Chapter 3

MB Architecture and Innervation

3.1 Introduction

The locust MB consists of 50,000 intrinsic neurons, the Kenyon cells (KCs). These neurons' somata are clustered in the inside of a cup-shaped area, the calyx, formed by the KC dendrites. KC axons project as a set of densely packed parallel fibers down the pedunculus of the MB and bifurcate into its two lobes: the ventrally projecting α lobe and the medially projecting β lobe. The current literature speaks little about the morphology of locust KCs and how it relates to the overall structure of the MB. So far, KCs have been described as having a primary neurite which branches at the periphery of the calyx into a number of secondary processes within the calyx. Their synaptic structures have been studied using electron microscopy and immunostaining: KCs have only post-synaptic specializations in the calyx [42], and both pre and post-synaptic specializations in the pedunculus and lobes [44].

The locust has been described as having a single calyx per MB; this calyx is divided into the ventral primary calyx and the dorsal accessory calyx. Weiss [101] described the primary calyx in orthopterans as having an annular central ring around the pedunculus which contains the fibers of KCs on their way to the pedunculus. He also described a thin internal zone of the primary calyx, containing the primary KC neurites, and that receives extrinsic fibers. PNs constitute the primary afferent input to the MB calyx. Their axons project profusely in the primary calyx. The accessory calyx input has been shown to originate from projection neurons of the globular lobe,

which neighbors the AL, and itself, receives input from the gustatory receptors [17].

Extrinsic cells of the MB are efferent cells with somata outside the MB and neurites within its neuropils (the lobes, pedunculus and calyx). These cells have been described briefly in one orthopteran family, the crickets, as being a heterogeneous population of which 16 have been morphologically identified [78]. In the locust, a few types of such extrinsic cells have been identified and briefly studied for functionality [47]. Some extrinsic cells in the locust MBs are GABA-ergic; there is also indirect evidence (NADPH-diaphorase immunoreactivity) of cell signaling via nitric oxide in the lobes [67].

As described in section 1.5.1, anatomical data and analysis of the locust olfactory system, especially as it pertains to the mushroom body (MB), are few. A number of functional studies have been performed on this system (as described in section 1.5.3) which describe information processing in the locust olfactory system in great detail. The aim of the current study is to describe both those structures whose functions have been described and help elucidate the areas to study next for functionality.

All methods described in chapter 2, (electrophysiology, intracellular fills, image processing and Golgi stains) were used to generate the data explored in the current chapter. In particular, the issues addressed include determining anatomical similarities and differences between locusts and other well studied insects (as described in section 1.3) as well as identification of MB calycal divisions, KC anatomical descriptions, KC and PN connectivity within the calyx, extrinsic cell identification and morphology, and peduncular and lobe structures.

This chapter describes three calycal divisions of the primary calyx and their relationship to KC dendrites and extrinsic cell processes. This classification adds a new division to the zones defined by Weiss [101], and subtracts one, namely the *zona interna*. Further, PN innervation of calycal divisions is described, and areas of connections of PNs to extrinsic cells outside of the MB are demonstrated. On a gross scale, the β lobe is shown to have two divisions based on KC axon morphology and those divisions are related to extrinsic cell morphology as well.

3.2 Results

3.2.1 Calyx Architecture

Calycal structure was studied by analysis of confocal stacks capturing the autofluorescence of the brain. The neuropils of the locust brain have considerable emission above 500 nm when excited by laser light of 488 nm. Different densities and compositions of structures leads to differences in their emission patterns. Therefore, it is possible to see the divisions between these structures in confocal images. The analysis of 100 stacks of intrinsic fluorescence of locust brains revealed the presence of four separable calyx structures. The previously defined primary calyx can be broken down into three parts: the outer-calyx (OC)(analogous to Weiss' *zona externa*), the mid-calyx (MC) (analogous to the central ring) and the inner-calyx (IC), which has not been previously described (figure 3.1). The OC extends in a three-quarters doughnut shape from the ventral surface of the brain and contains the dendrites of the KCs (figure 3.1 - yellow). The MC is a disc, relatively thin in the anteroposterior axis, that fills in the hole of the doughnut formed by the OC (figure 3.1 - orange). The IC forms two humps sometimes spherical and sometimes oblong - on the anterior surface of the MC (figure 3.1 - green). Both the MC and the IC contain the dendrites of KCs and their axons as they pass through to the pedunculus. The OC is the largest calycal neuropil by volume, forming about two-thirds of the primary calyx, followed by the MC and the IC. These data agree with previous information on the extent and position of the accessory calyx [8] [101].

3.2.2 KC morphology

The three divisions, OC, MC, and IC, are further identifiable by the extent and structure of the KC dendritic arbors. These cells were analyzed by intracellular fills with LY and AF (n = 79), as described in chapter 2. Figures 3.2(b) and 3.3 show one particular KC filled with LY, with one set of branches (red arrow) restricted to only the IC (figure 3.2[b]). This cell also arborizes extensively in the OC. Another KC,

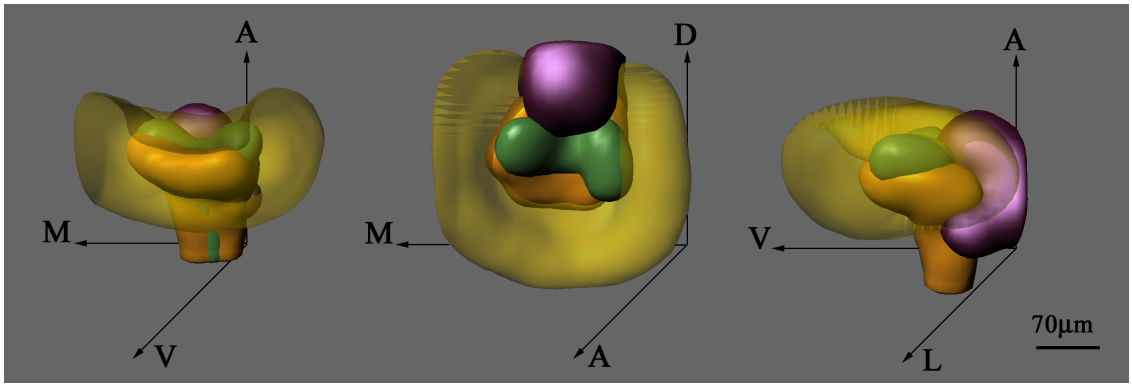
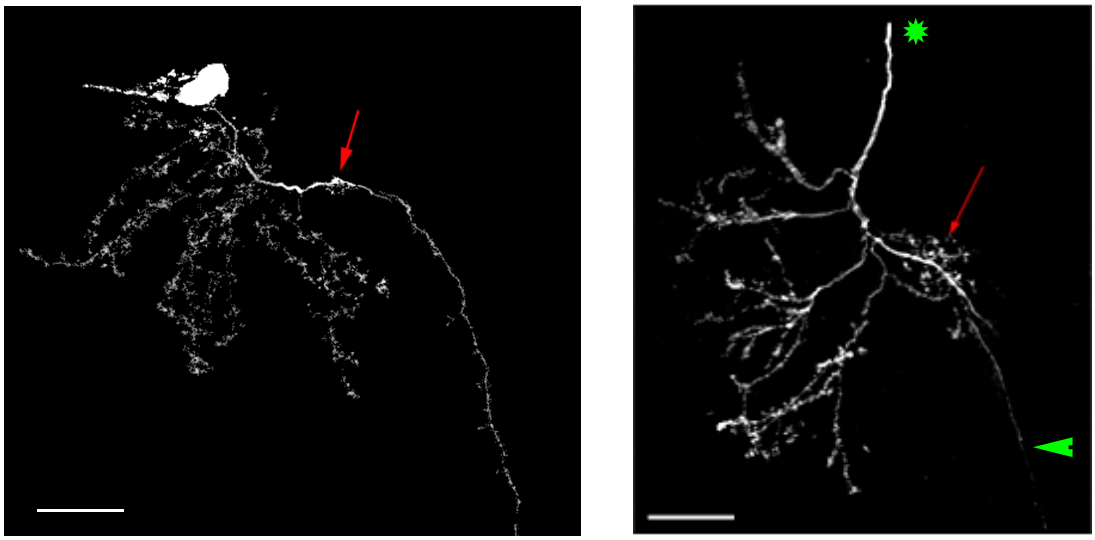


Figure 3.1: Three-dimensional Imaris reconstruction of the locust calyx showing newly defined divisions, depicted in three views. From left to right, facing out of page, are the ventral, anterior and lateral views (or front, top and side views respectively). The outer-calyx (OC) is depicted in yellow, the mid-calyx (MC) in orange, the inner-calyx (IC) in green, and the accessory calyx in purple. The dorsoventral axis is depicted by 'D' at the dorsal end and 'V' at the ventral end. The mediolateral axis is depicted by 'M' and 'L'. The anteroposterior axis is depicted by 'A' and 'P'. Scale bar is equal to 70 μm.

depicted in figures 3.2(a) and 3.4, sends a small specific set of short processes to the MC only (figure 3.2(a)), red arrow and figure 3.4, green star). This KC too has other extensive arbors in the OC. Of 79 KCs filled, all had some or all of their dendrites in the OC; 6% also had some dendrites restricted to the MC, and 13% also had some dendrites restricted to the IC. None had dendrites within the accessory calyx, perhaps because these cells lie deep within the perikarya or have properties which differ from KCs within the other three divisions, making them hard to fill using current methods.

Based on the above intracellular fills of KCs, at least three broad classes of cells can be defined based on calycal innervation: cells that arborize exclusively in the OC (OC-exclusive cells), those that arborize in the OC and MC (OC/MC cells), and those that arborize in the OC and IC (OC/IC cells). Other permutations (e.g., cells that projected to both the MC and IC) were not seen, though we cannot rule out their existence. The MC and IC also contain many passing axons of OC-exclusive cells as they project to the pedunculus. These in-transit processes have no spines or varicosities in the IC or MC, but the existence of synaptic contacts onto or from them cannot be excluded.

KC dendritic morphology differs within the three divisions, OC, MC, and IC. The IC houses dendrites that tend to be stubby and densely covered with spines and varicosities. Dendrites of the OC, by contrast, tend to be less densely covered with decorations and each branch tends to be much longer. MC neurites are generally short and covered with many varicosities and few spines. Golgi stains also confirmed both the newly defined calycal structures, as well as dendritic morphological differences between them. The red star in figure 3.5(a) shows the IC in a 15 μm thick Golgi stained section. The MC is shown in another Golgi section in figure 3.5(b), outlined by a red rectangle.



(**a**) (**b**)

Figure 3.2: Ray-traced reconstructions of two non-OC-exclusive KCs viewed from the ventral surface of the brain. These same two cells are reconstructed in the following figures within their respective calyx structures. (**a**) An OC/MC cell. Small cluster of MC stubby dendrites are indicated by the red arrow. Scale bar is equal to 15 μm. (**b**) An OC/IC cell. Cluster of IC projections is indicated by the red arrow. Note that the cell body is not shown; the green star indicates the primary process leading to the soma; the green arrow indicates the KC axon. Scale bar is equal to 30 μm.

3.2.3 PN Axons in the Calyx

To understand the possible olfactory connectivity and inputs to the various divisions of the calyx, PNs were also filled with LY. Of 150 attempts, 65 were successful PN

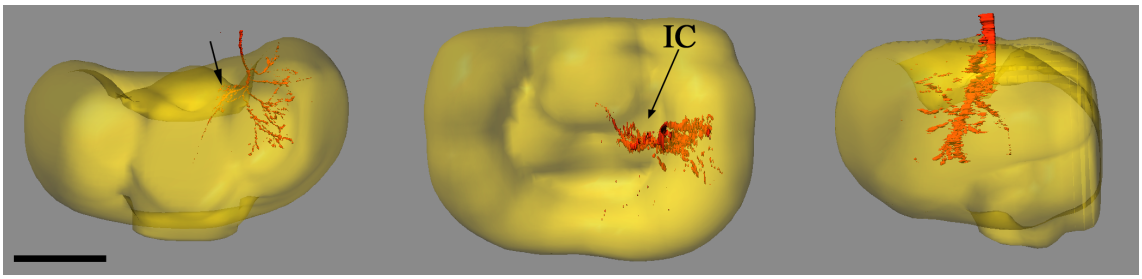


Figure 3.3: Three-dimensional Imaris reconstruction of a KC with branches in the IC (arrow) and OC. All calyx neuropil divisions are rendered in the same color. The same three views are depicted as in figure 3.1 - from left to right: front, top and side views. Scale bar is 100 μm.

fills with full AL projections, five of which were complete fills including calycal and lateral horn projections as previously described [8] and [42]. Nine more of these 65 cells showed partial fills in the calyx. In all 15 PN axon calycal fills — whether full or partial — PN innervation was restricted to the OC only. PN axon collaterals were never seen to innervate the MC, IC, or accessory calyx (figure 3.6). Of the 5 complete fills, PN axon collaterals covered the entirety of the OC; each PN then appears to have the potential to contact any KC that innervates the OC (also see section 4.4.2 and figures 4.52 and 4.53).

3.2.4 Peduncular Structure and KC Morphology

The pedunculus of orthopterans has been shown to consist of three separate fiber bundles [101]. Observations of the intrinsic fluorescence of this structure confirms this finding. There is a loose transformation between the calyx and the pedunculus structures; the IC and accessory calyx mostly flow into the center bundle of the pedunculus, while the OC and MC flow into its two outer bundles (figure 3.7). Autofluorescence also shows that these fiber bundles coalesce into one large bundle at the end of the pedunculus, distal to the calyx (3.8). Fibers then rearrange as they enter the lobes.

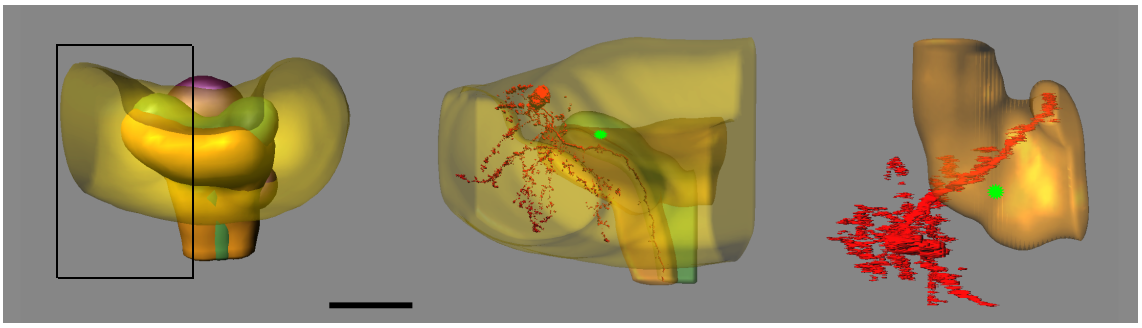
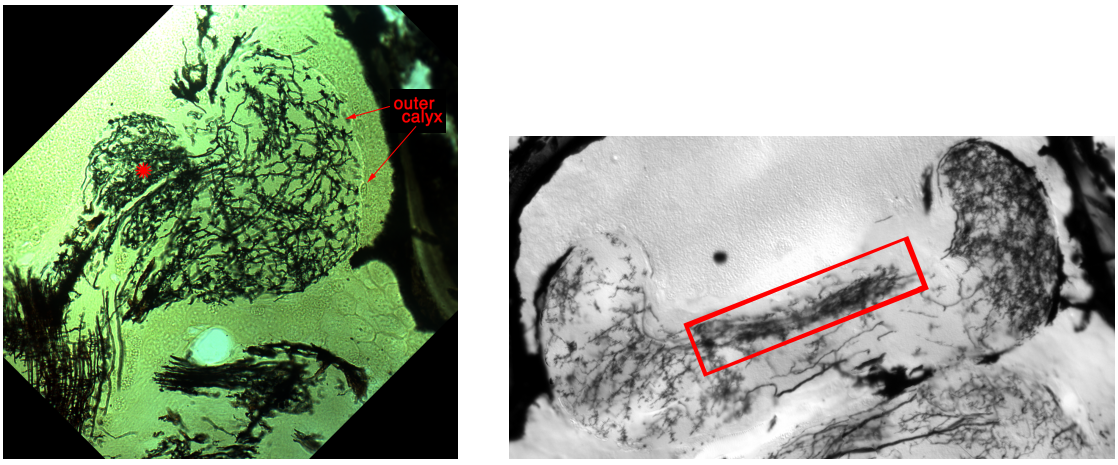


Figure 3.4: Three-dimensional Imaris reconstruction of half of the locust calyx showing a KC that innervates the mid-calyx and the outer-calyx. Left image shows the frontal view of the calyx with three divisions, in different colors. Black box shows the area of the calyx reconstructed in the right two images. Middle image shows the KC reconstructed with all calycal divisions: OC in yellow, MC in orange and IC in green, from the front view. The green asterisk shows the short MC dendrites just outside the OC. Right image shows the KC reconstructed with the MC only, viewed from the top, with the green asterisk again showing the position of the MC dendrites.



(**a**) (**b**)

Figure 3.5: Golgi-stained frontal sections of the locust MB depicting calycal divisions. (**a**) Lateral side of the calyx with a red star indicating the position of the IC. A clear division can be seen between this and the outer-calyx. (**b**) A superficial section of the calyx where the thin MC can be seen. Part of the MC is outlined by the red rectangle.

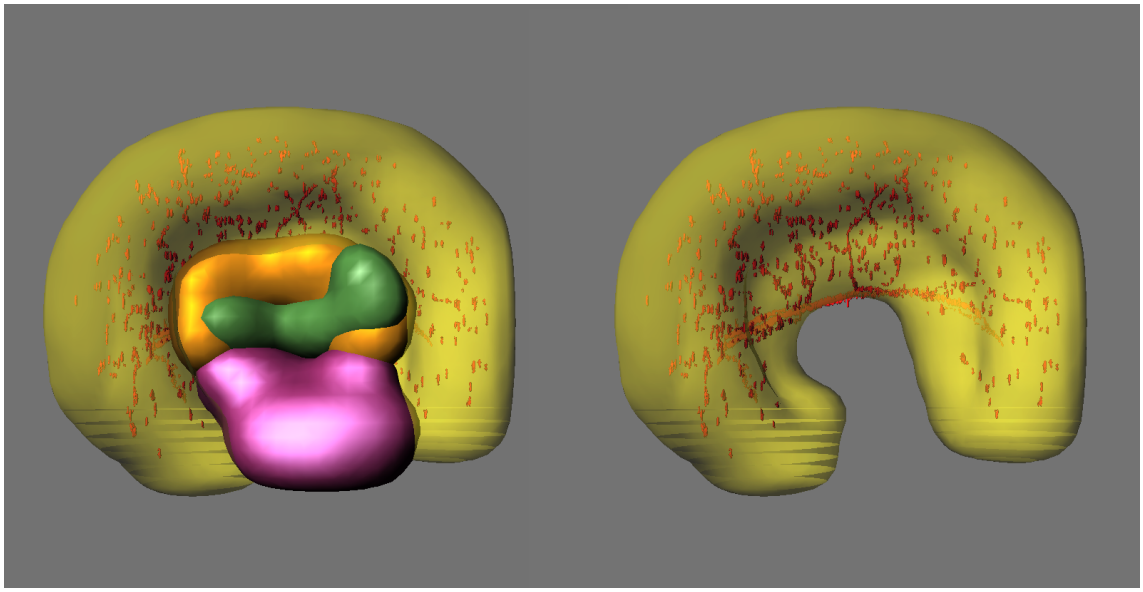


Figure 3.6: Imaris reconstruction of the calyx and two PNs innervating it, viewed from the anterior or bottom aspect. On the left, the OC is shown in yellow, the MC in orange, the IC in green and the accessory calyx in purple. The right image shows PNs in red innervating the OC exclusively.

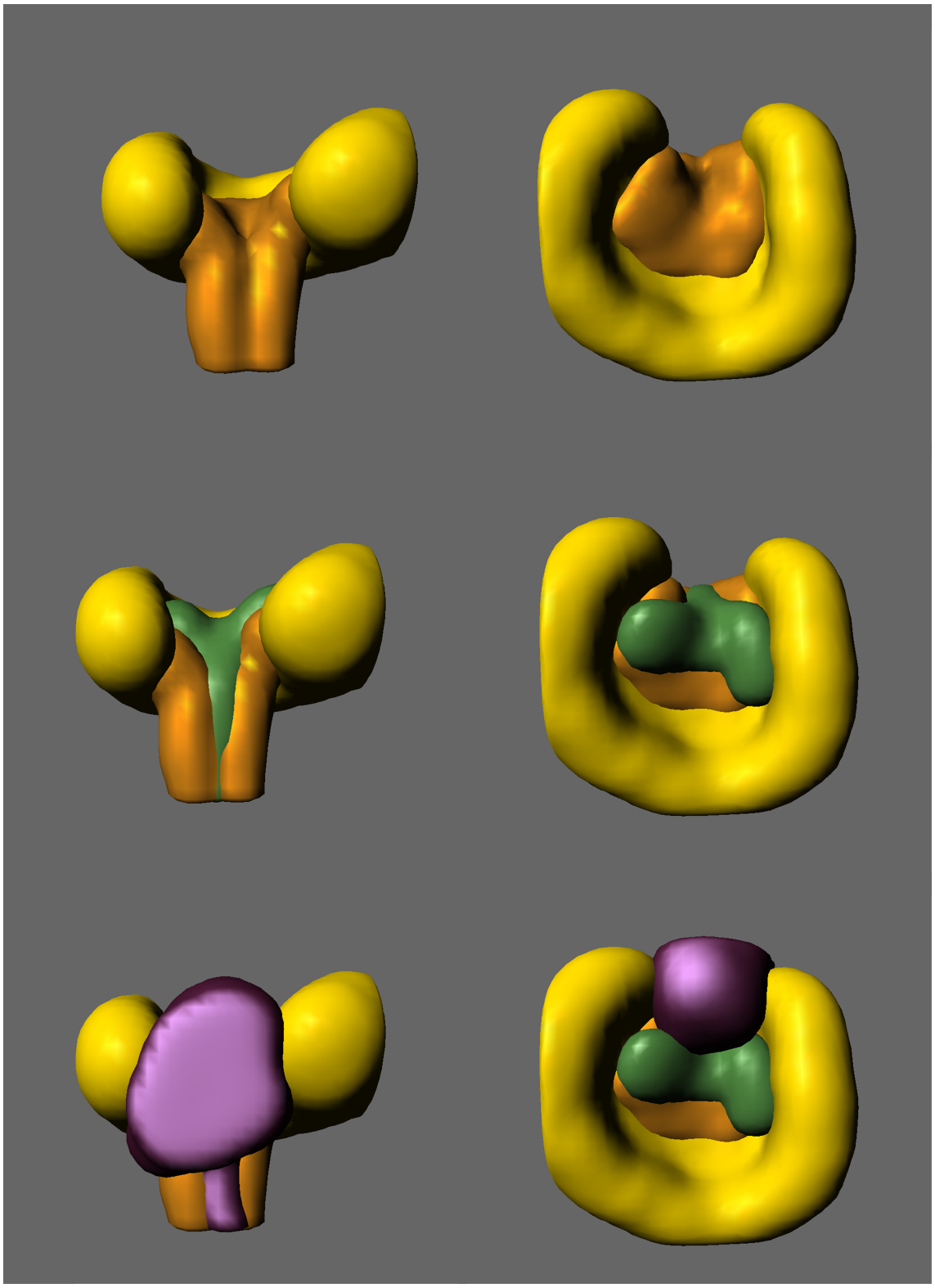


Figure 3.7: Calyx division relationship to the pedunculus. Each row shows the calyx and posterior portion of the pedunculus from the ventral or back aspect(left column) and posterior or top(right column) aspect. Top row shows the regions of the calyx (OC in yellow and MC in orange) that feed into the two peripheral fiber bundles (also in orange). The middle row shows the area of the central bundle that the IC feeds into, and the bottom row shows the accessory calyx (purple) and its peduncular innervation.

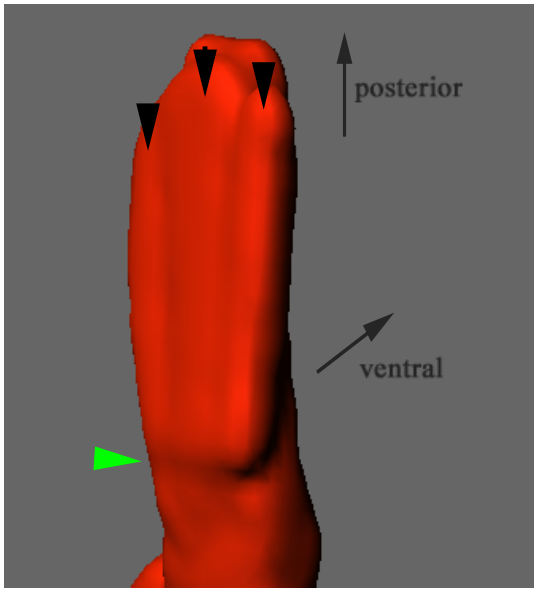
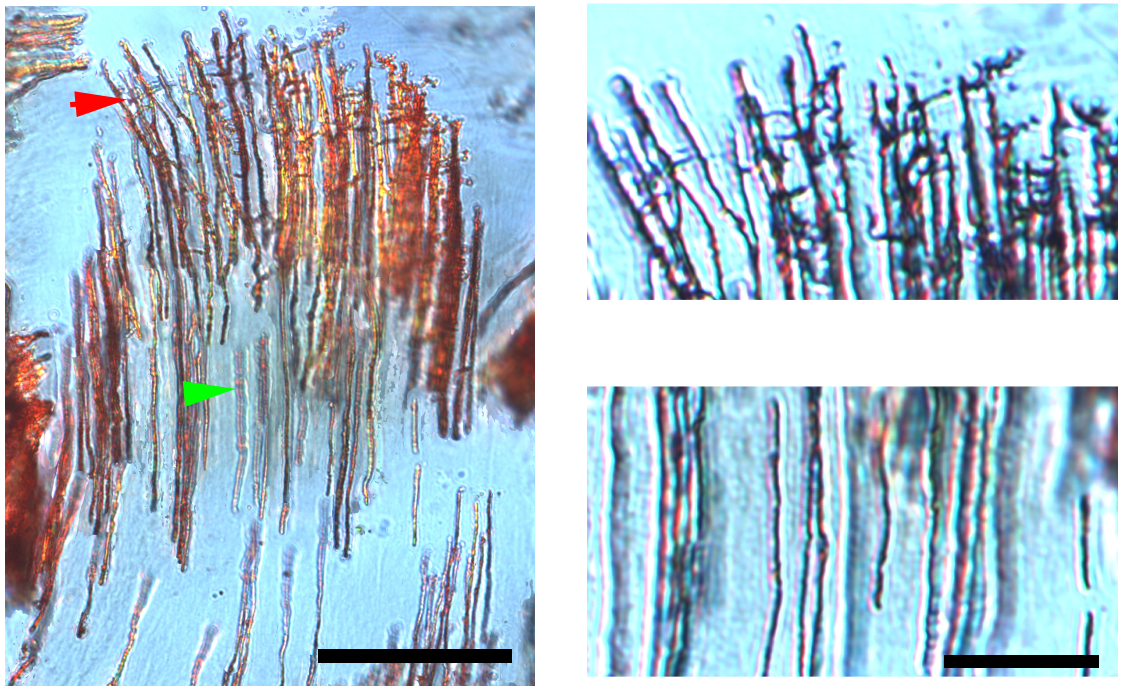


Figure 3.8: Imaris reconstruction of the pedunculus and β lobes viewed from the dorsal aspect. Three black arrows point to the three fiber bundles that emerge from the calyx. Green arrow shows the point at which the fibers join together.

Fills of the KCs conform loosely to the above stated transformation. KCs that innervate the IC always send axons down the central bundle of the pedunculus, while OC-exclusive and OC/MC cells send axons into all fiber bundles of the pedunculus. Golgi stains show that KCs of the accessory calyx send axons down the dorsal aspect of the core fiber bundle (figure 3.9).



(**a**) (**b**)

Figure 3.9: Slightly oblique 15 μm Golgi-stained section, showing a comparison of KC axons in the periphery of the pedunculus vs. the core. (**a**) Red arrow indicates peripheral spiny axons and green arrow indicates the core smooth axons. Scale bar is 20 μm. (**b**) High magnification images of the spiny peripheral axons (top) and smooth core axons (bottom). Scale bar is 5 μm.

Analysis of Golgi stains also reveals two distinct kinds of KC axon types. One set of axons are densely covered with spines, while the other set are smoother with fewer and shorter spines. This confirms results shown in 1996 by Leitch and Laurent [44]. It is further clear in Golgi stains that these two types of axons segregate into separate parts of the pedunculus. Spiny axons tend to occur on the periphery of the pedunculus, while smoother axons are found mainly in the core. This is most obvious in the posterior two-thirds of the pedunculus, distal to the calyx, and the lobes.

3.2.5 MB Lobe Structure and KC Morphology

Golgi stains indicate that structural divisions occur in the β lobe and coincide with KC axon morphology. Spiny axons segregate out to the ventral and superficially medial regions of this lobe, while smooth axons segregate out to the dorsal, lateral and core regions (figure 3.10). Figure 3.11(b) shows a Golgi stained section of the β lobe exhibiting spiny axons medially and smooth axons posteriorly. These two areas of differing KC axon morphology seem to be matched for volume. Spiny axons are seen to rearrange and cross-over in the heel of the MB as they enter into the lobes. Due to high staining density in Golgi sections, it was not possible to determine which KC soma and dendritic tree individual axons corresponded to. Intracellular fills of KCs down to the lobes were rare; a few fills of cells that innervated the IC and traveled in the pedunculus core crossed at the heel into the ventral β lobe with spiny axons. Intrinsic autofluorescence imaging reveals a non-fluorescent core in the spiny axon division of the β lobe. Neither KC nor extrinsic cells were found to innervate this area (figures 3.12 and 3.13).

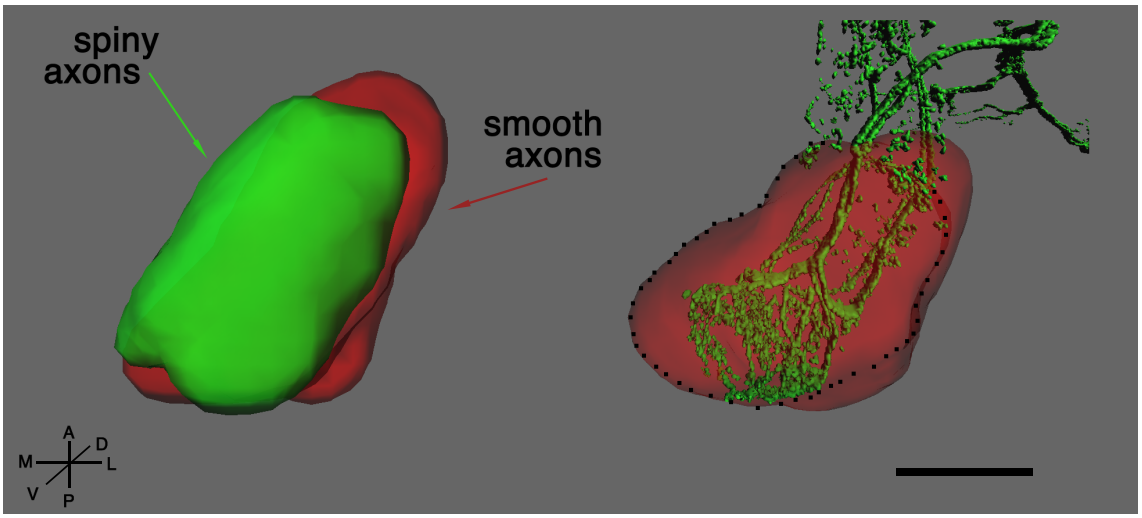
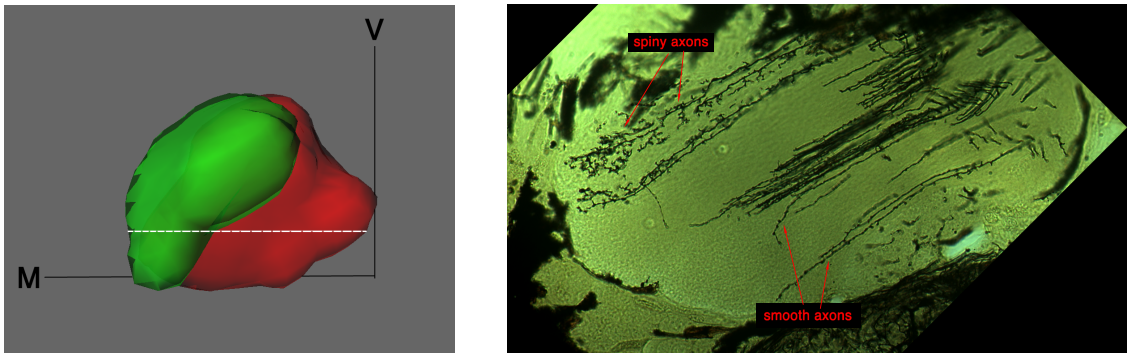
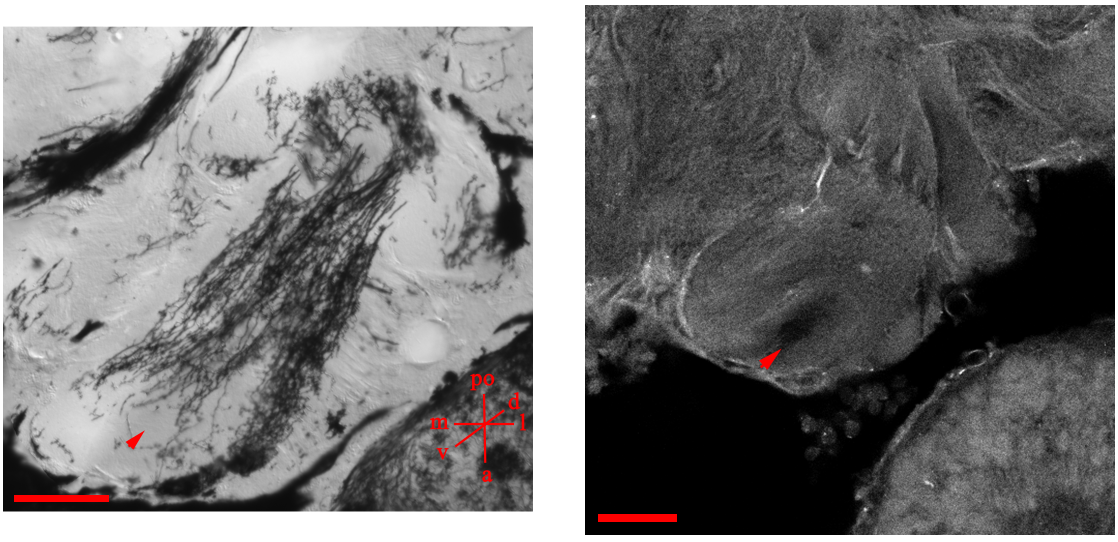


Figure 3.10: Three-dimensional reconstructions of the β lobe. Left image shows the two divisions of the β lobe based on innervation by spiny axons (green division) and smooth axons (red division). Right image shows the processes of a class II extrinsic cell, preferentially innervating only the spiny axon zone, outlined with dotted line. Scale bar is equal to 50 μm.



(**a**) (**b**)

Figure 3.11: KC axon morphology within the β lobe. (**a**) Imaris reconstruction of the β lobe viewed from the black arrow shown figure 3.10. White dotted line shows the position of the Golgi section in (b). (**b**) Golgi-stained section of the β lobe showing spiny axons at the medial edge and smooth axons at the posterior edge.



(**a**) (**b**)

Figure 3.12: The spiny axon division of the β lobe exhibits a non-fluorescent core which KCs do no innervate. Scale bars are 50 μm. (**a**) 15 μm Golgi section showing a superficial slice of the spiny axon division. Red arrow indicates core. (**b**) 3 μm optical slice of intrinsic fluorescence showing the beta lobe at the same angle as in part (a). Red arrow indicates core.

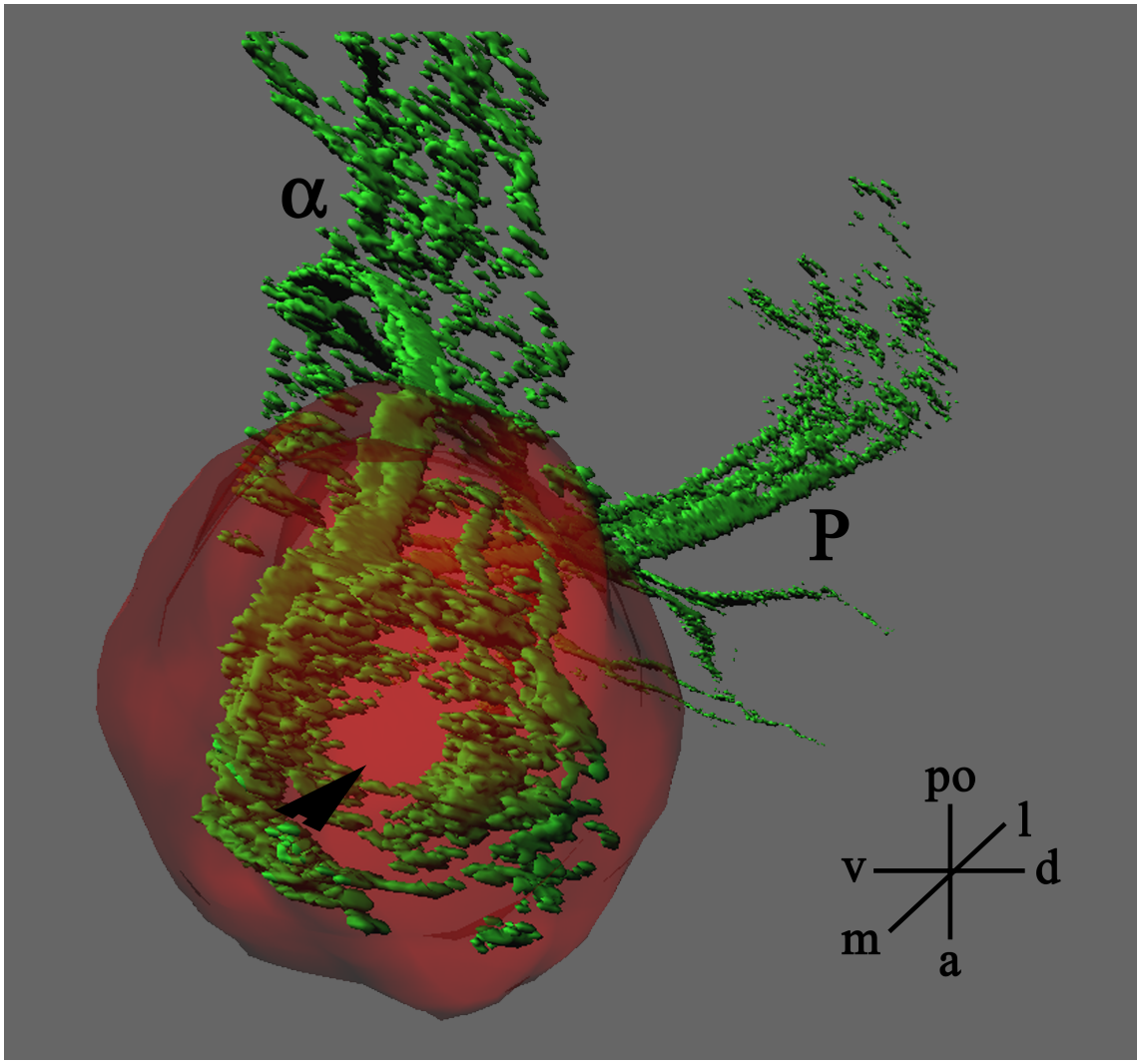


Figure 3.13: Imaris reconstruction of two CII extrinsic cells within the beta lobe. Black arrow indicates the core of the spiny axon division shown in figure 3.12. P: peduncular processes; α: α lobe processes.

A concerted study of α lobe structures and KC axon projections within it is not feasible, given the current data.

3.2.6 MB Structure and Extrinsic Cells

LY fills of odorant responsive cells recorded from dendrites in the β lobe revealed several extrinsic cell morphologies. Here, two sets of cells are defined — class I and II — based on their morphological similarities within and across individual animals (figures 3.15 and 3.14). Table 3.1 lists the characteristics used to define each class of cells, including the position of their somata, the routing of each of two secondary neurites, and peduncular innervation. Other cell morphologies were witnessed, but not repeated across individuals (for further details, see section 4.3). All extrinsic cells with verified odorant responses tended to have large and elaborate arbors. Often, the cells innervated both lobes, as well as some or all of the pedunculus. Most cells also contained neurites in the lateral horn. Soma positions varied widely; class I somata were located in the superficial pars intercerebralis, while class II somata were just posterior to the optic stalk. Other extrinsic cell somata were also seen near the calyx on the lateral side of the brain and within the tritocerebrum.

Table 3.1: Characteristics of Extrinsic Cell Classes

Characteristic	Class I	Class II
Soma Location	Pars Intercerebralis	Lateral Horn
Secondary Neurites (1)	β lobe to LH lobe	β lobe to pedunculus
Secondary Neurites (2)	α lobe to pedunculus	α lobe
Peduncular Innervation	6 major processes central fiber bundle	2 major processes medial and core fiber bundles

The class I extrinsic cells (CI) innervate the alpha lobe sparsely, and the β lobe more densely. The primary neurite splits into three secondary neurites just above the central complex: one branch innervates the β lobe, one innervates the α lobe, and one branches into the pedunculus (figure 3.14). β lobe projections consist of a mesh of fine processes concentrated at the bulbous tip of the β lobe. Peduncular projections

are also dense and fine, and proceed midway up the pedunculus core.

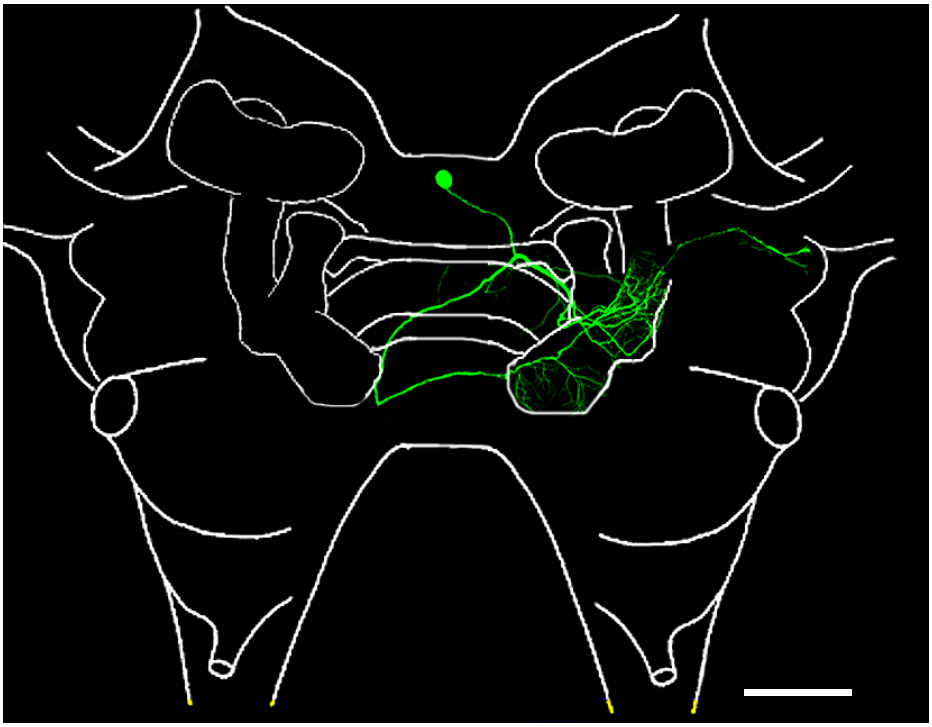


Figure 3.14: Schematic of the locust brain, showing the position of a class I extrinsic cell. Ray-traced reconstruction of a single LY-filled cell overlaid on a schematic of the locust brain. Soma lies in the pars intercerebralis, between the two MB calyces. The cell's processes innervate the MB lobes and the posterior end of the pedunculus. An extension of the β lobe process innervates the LH. Sparse fibers are seen in the midbrain area. Scale bar is equal to 200 μm.

The class II extrinsic cells (CII) innervate the MB pedunculus and lobes extensively, as well as parts of the lateral protocerebrum. Of five fills, one pair was seen to innervate both the IC and MC as well (figure 3.16). Each CII cell has a large diameter fiber that innervates the shell of the β lobe in a c-shape, facing posteriorly. From this 'c' a fine mesh of fibers are sent into the β lobe. This β lobe innervation coincides with the region where KC spiny axons innervate. CII cells were never seen to innervate the division of the lobe that house the smooth axons (figure 3.10). Two medium diameter processes also branch out from the large fiber and project up the pedunculus, parallel to its long axis, one running in the medial fiber bundle and the other in the lateral edge of the central bundle (figure 3.16). CII cells were not seen

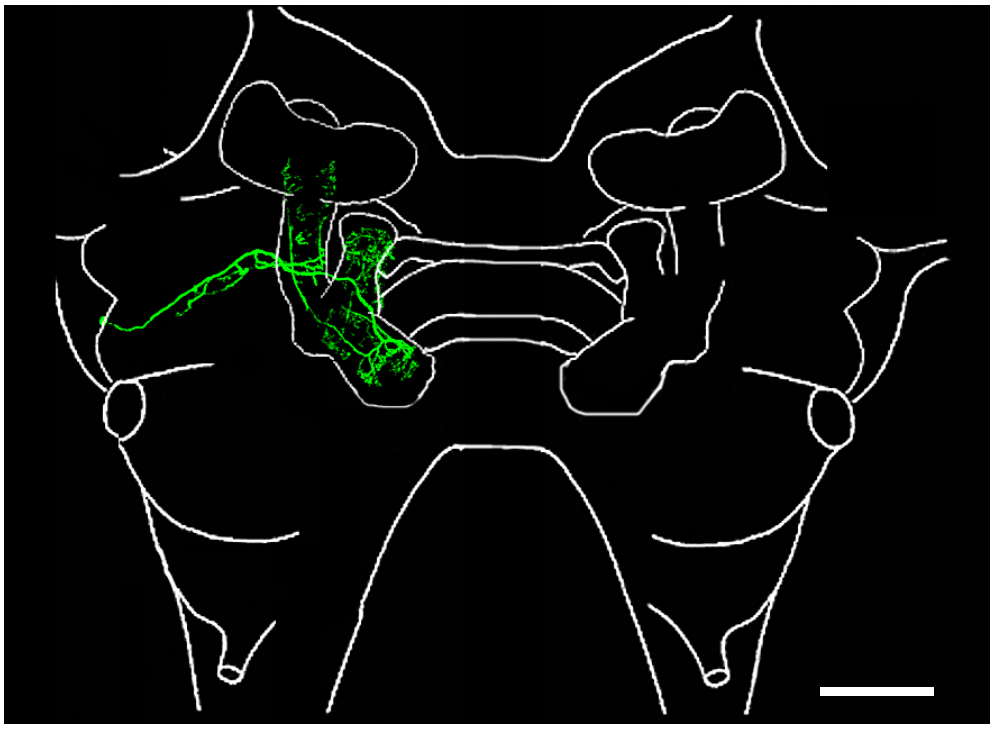


Figure 3.15: Schematic of the locust brain, showing the position of a class II extrinsic cell. The cell image is a ray-traced reconstruction of one cell filled with the dye LY, overlaid on a brain schematic. Soma lies in the LH, and processes are sent to the MB lobes, pedunculus, IC and MC. Diffuse processes are also seen in the LH, off the cell's primary neurite as it passes to the MB. Scale bar is equal to 200 μm.

to innervate the lateral fiber bundle. The medium diameter fibers running up the pedunculus send numerous branches into the core of the fiber bundles, perpendicular to the long axis. These are the processes that continue into the IC and MC from some of these cells (figure 3.17). It is not possible from the current data to determine if these processes perform an input or output function. Further analysis of the fine fibers of CII cells in Golgi stains revealed numerous spines and varicosities on these collaterals, although the medium diameter fibers from which they project have few or no visible decorations.

One double fill in which two CII cells are filled concurrently (figure 3.16) in one individual reveals that this class of cells may be repeated several times at slightly offset positions (figure 3.18). Bodian stains reveal a bundle of 10-15 large processes resembling CII cells at the α and β lobe junction. These processes can be followed

to a group of somata in the position where CII somata are seen to occupy; the large diameter processes can also be followed into both lobes of the MB, and are reminiscent of CII processes in those areas.

3.2.7 Lateral Horn

As mentioned above, some extrinsic cells neurites innervate the lateral horn of the brain, an area just posterior to the optic stalk (figures 3.15 and 3.14). This bulbous region is visible via fluorescent confocal microscopy. This bulb occurs relatively deep within the brain about 200 μm from the ventral surface.

The β lobe branches of the CI cells project to, and terminate in this bulb of the LH. These branches do not contain the pre-synaptic-like varicosities that are seen in this cell's projections to the calyx, suggesting that perhaps the LH provides input to CI cells. CII cells also send sparse branching fibers into the LH. These neurites are split off from the primary neurite in close proximity to the cells' somata. It is unclear if these processes are input or output areas for CI cells.

The majority of the innervation of the LH seems to consist of PN axon terminals. Figure 3.19 shows an intracellular LY fill of three PNs stained concurrently. The axons of these cells project through the base of the calyx (branching profusely in the OC) and end in the LH where they each branch into several fibers (for a full discussion of the LH, see section 4.4.2 and figure 4.54). Golgi and Bodian stains also confirm that PN axons project to and end as a group in the deep bulbous structure of the LH. From these stains, it seems likely that all or at least the majority of PN axons terminate in the LH.

3.3 Discussion

As outlined in section 1.3, honeybee and cockroach MB calyces have been shown to have calycal divisions as defined by afferent input. It is now clear that in the locust as well, calyx divisions occur. The locust MC morphology is reminiscent of the cockroach calycal division that receives visual input: both are a narrow band of calyx

situated centrally. KC intracellular fill data now allows the partitioning of locust KCs into classes based on area of innervation.

Other cells in the locust brain show preferential innervation of these calycal areas; PNs innervate only the OC, leading to the possibility that the OC is the olfactory area of the calyx like the lip region of the honeybee calyces. Some extrinsic MB cells of the locust brain are seen to preferentially innervate only the MC and IC, though their functional polarity there — whether input or output — is unknown. The accessory calyx has already been shown to receive mainly gustatory input [17].

In *Drosophila*, Strausfeld [89] has postulated that, though the calyx looks like a single entity, it may actually be a pair of fused calyces as in the honeybee and cockroach. This, he suggests, is evidenced by the bilateral symmetry of the calyx, both structurally and immunohistochemically. In the locust too, the primary calyx (here consisting of OC, MC and IC) has been described as a single entity; bilateral symmetry, especially between the lateral and medial IC halves, as well as a lack of KC cross-over in the calyx indicate that the calyx could be the emergence of the two calyces that appear in other insects. The peduncular region proximal to the primary calyx does indeed consist of two merging fiber bundles from each side of the calyx. The central fiber bundle itself is bipartite at its calycal end, where KC fibers from each half of the calyx enter their ipsilateral side. This suggests that the two halves of the calyx are at least partially separate structures. The PNs too treat the OC as two structures; the PN axons send collaterals to the posterior edge of the OC on the medial side, sending separate sets of collaterals to each side of the OC.

Like the other insects so far described, as well as the orthopteran crickets [78], locust KC axon morphology comes in two varieties: smooth and spiny. Also, like those others, there is rearrangement of these axons upon entry into the lobes in the locust. The β lobe contains a visible structure in the form of light and dark intercalated regions reminiscent of other insect species. This evidence suggests that there may be a pattern from calycal innervation to lobe innervation. Further experiments are required to identify where innervation to these stripes comes from and how that relates to the KCs.

The patterns of extrinsic cell innervation of the MB further supports the idea that the stripes in the β lobe are functional units. The stereotypical patterned way in which CII processes project into this lobe coincides with the stripes and suggests that there are particular KC axons that the CII cell is connecting to. Again, this is similar to results obtained via immunohistochemistry in honeybee and cockroach.

One important finding of this section is the possibility of feedback to the KCs. Feedback may occur at their output in the pedunculus, lobes and calyx, especially in the MC and IC. CII cells could be feeding back to the calyx and pedunculus to modulate KC outputs which CII cells could be monitoring from connections with KCs in the lobes. CI cells may be comparing unprocessed PN olfactory information from the LH and processed olfactory information from the KCs in the β lobe, and then modulating KC output via connections in the α lobe and pedunculus.

It is possible that either or both classes of extrinsic cells defined here are GABA-ergic. Leitch and Laurent [44] showed large diameter GABA fibers in the peduncular periphery in a location consistent with CII fibers there. The lobes and calyces also contain GABA positive processes. Further, GABA stains (data not shown) reveal that GABA immunoreactive somata occur both in the pars intercerebralis, the location of CI somata, as well as in the lateral protocerebrum where CII somata are. The modulatory function of these cells may thus be via inhibition. (For further analysis of the possible neurotransmitter and neuromodulator content of extrinsic cells, see section 4.3).

Further studies to identify other inputs to calycal divisions, as well as identifying and recording from KCs specific to each division in response to various sensory stimuli are required to establish how and why these divisions have developed. Experiments that use stimulation in the β lobe will help determine what and if there is feedback from extrinsic cells to the calyx for modulation of KC output, or if extrinsic cell innervation of the calyx is purely dendritic. Further, immunochemistry with different neurotransmitters and neuromodulators will also help to shed light on functional regions and cells within the locust brain.

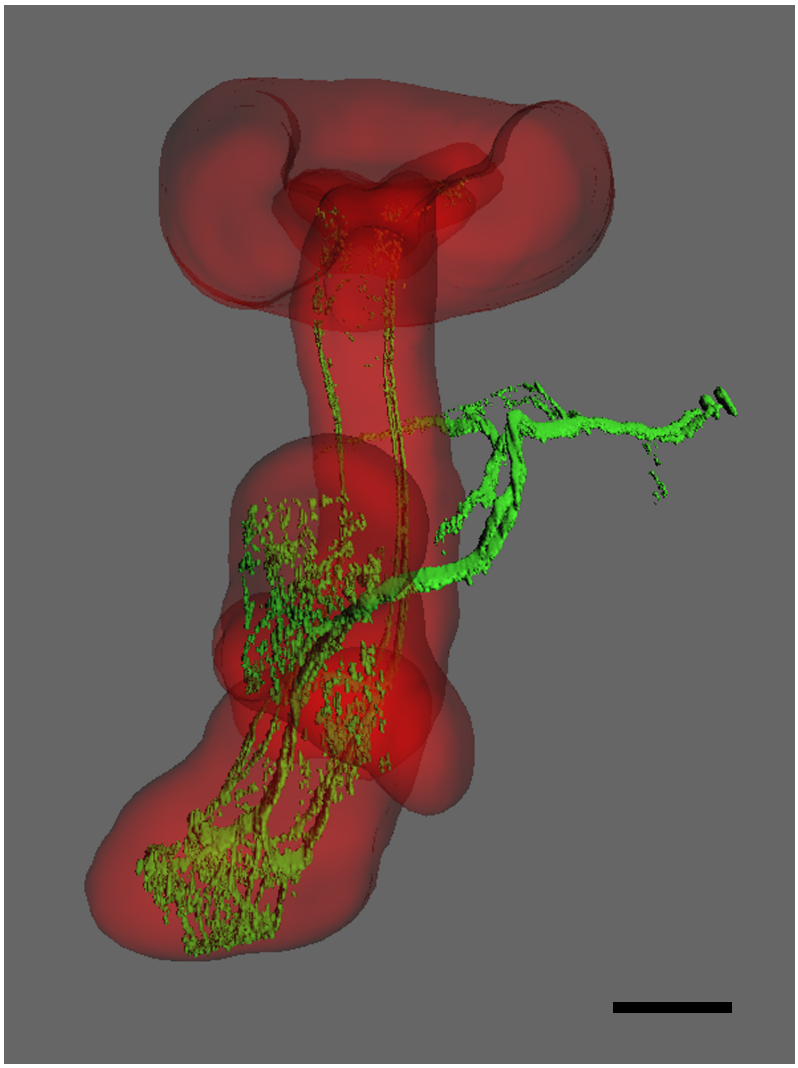


Figure 3.16: Reconstruction of a pair of CII cells (green) along with the MB neuropil (in red), from the frontal view. Extensive innervation of both lobes and pedunculus is visible. LH, MC and IC innervation are also visible. Scale bar is equal to 100 μm.

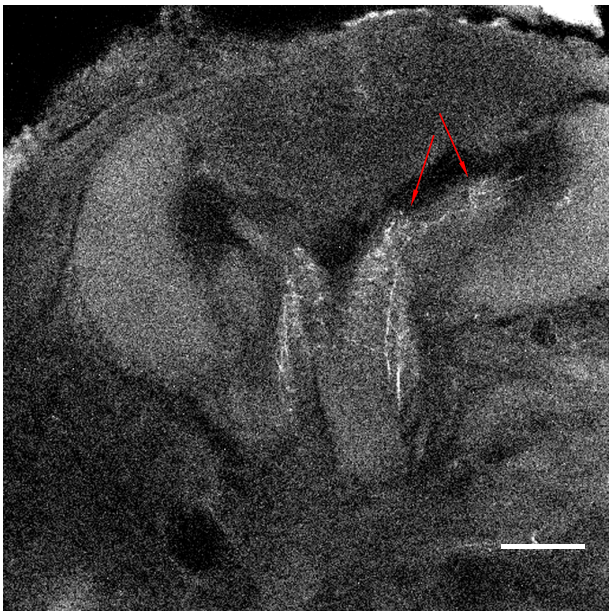


Figure 3.17: 1 μm confocal image of the calyx from a stack of the pair of the LY-filled CII cells depicted in figure 3.16. Red arrows indicate the processes of the CII cells innervating the IC. Scale bar is equal to 100 μm.

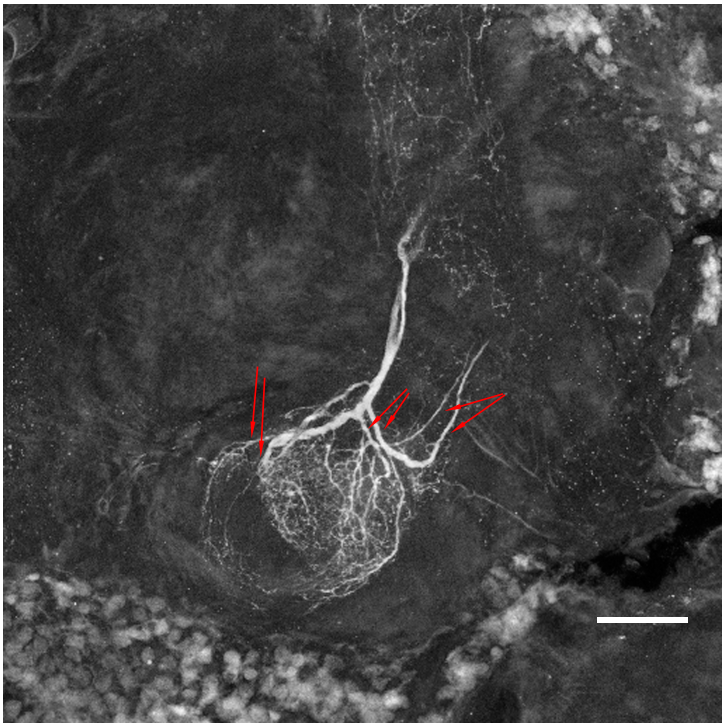


Figure 3.18: 20 μm thick ray-traced projection of several images from a confocal stack of the β lobe of the pair of filled CII cells depicted in figure 3.16. Pairs of red arrows indicate pairs of identical fibers from each cell. Scale bar is equal to 25 μm.

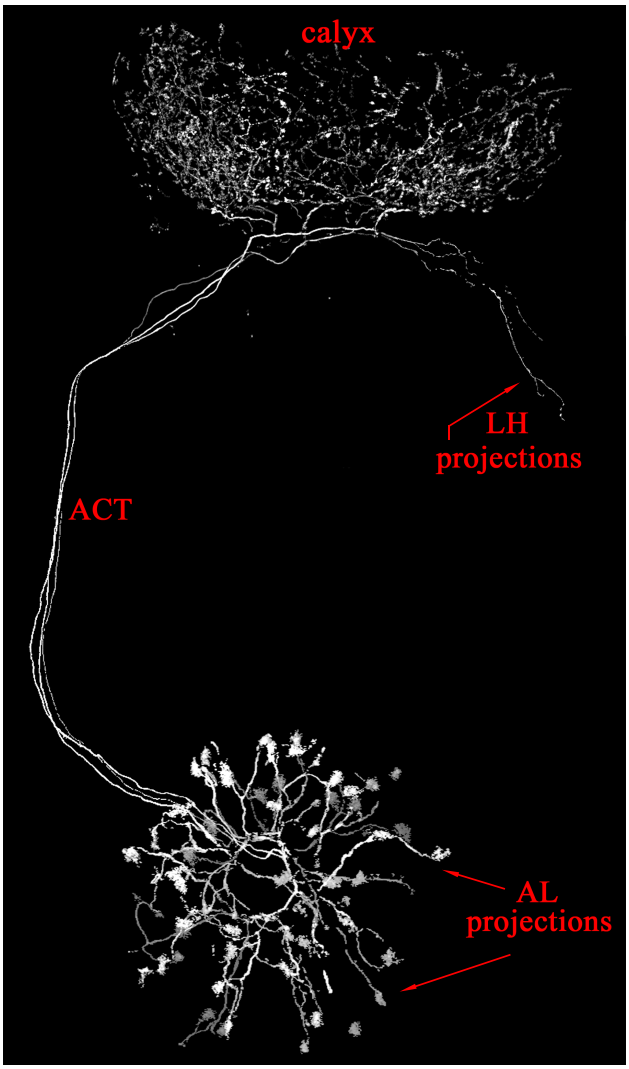


Figure 3.19: Ray-traced reconstruction of three PNs filled with LY, showing the entirety of processes with innervated neuropils labeled. Frontal view.

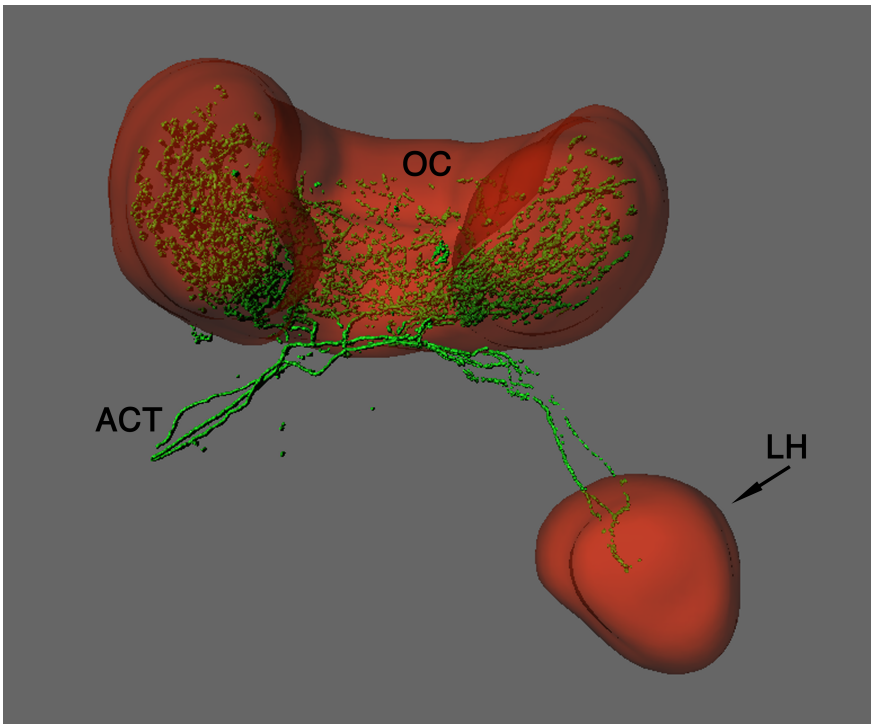


Figure 3.20: Three-dimensional Imaris reconstruction of the set of PNs in 3.19, including the OC and the LH. The red bulb that is labeled as the LH is a defined area visible autofluorescently, and in both Bodian and Golgi stains. This same bulb is innervated by both PN terminals (as shown) and extrinsic cell processes.