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GABAergic SYNAPTIC CONNECTIONS IN MUSHROOM BODIES OF INSECT BRAINS*

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Distribution and synaptic connections of GABA fibres in neuropil parts of the mushroom bodies in brains of crickets *(Gryllus bimaculatus)* and bees *(Apis mellifera)* were investigated by immuno-light and electron microscopy. In the inner calyx neuropil of cricket mushroom bodies, GABA fibres are pre- and postsynaptically connected with proximal Kenyon cell dendrites, indicating synaptic contacts differing from those of the Kenyon cell dendritic tips in the peripheral microglomeruli of the calyces. A more complex interaction of GABAergic fibres and Kenyon cell dendrites than assumed before is shown. In the mushroom bodies of bees, dendritic like strata of GABA fibre projections contribute to the subcompartmental layers of the vertical lobe. The GABA-immunostained fibre profiles exhibit pre- and postsynaptic sites as well and can therefore not be considered purely postsynaptic dendritic neuron parts. The micromorphology and synaptic contacts of the dendrites and dendritic like arborizations are seen as parts of local circuits within mushroom bodies.

Keywords: Insects – mushroom bodies – GABA – immunocytochemistry – synapses

INTRODUCTION

Numerous studies have investigated structure, development and functions of the mushroom bodies (MBs), a prominent multisensory neuropil in insect brains [2, 14], crucially involved in learning and memory [8]. The paired MBs consist of intrinsic local neurones, the Kenyon cells, and extrinsic projection neurones (PNs), connecting the MBs with other brain areas. Though there is an increasing information on the

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structure of MBs and their equipment of identified neurones with transmitters [1, 3, 4, 5, 9, 16, 17, 20], synaptic connectivity of MB nerve cells is poorly understood. Special attention has been focussed on the distribution, synaptic contacts and physiology of the so-called GABAergic feedback neurones [1], connecting the calyces, which represent the main input site of the MBs with the vertical (α-lobes) and medial lobes (β-lobes), considered the main output areas of the MBs [14]. Using immunocytological light and electron microscopy we found out a more complex GABAergic connectivity at KC dendrites in the calyces of the cricket, and pre- and postsynaptic sites of dendritic like GABAergic arborizations in the MB lobes of bees.

MATERIAL AND METHODS

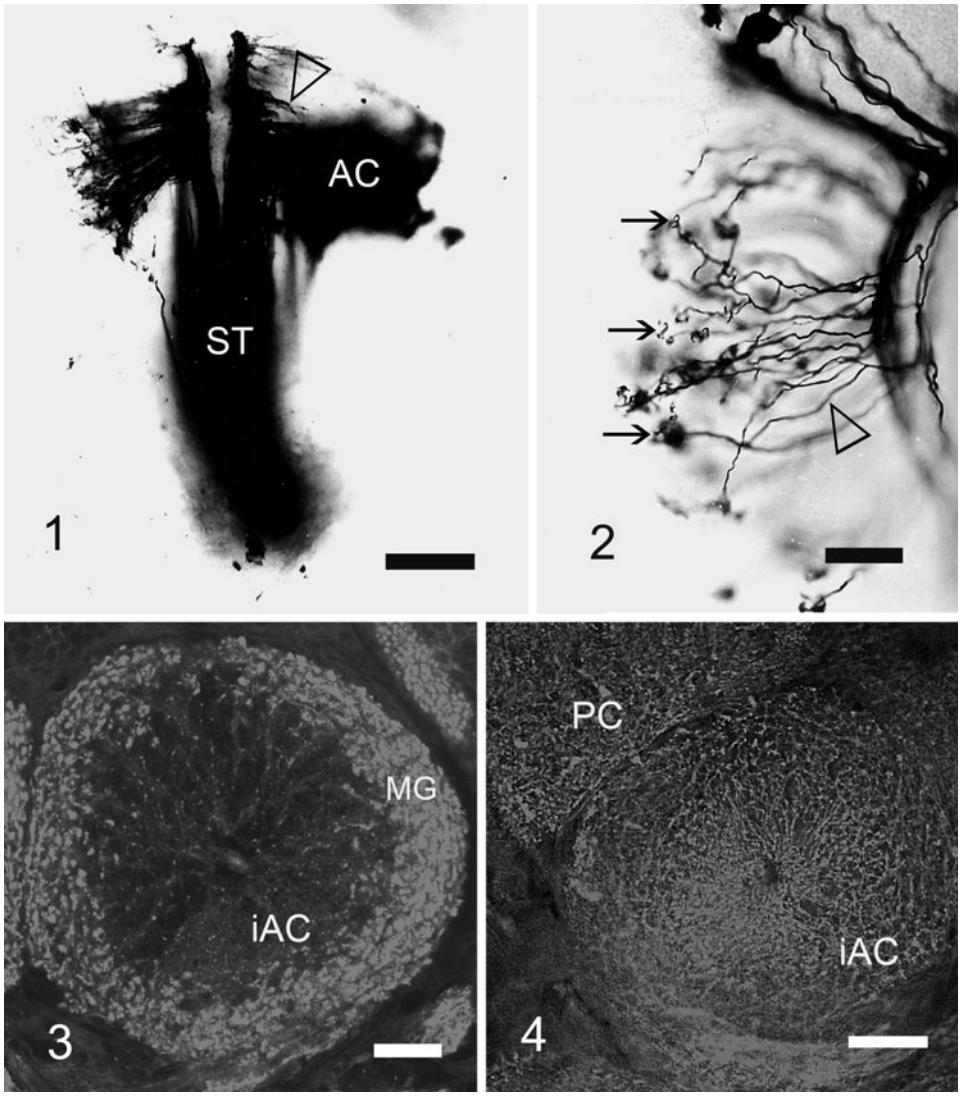
Staining experiments were carried out with adult male and female crickets (*Gryllus bimaculatus*), aged one week and with forager honeybees (*Apis mellifera*), from the institute's breeding colonies. Brains were fixed and processed for immuno-light and electron microscopy according to protocols, given elsewhere [3, 16]. We employed anti-GABA-antiserum from Incstar, Stillwater USA, and anti-GABA-KLH (keyhole-limpet-hemocyanin), kindly provided by Drs. J. Hildebrand and T. Kingan, Tucson, USA; anti-*Drosophila*-synapsin I: SYNORF1 (a gift from Drs Buchner and Hofbauer, Würzburg, Germany). For immuno-light and -electron microscopy serial brain sections (paraffin technique or vibratome sections) were used. Golgi-impregnations of cricket brains delivered KC images [13]. Immunostained slices were inspected by conventional light, fluorescence or confocal microscopy, and digitized pictures were further processed with Adobe Photoshop program.

RESULTS

Mushroom body calyces of the cricket

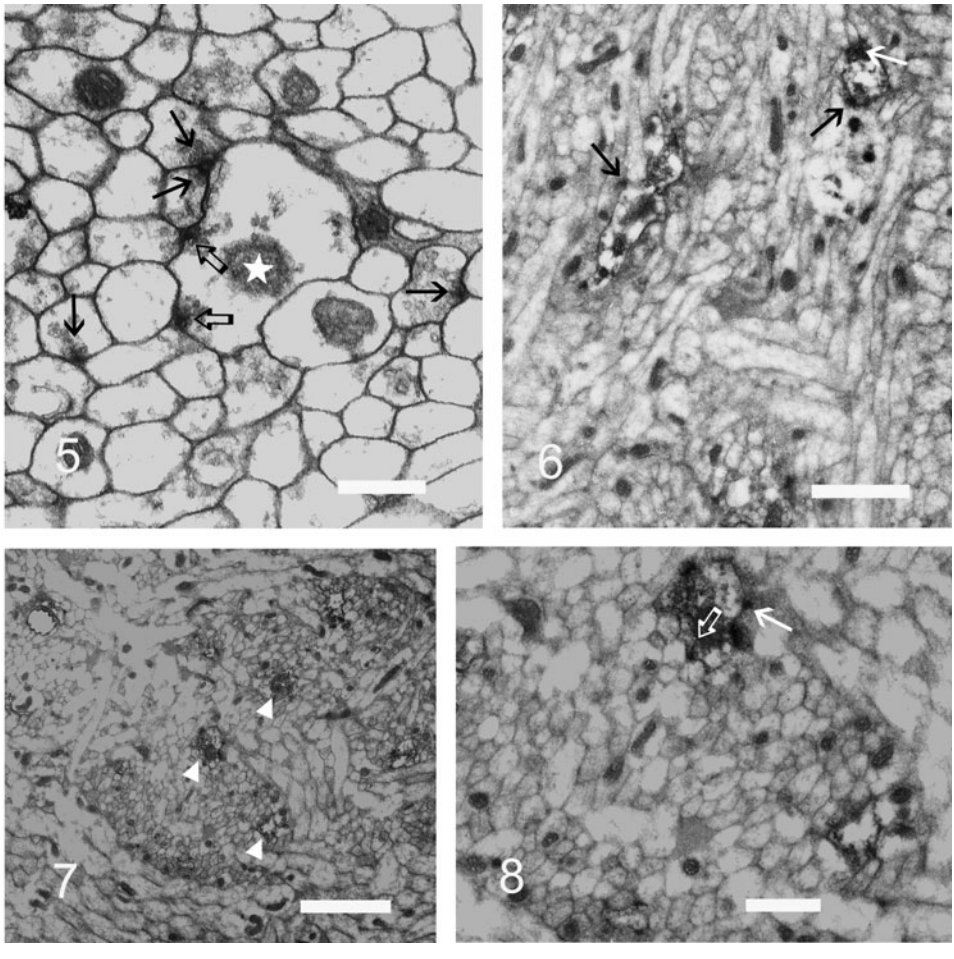
Cricket MBs show distinct subcompartmental organization of the calyces, stalk and lobes, based on the arrangement of KC populations (KC I-KCIII types), classified from Golgi-impregnations [13]. The calyces (Figs 1–8) are divided into an anterior (AC) and a posterior calyx (PC). The AC harbours KC I and II dendrites, whereas KC III dendrites are restricted to the PC.

The cup shaped AC (Figs 3, 4) is further divided into an inner part where the proximal KC dendrites are gathered into fascicles, radially projecting into the outer microglomerular layer. In this layer, KC dendritic spines (Figs 1, 2) synapse with presynaptic extrinsic boutons. Anti-*Drosophila*-synapsin immunostaining reveals small spots indicating putative presynaptic sites in the inner AC (Fig. 3) and in the stalk. PC and AC show a dense network of GABA-immunopositive fibres (Fig. 4) of different origins [17]. Confocal images show GABAergic boutons in the microglomerular layer and blebs in the inner AC calyx (Fig. 4) and in the stalk.



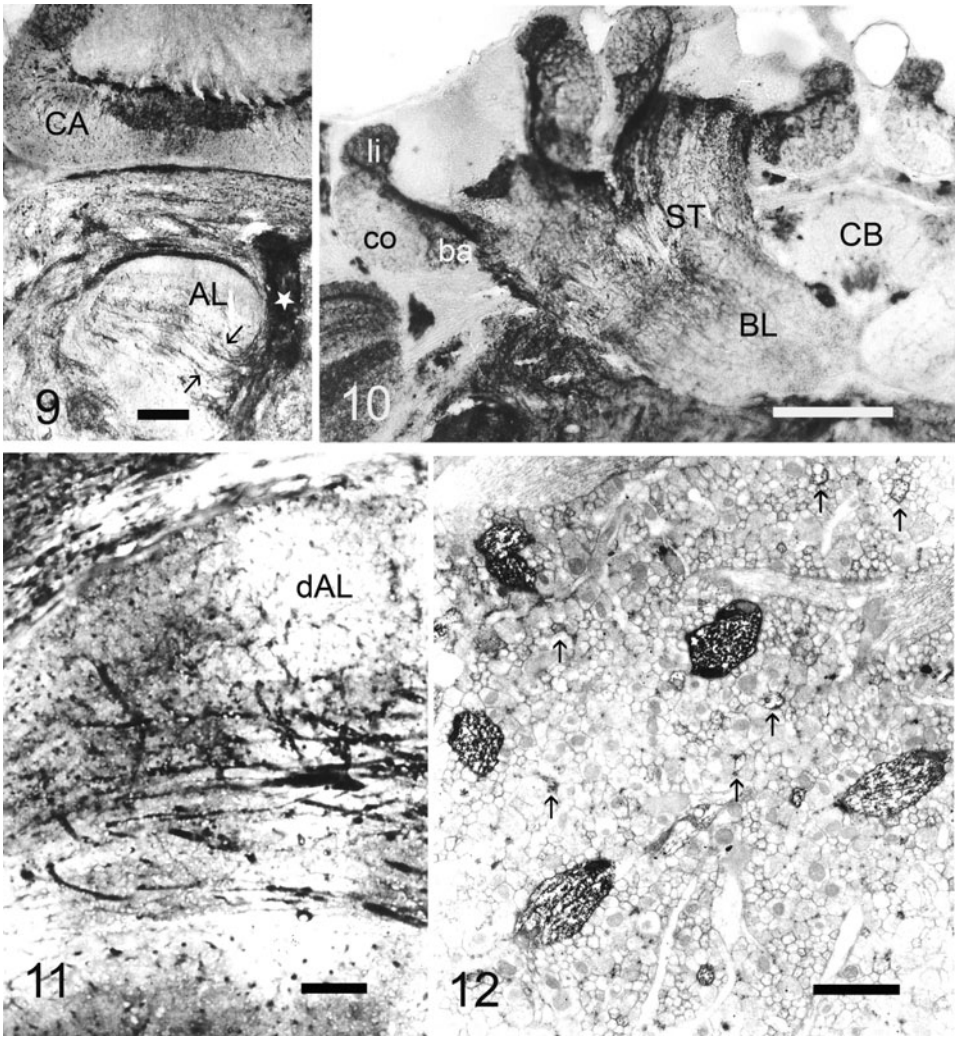
Figs 1–4. Cricket, mushroom bodies, light microscopy. Figs 1, 2. Golgi impregnations of Kenyon cells, frontal sections; dendrites with claw-like terminals (arrows) restricted to the peripheral anterior calyx (AC) emerge from axon bundles descending to the stalk (ST); the tiny proximal dendrites (triangles) do not exhibit blebs. Figs 3, 4. Laserscan images of the anterior calyx, horizontal sections. Fig. 3. Synapsin immunostaining marks presynaptic elements; note peripheral ring of densely packed boutons in the microglomerular layer (MG) and presynaptic small spots in the inner anterior calyx (iAC), corresponding to the zone with proximal Kenyon cell dendrites. Fig. 4. GABA immunostained fibres in the AC, which radially project to the peripheral layer of microglomeruli; GABA staining is also seen in the posterior calyx (PC). Scale bars Figs 1, 3, 4: 50 μm; Fig. 2: 25 μm

Synaptic complexes are detected in the peripheral microglomerular layer as well as in the inner parts of the AC (Figs 5–8). In the latter, small presynaptic profiles (diameters 0.1–0.4 μm) exhibit dyadic synapses to other fibre profiles. Reciprocal synaptic coupling of larger fibre profiles and other small profiles, among them proximal KC dendrites, is often encountered (Fig. 5). The radial KC dendritic fascicles always contain some GABA-immunostained profiles synapsing with immuno-negative fibre profiles, considered KC proximal dendrites with *en passant* synapses

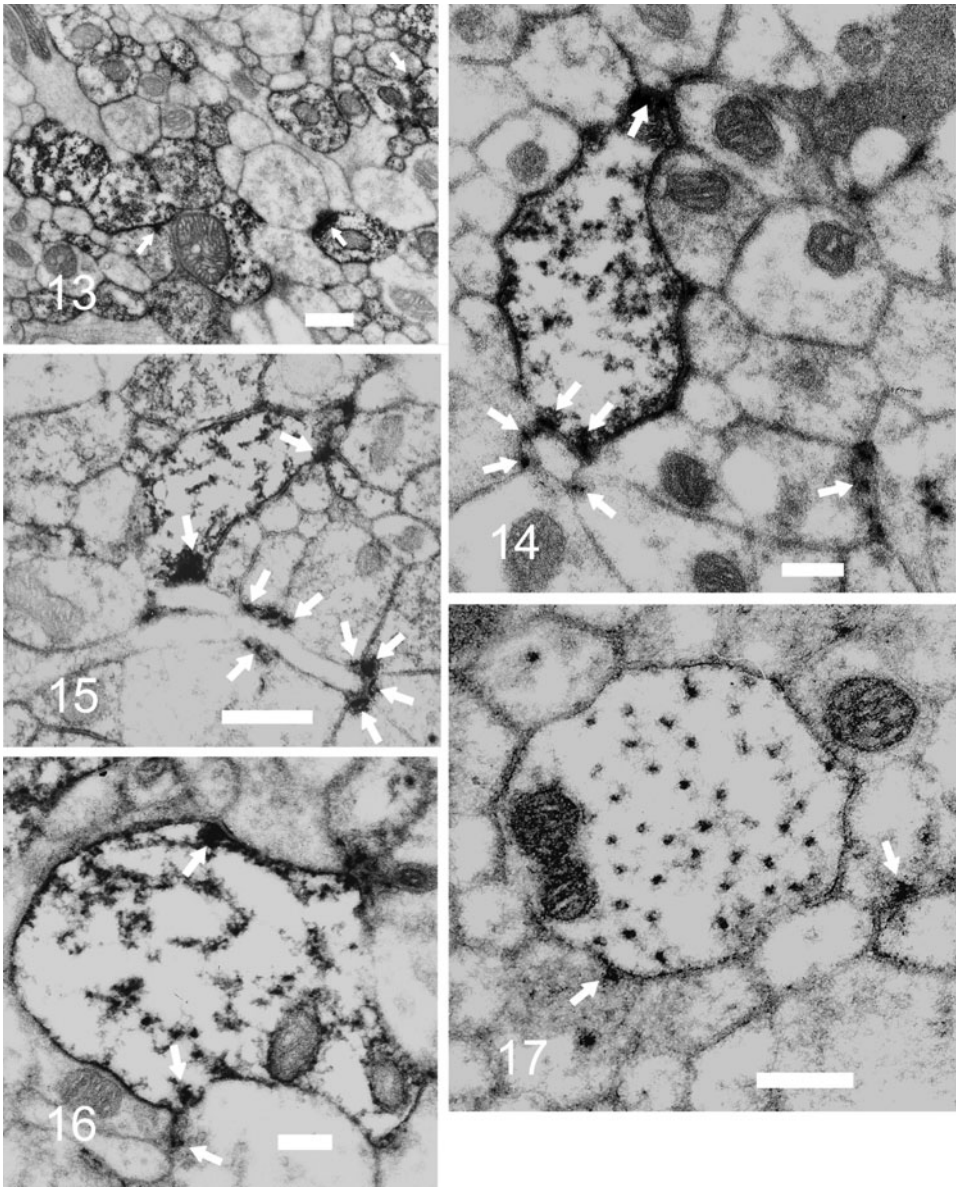


Figs 5–8. Cricket, mushroom bodies, inner anterior calyx. Fig. 5. Conventional transmission electron micrograph; a profile (star) with presynaptic sites (thick arrows) receives synaptic inputs (small arrrows) by surrounding fibres; note reciprocal synapses. Figs 6–8. Electron micrographs of GABA immunostained fibres. Fig. 7. Fascicles of unstained Kenyon cell dendrites and GABA profiles (triangles). Fig. 8. Magnification from Fig. 7, presynaptic sites marked by arrows. Scale bars Figs 5, 8: 1 μm; Fig. 6: 2 μm; Fig. 7: 4 μm

(Figs 6–8). This mode of synaptic circuit differs from the presynaptic coupling of GABA profiles to KC dendritic spines, which do not show presynaptic organells in the peripheral microglomeruli.



Figs 9–12. Bee, mushroom bodies, GABA immunostaining, Figs 9–11 light microscopy, Fig. 12. electron microscopy, frontal sections. Fig. 9. The protocerebral-calycal tract (star) forms dendritic like projections (arrows) in strata of the α-lobe (AL); the ascending massive arbor of the tract supplies subcompartments of the calyx (CA). Fig. 10. Differential GABA immunostaining in the subcompartments lip (li), collar (co) and basal ring (ba) of the calyx, the stalk (ST) and β-lobe (BL); central body (CB). Fig. 11. Arborizations of GABA fibres in the dorsal α-lobe (dAL) form small profiles (black dots) running in parallel to small Kenyon cell axons (compare Fig. 12). Fig. 12. GABA fibres of diverse diameters (arrows) are scattered among a majority of unstained small profiles. Scale bars Figs 9, 11: 20 μm; Fig. 10: 50 μm; Fig. 12: 2 μm



Figs 13–17. Bee, mushroom bodies, α-lobes (Figs 13–15, 17), β-lobe (Fig. 16), GABA immunostaining, electron microscopy. Figs 13. GABA fibre profiles with input and output sites (presynaptic arrows). Fig. 14. Presynaptic sites marked with arrows; convergent input of unstained fibres and a GABA immunostained profile to an unstained element. Fig. 15. Presynaptic sites marked by arrows; synaptic output sites of a GABA stained profile. Fig. 16. Presynaptic sites marked by arrows. A GABA immunostained fibre shows output sites. Fig. 17. Presynaptic sites marked by arrows; a GABA immunostained fibre receives input from an unstained profile. Scale bars Figs 13, 15: 0.5 μm; Figs 14, 16, 17: 0.25 μm

Synaptic contacts of GABA fibres in the mushroom body lobes of the bee brain

The extrinsic GABA fibres found in all MB neuropil compartments exhibit different intensity of immunostaining, distribution and morphology (Figs 9–12). About 110 GABA neurones [1] are part of a massive bifurcating protocerebral-calycal tract (PCT) which first invades the vertical lobe to form dense dendritic-like layered fields, and minor projections are found in the the median lobe. The dendritic like structures of GABA fibres contribute to the strata of KC axon populations [18]. The other branch of the ascending PCT supplies the calycal subcompartments with GABAergic fibres [5, 15]. In the vertical lobe, GABA-immunostained fibres (Figs 12–15, 17) representing a minority among abundant immuno-negative fibre profiles form fine terminal branches (with diameters below 0.1 μm). These immunonegative fibres, presumably mainly KC profiles, filled with synaptic vesicles and presynaptic sites, converge to postsynaptic fibres without synaptic organelles. The GABA profiles with synaptic sites distributed in the dendritic like strata were encountered in three forms: as presynaptic elements with dyadic synapses, entoured by postsynaptic immuno-negative profiles, which often exhibit other input synapses; furthermore as mere postsynaptic fibres, and, finally, with pre- and postsynaptic specializations, coupled to unstained fibre profiles, presumably many of them KC fibres (Figs 13–17). From our approach we cannot decide whether these modes of synaptic connectivity have to be attributed to three different types of GABA neurones. Inspection of some samples also revealed pre- and postsynaptic GABA fibres in the median lobe (Fig. 16) and in the stalk. Our morphological observations do not allow to propose a purely and predominant postsynaptic status for all GABAergic arborizations in the lobes and stalk of MBs of the bee.

DISCUSSION

The discussion concentrates on the synaptic connectivity of KC dendrites in the cricket MB calyx neuropil and on dendritic like strata of GABA fibres in the lobes of bee MBs. KC dendrites are known to receive massive input from presynaptic boutons of projection neurones of diverse brain parts [11, 14]. These boutons, surrounded by and coupled to tiny KC dendritic endings, are central elements in the so-called microglomeruli of calyces. In addition, GABAergic boutons presynaptic to the KC spines contribute to a single microglomerulus [20], as found in all species so far investigated. The microglomeruli therefore show complex synaptic circuitry. The scheme remains uncomplete, because other extrinsic constituents shown by transmitter immunocytochemistry [9, 14] cannot yet be incorporated in terms of synaptic connectivity. The current scheme of microglomeruli presents the KC dendrites as purely postsynaptic elements. Putative synaptic sites shown by synapsin immunocytochemistry do however occur in the inner anterior calyx neuropil (this study), where proximal KC dendrites are gathered into fascicles. Immunocytochemistry now shows

pre- and postsynaptic sites connecting KC and GABA fibres, among the latter elements which must not be feedback nerve cells [19]. We tentatively suggest for one functional mode a selective inhibitory effect of GABAergic elements on KC dendrites at different levels, and on other extrinsic elements, not yet identified. Presynaptic coupling of a KC dendrite to a GABA fibre could serve for inhibition by reciprocal connectivity and for divergent GABAergic modulation of other proximal postynaptic KC dendrites.

A dendritic nature has been assigned to stratified arborizations of extrinsic neurones in the lobes, including so-called feed back and GABAergic nerve cells [6, 7, 10, 14]. Synaptic connectivity of GABA fibres in the lobes cannot be interpreted to serve exclusively for selective recurrent information flow via the PCT back to KC dendrites in the calyces, initiated by activated sets of presynaptic convergent Kenyon cell fibres in the lobes. Presynaptic sites of GABAergic fibres in the lobes point to output to KCs and other, extrinsic fibres. Electron microscopy thus provides insight into synaptic circuits at a level of resolution still not depicted by light microscopy and not generally accessible for physiological approaches. We see the KC dendrites and the dendritic like projections in the MB lobes as parts of local microcircuits, originally proposed for vertebrate and invertebrate nervous systems from ultrastructural studies [12].

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