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The insect mushroom body, an experience-dependent recoding device

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ABSTRACT

The insect mushroom body is a higher order integration center involved in cross-sensory integration and memory formation. The relatively large mushroom bodies of social Hymenoptera (e.g. bees) have been related to the demands of a social system and the neural processes required to allow the animal to navigate in an ever-changing environment. Here I review studies aiming to elucidate the neural processes that take place at the input and the output sites of the mushroom bodies and that underlie cross-sensory integration, associative learning, memory storage and retrieval. Highly processed sensory information is received at modality-specific compartments of the input site, the calyx. The large number of intrinsic neurons of the mushroom body receive multiple sensory inputs establishing combinations of processed sensory stimuli. A matrix-like memory structure characterizes the dendritic area of the intrinsic neurons allowing storage of rich combinations of sensory information. The rather small number of extrinsic neurons read out from multiple intrinsic neurons, thereby losing their sensory coding properties. The response properties of these neurons change according to the value of stimulus combinations experienced. It is concluded that the mushroom bodies transform the highly dimensional sensory coding space into a low dimensional coding space of value-based information. A model of such an experience-dependent recoding device is presented and compared with the available data.

1. Introduction

Insect brains contain a pair of neuropils composed of the processes of densely packed neurons, whose name "mushroom bodies" (MB) reflects their peculiar structure. Right from the time of their discovery these structures were connected to higher order neural functions of the insect brain. Kenyon (1896) who subsequently provided the first anatomical analysis of the structure put it in these words: "Ever since Dujardin (1850) discovered the mushroom bodies and pointed out the relation between their size and the development of insect intelligence, nearly every writer on the subject of the hexapod brain who has referred to the matter of intelligence has recognized the fact". However, even with the brilliant work performed to date in *Drosophila* (Davis and Han, 1996; Heisenberg, 2003; Perisse et al., 2013a), direct evidence that higher order neural functions are related to insect intelligence in a more general sense is still rather scant.

Several approaches have been perused in unraveling the potential involvement of the mushroom body (MB) in higher order integration, (1) a comparative approach searching for correlations between size and structure of the MB on the one side and life history of the respective species on the other; (2) an ontogenetic approach relating age and experience dependent alternations of the MB's structure to increasing behavioral traits; and (3) functional analysis of neural components and networks of the MB in order to elucidate the intrinsic neural operations with respect to behavioral demands. We have entertained the latter approach but I will first address the other two highly informative approaches. von Alten (1910) and Howse (1975) described a correlation between the social lifestyle of *Hymenoptera* and MB volume and its intrinsic organization: a more complex social life appears to be related to relatively larger and more structured MBs. Ehmer et al. (2001) found a correlation between MB and the social dominance of queens in paper wasps. It was also observed that parts of the MB in honeybees increase in volume with age and experience (Durst et al., 1994; Fahrbach et al., 1995; Withers et al., 1993) although no neuroblasts survived adult emergence (Ganeshina et al., 2000). Similar observations were reported for other *Hymenoptera*, e.g. ants (Gronenberg et al., 1996) and wasps (O'Donnell et al., 2004). Stieb et al. (2010) were able to trace this structural synaptic plasticity in the MB calyx to visual experience rather than to age dependence in the fast running desert ant *Cataglyphis*. An across species comparison in butterflies (Snell-Rood et al., 2009) revealed that the volume of the MB calyx was positively related to experience with the respective plant host. At the family level, the relative volume of the MB calyx and the antennal lobes following learning was positively related to overall success in finding the plant hosts.

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MB development in honeybees is influenced by the social environment in the first 8 days of adult life, with different environments having markedly different effects on MB size. Volume changes induced by the transition from within hive and foraging activities result from the increased branching pattern of dendrites in the visual region of the MB, the collar, leading to greater number of dendritic spines (Farris et al., 2001). However, honeybee queens also have relatively larger MBs although they do not forage. The idea that the juvenile hormone controlling the development of queens and the behavioral changes connected to this transition may directly influence MB volume in honeybees could not be corroborated (Fahrbach et al., 2003). Summarizing the result of several studies in honeybees Maleszka et al. (2009) concluded that MB volume adaptation is not limited to a particular time of adult life but rather it is influenced by the animal's behavior throughout its life.

An interesting but rather speculative hypothesis relates the evolutionary background of MB development to the necessity to precisely locate multiple places in the environment, as is the case in a parasitoid lifestyle, and may have served as a preadaptation for a social life which requires the insect to return to nest site (Farris and Schulmeister, 2010). Thus it is argued that the structural elaboration of the MBs was driven by the cognitive demands of host-finding behavior in parasitoids rather than sociality in Hymenoptera. As will be shown below there is still no clear evidence that the MBs are involved in the neural processes of spatial cognition, although multiple observations do suggest such a relation.

A first tentative indication that the MBs are involved in memory storage came from the finding that the time course of retrograde amnesia induced by cooling the calyces matches the time course of cooling the whole animal (Menzel et al., 1974). Around the same time the MBs in *Drosophila* were proven to be closely associated with olfactory learning (Heisenberg et al., 1985). Recently Hourcade et al. (2010) established a close relationship between synaptic structures in the olfactory input part of the MB, the lip region, and consolidation of olfactory memory in the honeybee. They showed that the density in presynaptic complexes, the olfactory microglomeruli, increases as a specific olfactory long-term memory is formed, while the volume of the neuropil remains constant. Stable structural synaptic rearrangements, possibly including the growth of new synapses, appear to underlie storage of stable memory in the insect and mammalian brain and require gene transcription. Visual learning involved in memorizing the hive location during initial orienting flights may also involve transcription-dependent synaptic reorganization. Kiya et al. (2007) showed that neural activity, as assessed by expression of an immediate early gene called kakusei, of a MB neuron subtype defined by the size of their somata, the small-type Kenyon cells (KCs), is prominently increased in the brains of dancer and forager honeybees. A different subtype, the small-and large-type KCs, increase in the brains of re-orienting workers.

Malun et al. (2002) interfered with the normal development of the MB using hydroxyurea to block differentiation of MB neuroblasts in early larvae. Side-specific classical discriminative olfactory conditioning of the proboscis extension response (PER) in the adult bee was then applied to address the question of whether reduced MB structures on one side of the brain interfere with this form of olfactory learning. All their experimental groups learned equally well to discriminate and respond to a rewarded (CS+) but not to an unrewarded (CS−) conditioned stimulus during acquisition and retention tests. Thus, partial MB lesions do not affect this form of elemental olfactory learning. However, more complex forms of olfactory learning were impaired.

Learning and memory formation as a component of intelligence is certainly closely related to MB function. Here I shall address the question of how memory is stored at the input and the output sites of the MB. A comparison of the coding strategies of these learning-dependent networks will allow us to propose a general re-coding scheme of the MBBs.

2. Methods

2.1. Preparation of the animals, odor stimulation and conditioning

Foraging (female) honeybees (*Apis mellifera carnica*) were caught at the entrance of the hives 24 h prior to an experiment. They were fed 30% sucrose solution and kept overnight under 12 h light and 12 h dark cycle in a humid box at approx. 25–27 °C. Next day they were cold-anesthetized on ice and fixed inside plastic restraining tubes such that only the mandibles, proboscis, and antennae could move freely (Bitterman et al., 1983). For Ca^{2+} imaging and electrophysiological experiments, the scapes of the antennae were fixed onto the head using the low temperature melting wax eicosane (Sigma) such that only the flagellae could move. This procedure did not interfere with odor learning. Odor stimulation was computer-controlled, using a stimulus device with separate channels for each odor as described elsewhere (Galizia et al., 1997). Depending on the particular experiment, up to seven different odors (1-octanol, 1-hexanol, 1-nonanol, 1-heptanol, heptanal, octanal, limonene) were used. In some of the experiments two of the odors were used for differential conditioning, a third as a control odor, and the learned behavior was monitored concurrently with the recordings. The olfactomter was placed in front of the bee such that the end of the outlet was at a distance of approximately 5 cm to the bee's head. A constant airstream (speed 1.5 m/s) was sent through a Teflon tube (6 mm in diameter). The control of magnetic valves via Spike2 software (Cambridge Electronic Design, Cambridge, UK) allowed the addition of a particular odor to the airstream. An exhaust pipe behind the animal ensured that odor did not accumulate.

Color illumination as a context stimulus was provided by a light guide connected to a lamp with filters for green (Schott, Mainz, Germany; Filter VG), yellow (GG 570), and blue light (BG 12). The exit of the light guide was placed in front of the bee beside the odor pump. All stimuli were controlled by the Spike2 software. In most of the experiments three odors (sometimes five) were used as stimuli (stimulus forward-paired with sucrose feeding: CS+, specifically unpaired stimulus: CS−, novel stimulus or stimuli: not presented during conditioning). An experiment usually consisted of five conditioning trials and one or more test trials (extinction trial) at different intervals after the last trial. PER occurring to the conditioned stimuli in the course of conditioning allows the characterization of behavioral learning. To detect and quantify a PER the muscle (M17) innervating the proboscis was recorded. Spike trains of the muscle activity during training sessions and/or test sessions were used to quantify PER.

2.2. Ca^{2+} imaging

Foraging honeybees were prepared for the experiments as described previously (Szyszka et al., 2005). Briefly, clawed KCs were selectively stained with the dextran-conjugated calcium indicator Fura-2 (10,000 MW; Molecular Probes, Eugene, USA) which was injected into the axons of clawed KCs in the ventral alpha lobe. MB extrinsic neurons (ENs) of the protocerebral calycal tract (PCT) were stained in the same way by injecting the dye at the point of exit from the alpha lobe (Haehnel and Menzel, 2012). Ca^{2+} imaging of these KCs was performed by ratiometric measurements of the Ca^{2+}-sensitive dye FURA-2 excited at 340 nm and 380 nm. Emitted fluorescence was measured using a 410 nm dichroic mirror and a 440 nm long pass filter. Images were recorded with a sampling rate of 6 Hz using a TILL-Photonics imaging setup (Till Vision,