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Review article

Fine structure of synaptic sites and circuits in mushroom bodies of insect brains

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ABSTRACT

In the insect brain, mushroom bodies represent a prominent central neuropil for multisensory integration and, crucially, for learning and memory. For this reason, special attention has been focused on its small chemical synapses. Early studies on synaptic types and their distribution, using conventional electron microscopy, and recent publications have resolved basic features of synaptic circuits.

More recent studies, using experimental methods for resolving neurons, such as immunocytochemistry, genetic labelling, high resolution confocal microscopy and more advanced electron microscopy, have revealed many new details about the fine structure and molecular contents of identifiable neurons of mushroom bodies and has led to more refined modelling of functional organisation. Synaptic circuitries have been described in most detail for the calyces. In contrast, the mushroom bodies' columnar peduncle and lobes have been explored to a lesser degree. In dissecting local microcircuits, the scientist is confronted with complex neuronal compartmentalisation and specific synaptic arrangements. This article reviews classical and modern studies on the fine structure of synapses and their networks in mushroom bodies across several insect species.

1. Introduction

Structural analysis of neural tissue beyond the limits of light microscopic studies was first established by electron microscopy. Those early, but high quality ultrastructural images of nervous tissue (De Robertis and Bennett, 1955; for review, see De Camilli et al., 2001) already made it possible to postulate general principles regarding the organisation of neuropil, including descriptions of synapses, both in vertebrates and invertebrates. Synaptic connectivity amongst several neural partners in immediate vicinity led to a fundamental concept in neuroscience; that of complex local circuitry and hence a novel view of functional integration (for reviews, see Pearson, 1979; Schmitt, 1979; Shepherd and Grillner, 2010).

Thus, compared to studies only relying on light microscopy, electron microscopy heralded a unique contribution towards understanding the organisation of neural networks. And, in very recent times analysis of fine structure at a resolution in the nanometre range comprises a wide range of techniques, complemented by computer assisted data analysis, that make it possible to directly visualise synaptic molecular complexes and their dynamics (Fahrbach, 2006; De Camilli et al., 2001; Berry et al., 2008; Burette et al., 2012; Pech et al., 2015).

Publications describing the general organisation of insect nervous tissue, such as those of Burrows (1996), Meinertzhagen (1996) and Strausfeld and Meinertzhagen (1998), have profoundly contributed to our understanding of circuit complexity in their emphasis on descriptions of uniquely identifiable neurons in the context of their synaptic and electrophysiological properties (e.g. Burrows, 1996). The property of uniqueness has usually been ascribed to large nerve cells that provide intersegmental connections amongst ganglia and between the brain and ganglia.

Such relatively large neurons are, however, far outnumbered by local neurons that connect circumscribed neuropils in the brain and ganglia, and these neurons are further outnumbered by small local neurons, such as found in the optic lobes, the brain's central complex and, particularly, in the mushroom bodies (Rensch, 1959; Witthöft, 1967; Strausfeld, 1976, 2012; Schürmann, 1987). These tiny local 'isomorphic' neurons, which are constrained to a specific neuropil, may still be considered as a unique type of neuron,

Abbreviations: DCV, dense core vesicle; SV, synaptic vesicle.

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although rather than being unique in terms of their singularity, they are unique with regard to their sharing the same general form and connectivity (Tanaka et al., 2008). And it is in the mushroom bodies that this type of uniqueness is most pronounced.

Mushroom bodies have been variously studied across a variety of insects: cockroaches, locusts, crickets, bees, ants, butterflies and the fruit fly *Drosophila melanogaster*. While the nervous systems of insects generally conform to an ancestral ground pattern, the brains of these so called 'model species' show considerable species-specific traits, especially in their brains, and such differences are particularly reflected in the organisation of their mushroom bodies (Heisenberg, 1998; Strausfeld et al., 1998, 2009, 2012; Farris and Sinakevitch, 2003; Fahrbach, 2006; Groh and Rössler, 2011). For example, the fruit fly can be considered as highly specialised in that it has relatively small mushroom bodies in comparison to its other brain neuropil areas. Cockroaches and many Hymenoptera, such as honey bees, represent the other extreme, having massive mushroom bodies with obvious divisions into specific subunits (Sánchez, 1933; Mobbs, 1985; Schürmann, 1987; Strausfeld, 2012).

Mushroom bodies (for nomenclature of brain neuropils, tracts and cell types see Ito et al., 2014) are paired protocerebral neuropils that comprise several different classes of neurons (for examples see Figs. 1 and 2). The bulk of the mushroom body is composed of "intrinsic neurons", so named because all of their integrative processes lie within the volume of the mushroom body. Kenyon cell somata typically reside above the calyces. Some additional types of intrinsic neurons have somata distant from mushroom bodies (Ito et al., 1998; Strausfeld, 2002; Tanaka et al., 2008; Liu and Davis, 2009). Processes of extrinsic neurons invade specific parts of the mushroom bodies connecting these with other regions of the brain's protocerebrum (Figs. 1 and 2). A mushroom body's neuropil is divided into parts: paired calyces, their peduncle which then provides a vertical and medial lobe (Strausfeld, 2002). The calyces and lobes are further subdivided into subcompartments and even smaller volumes designated by arrangements amongst parts of intrinsic and extrinsic neurons (Fig. 2B–E), the latter reaching the lobes by either single neurons or in groups within a discrete tract (Mobbs, 1982, 1985; Ito et al., 1998). Inputs to the calyces, called "projection neurons" reach the neuropil by discrete tracts and contribute within the calycal neuropil characteristic synaptic complexes (for details see Chapter 3.2) that are distinct from the synaptic organisation of extrinsic neurons in the lobes (Leiss et al., 2009a,b; Butcher et al., 2012). All mushroom body neuropil compartments are equipped with synapses (Figs. 3 and 4).

Detailed knowledge about mushroom body fine structure is indispensable for reviewing classical work done before the advent of electron microscopy and more recent and current research. The present review thus focuses on the structural and ultrastructural investigations of synapses, synaptic circuits and the texture of mushroom body neuropils. The review is restricted to mushroom bodies of imagos and deals only briefly with dynamic features of neurons. Information about the evolution, development, growth and plasticity of mushroom bodies can be found in many other publications (e.g. Heisenberg et al., 1995; Farris et al., 2001; Farris and Sinakevitch, 2003; Groh et al., 2006; Groh and Meinertzhagen, 2010; Groh and Rössler, 2011; Zhao et al., 2008; Dobrin et al., 2011; Pasch et al., 2011; Strausfeld, 2012; Eickhoff and Bicker, 2012; Pech et al., 2015; Montgomery et al., 2016).

Analyses of mushroom bodies based on classical and modern methods have led to many varied and interesting hypotheses about the functional significance of these prominent brain regions. For studies on functions, the reader is referred to studies of Erber et al.

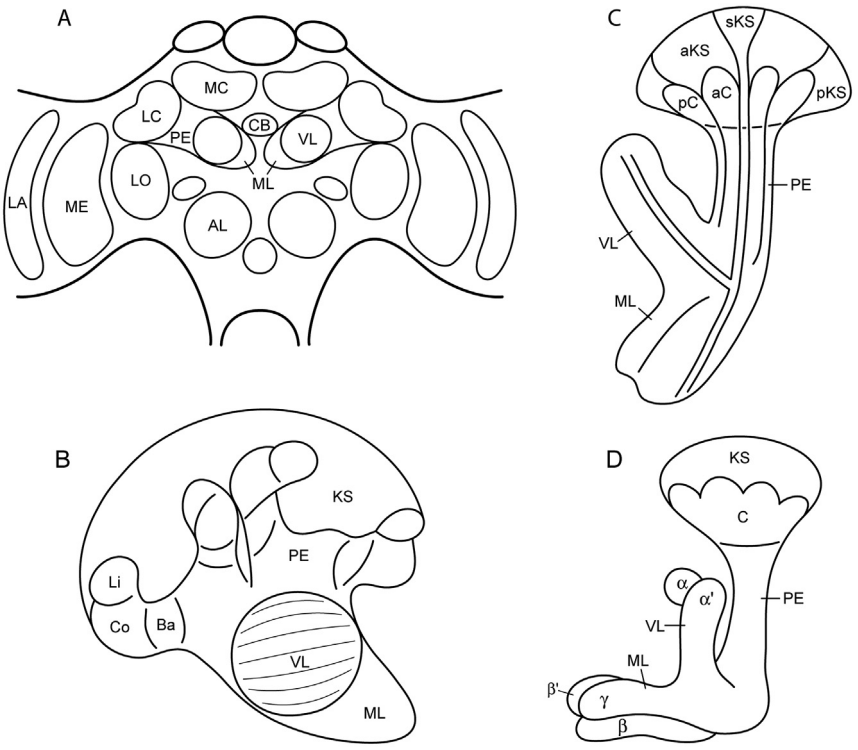

Fig. 1. Schematic diagrams of mushroom bodies with main neuropil areas and subdivisions. A, B. Brain and mushroom bodies of worker bee *Apis mellifera*. C. Cricket *Gryllus bimaculatus*; mushroom bodies. D. *Drosophila melanogaster*, mushroom bodies with α,α′,β,β′,γ subdivisions, after Fahrbach (2006). Abbreviations: LA lamina, ME medulla and LO lobula of optic lobe, AL antennal lobe, CB central body, LC lateral and MC medial calyx, KS Kenyon cell somata layer, aKS anterior layer, pKS posterior layer of Kenyon cell somata, aC anterior and pC posterior calyx, Li lip, Co collar, Ba basal ring of calyx, PE peduncle, ML medial lobe, VL ventral lobe.

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