermost one (Fig. 1c). Taken together, glomeruli clusters 1–3 supply middle and inner lip layers via the lateral antenno-cerebral tract whereas the outer lip layer is supplied by glomeruli clusters 4 and 5 via the inner antenno-cerebral tract (Fig. 1m). Our evidence is not sufficient to assign a single lip layer to each of the five glomeruli clusters because we were not able to fill each cluster individually without tracer leakage into neighboring clusters. However, we suggest that glomeruli clusters do indeed supply individual layers in the lip. Compared to bees, the organization of the lip region appears to be more complex in ants.

Visual input to the calyx

Neurons originating from the lobula and medulla supply the calyx' collar. In honey bees, five outer layers of the collar (Fig. 1k) receive alternating input from neurons originating from the dorsal or ventral medulla, respectively. Each layer in the collar is supplied by a different class of medulla neuron that differ not only in their dorso-ventral distribution but also in the length of their dendritic branches probing the medulla [3]. Axons of these medulla neurons form the anterior superior optic tract (asot) and the anterior inferior optic tract (aiot; [16]; Fig. 1k, m). The innermost layer of the collar, marked by an asterisk in Fig. 1k, is supplied by neurons originating from the lobula and carried by the lobula tract (lot; Fig. 1m). These visual interneurons are described in more detail in [3].

In all ants, the collar is smaller and the lip region is larger compared to honey bees, corresponding to the much smaller optic lobes in ants. In carpenter ants, the collar comprises only three layers (Fig. 1m): two receiving input from the medulla and one receiving input from the lobula. The layers receiving input from the medulla are less well separated compared to the layers in bees, hence segregation of input from the dorsal vs. ventral medulla is gradual with considerable overlap. We did not find an anterior inferior optic tract (aiot) in ants. Like the entire visual system, visual input to the mushroom bodies is analogous yet reduced and less complex in ants compared to honey bees.

The basal ring is characterized by coarser neuropile texture compared to the lip and collar. In bees (Fig. 1n), it receives segregated input from the antennal lobes



(inner half), the lobula (middle layer) and the medulla (outer layer). In ants the basal ring is sometimes hard to discern. It also receives visual and antennal input, but it is not clear whether input from the medulla and lobula are segregated.

DISCUSSION

The mushroom bodies are involved in integration of multimodal information, learning and memory, and motor control [reviewed in 11, 18]. In most insects, they receive olfactory input from the antennal lobes. Direct visual input to the mushroom bodies is only prominent in Hymenoptera, and it is likely that it corresponds to the visual learning and orientation abilities of many bees, wasps and ants. Visual information is kept segregated at the input level of the calyx (at least 6 different visual input channels in the bees' collar). In the absence of physiological evidence and judging from behavioral experiments [14] we suggest that different layers in the collar process different visual qualities, such as texture, color or movement.

Like the collar, the lip is subdivided and comprises at least three layers in bees and probably more in ants. Together, the design and stratification pattern of the calyx reflects a species' behavior and sensory environment: bees and wasps fly and strongly rely on vision; their optic lobes and their mushroom body collars are large and comprise many different layers [the latter has only been demonstrated for honey bees [3, 9] and bumblebees]. In contrast, ant workers do not fly and in most species rely much less on vision than on olfaction and touch. Correspondingly, their optic lobes and collar regions are small but their lip regions are large and more complex.

In no case do we know about the functional differences between neurons supplying different calyx layers. Obviously, all visual input neurons respond to simple light and some to movement stimuli, but more complex stimuli have not been tested [7]. Likewise, most antennal lobe projection neurons in bees respond to odors and many

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Fig. 1. Antennal lobe input to the lip region in *C. ocreatus* (a–j). **a** Two major tracts, the lateral (*lact*) and the inner antenno-cerebral tract (*iact*) connect the antennal lobe *al* to the calyx' (*ca*) lip region. **b** Antennal input to the lip is layered; **c** injecting tracer in the anterior glomerular cluster (cluster 1 in **d**) only stains the inner part of the lip. Antennal lobe glomerular clusters 1–5 shown in **d** (anterior section) and **e** (posterior section). **f** Schematic showing glomerular clusters 1–3 connecting to the inner part of the lip and clusters 4 and 5 to the outer layers. **g** Filling glomeruli cluster 4 results in labeling of the outer lip layer (lip enlarged in **h**). **i** Filling glomeruli cluster 2 results in labeling of middle layers of the lip (lip enlarged in **j**). **k** Terminals of visual interneurons in the honey bee's collar *co* are labeled from the dorsal (*grey* terminals) and ventral medulla (*white* terminals). *Arrows* indicate cell bodies of some of the medulla neurons; *asterisk* denotes part of collar that receives input from the lobula (not labeled); *asot* anterior superior optic tract. **l** Lip and collar of a carpenter ant's calyx; layers in collar labeled from the medulla and lobula; **m** Schematic showing major tracts supplying the honey bee's calyx *ca* with input from the optic lobes medulla *me* and lobula *lo* (*aiot* anterior inferior optic tract, *lot* lobula tract) and from the antennal lobe (*lact*, *iact* lateral and inner antennal cerebral tract, respectively); **n** Different input layers in the calyx' lip, collar and basal ring. *b.r.* basal ring; *pe* peduncle, *ml* median lobe, *cal* calyx; scale bars 50 μm

also to mechanosensory stimuli or to sugar water [13], but general differences between neurons originating from different glomeruli clusters are not known except for sex-specific macroglomerular complexes which process pheromone information (reviewed in [12]). It is not even known whether the clustering of glomeruli has any functional significance; one study suggests that similar odors may be processed, but that temporal aspects are different between different glomeruli clusters [1]. In analogy to visual input segregation we suggest that antennal input to the lip may be segregated according to broad odor classes that may have different biological meaning, such as food odor, pheromones or aversive odors. In summary, we think that input to the mushroom bodies is not only modality specific but is segregated into many different, behaviorally relevant channels of sub-modalities that may support the associative function ascribed to the mushroom bodies, such as linking a particular color or pattern with food odor. The basal ring receives collaterals from the same neurons that supply the lip or collar, hence it does not process different kinds of sensory information. However, the basal ring comprises a different set of Kenyon cells [19], which are postsynaptic to the sensory input neurons. The basal ring might thus extract different information from the same input neurons (such as stimulus quality or amplitude, temporal aspects or coincidence of input).

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