Chapter 4

Brain Atlas

4.1 Introduction

This chapter presents an atlas of cells of the locust olfactory system. Olfactory neurons are complicated and extensive cells, often innervating multiple brain regions at varying depths. Thus, a two-dimensional drawing of these cells is often hard to interpret, especially if the data is to be used to judge areas to study using electrophysiology where a precise targeting of cells is required. One goal of this chapter is to present locust anatomical data in an accessible way; cells are presented in relation to their underlying brain structures reconstructed in three dimensions.

Specifically, three sets of cells are presented: the Kenyon cells, the mushroom body extrinsic cells and the projection neurons. The data was collected using intracellular fills with the fluorescent dyes, Lucifer Yellow and Alexa Fluor and imaged with a confocal microscope. The cells were isolated in stacks of images using *nimage* (as described in chapter 2). In a few instances, Bodian stains and GABA immunochemical stains were also considered.

In general, for each set of cells and each class within these sets, data is presented in two ways: three-dimensional Imaris reconstructions and ray-traced projections (produced with ImageJ). Ray-tracing projects the data based on pixel brightness and depth within the stack so that deeper structures are dimmer, producing depth in one-dimensional images. Imaris reconstructions are used to present the cells within traced brain structures. This method filters and smooths the data.

Each of these two methods has its own advantages, and each complements the other. In general, cell details are better viewed in ray-traced projections because the data is not filtered or smoothed in any way. However, to adequately present brain structures, Imaris projections are essential. Gaussian smoothing presents a 'solid' version of brain structures that appears smooth and continuous. The disadvantage to this method is that the cells themselves are also smoothed, leading to the loss of detailed information.

Three sections present the three cell classes using the above methods. KC are shown in relation to their calycal innervation, as individuals and as groups of two to four cells stained together for comparison of dendritic fields and innervation. Two classes of extrinsic cells as defined in chapter 3 are illustrated in detail and related to immunohistochemical data (both new and from existing literature). Several other extrinsic cells are also shown. Finally, PNs are considered — both as individuals and as groups of two or three — first, with respect to their innervation of the AL, and then, with respect to their projections within the calyx and LH. A final reconstruction shows the entire circuit described in this chapter with multiple cells of each type overlaid on brain structures.

4.2 Kenyon Cells

The purpose of this section is to catalog the variety of morphological types of KCs based on their dendritic morphology as it pertains to calyx zone innervation. The calyx regions, defined and described in chapter 3, are the outer-calyx (OC), the mid-calyx (MC) and the inner-calyx (IC). There are three KC types defined here that are associated with these zones. The following three subsections present individual cells of each type: the OC-exclusive cells, the OC/MC cells and the OC/IC cells. A separate subsection presents reconstructions showing multiple KCs within the same calyx for direct comparison of dendritic fields and sampling within the calyx. Finally, reconstructions of entire KCs, including calyx, peduncular, and lobe innervation are presented both in three-dimensions within brain structures and as ray-traced recon-

structions.

OC-exclusive cells provide the largest class of cells. As their name suggests, these cells' dendritic branches are confined exclusively to the OC. The OC is the most voluminous calyx division and a large number of cells filled fit this category. Of 79 total filled cells analyzed in this sections, 59 (75%) are OC-exclusive cells, 10 (13%) are OC/IC cells and five (6%) are OC/MC cells. Twenty five OC cells, four OC/IC cells and four OC/MC cells are presented.

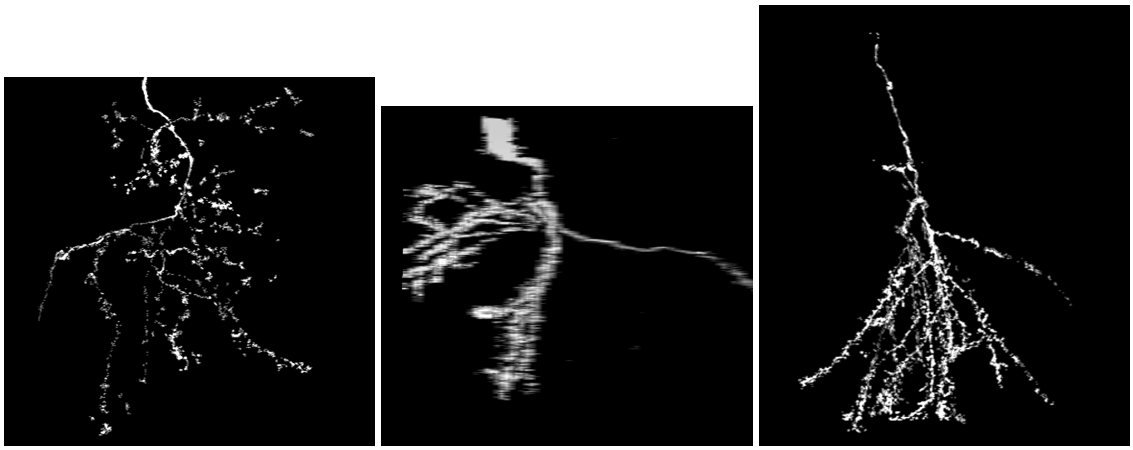
For each type of cell, data is presented first in the form of three-dimensional Imaris reconstructions for representative cells of each type. Then, ray-traced reconstructions show the details of individual cells from three aspects: ventral, anterior and lateral (see figures 1.5 and 1.6).

4.2.1 Kenyon Cells of the Outer-Calyx

The outer-calyx exclusive cells are a morphologically heterogeneous class of cells. The number of secondary branches in these cells ranged from 10 to 40, with a dendritic span that could cover anywhere from 1/8 to 1/2 of the calyx. Spines and varicosities were counted in eight cells, although the quality of data makes this number only a rough estimate. For eight cells, an average of 132 such decorations were counted, with a range between 87 and 181.

In order to present the data in a more clear way, the OC-exclusive cells are grouped roughly by overall morphological similarities, as judged by eye. These are not strict divisions by any means, and they are only made for clarity of presentation. Three groupings are presented: cells whose dendritic branches are split into two major sections (bipartite cells); cells whose dendritic trees form a fan-like shape; and cells with one pyramidal dendritic tree. It is important to note that taken as a group, the three types of KCs form a gradual progression from single-tree to bipartite to fan-shaped. Many cells do not fit neatly into one category.

Fan-shaped KCs tend to have the largest overall span, fanning out in one of the three dimensions to cover up to $1/5th$ of the OC. Usually, a number of branches



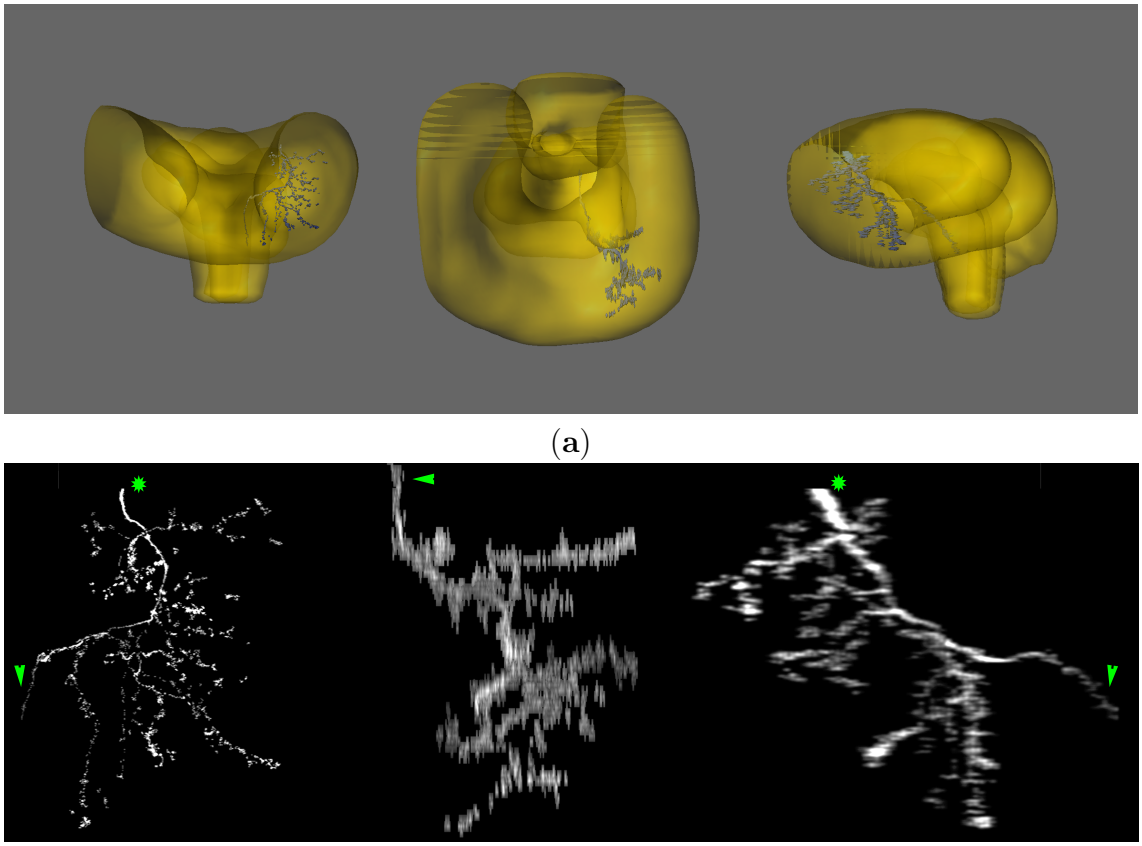
(**a**) (**b**) (**c**)

Figure 4.1: Comparison of the three types of OC-exclusive cells, shown from the angle that best depicts classification. (**a**) An OC-exclusive fan-shaped cell viewed from the front or ventral aspect. This cell is also shown in figure 4.2. (**b**) An OC-exclusive bipartite cell viewed from the side or lateral aspect. This cell is also shown in figures 4.5(b) and 4.6(b). (**c**) An OC-exclusive single-tree cell viewed from the front or ventral aspect. This cell is also shown in figure 4.12(b).

project from an S-shaped primary neurite that becomes the peduncular projection at one end. Five fan-shaped cells are shown in figures 4.2 to 4.3.

The most frequently encountered cells fit into the bipartite category. Bipartite cells tend to be of medium span (covering about 1/8 of the OC), although large (figure 4.6) and small (figure 4.9) spans do occur as well. These cells are seen to innervate the center of the OC rather than the lateral or medial aspects of it. Figures 4.5 to 4.9 show 10 bipartite cells.

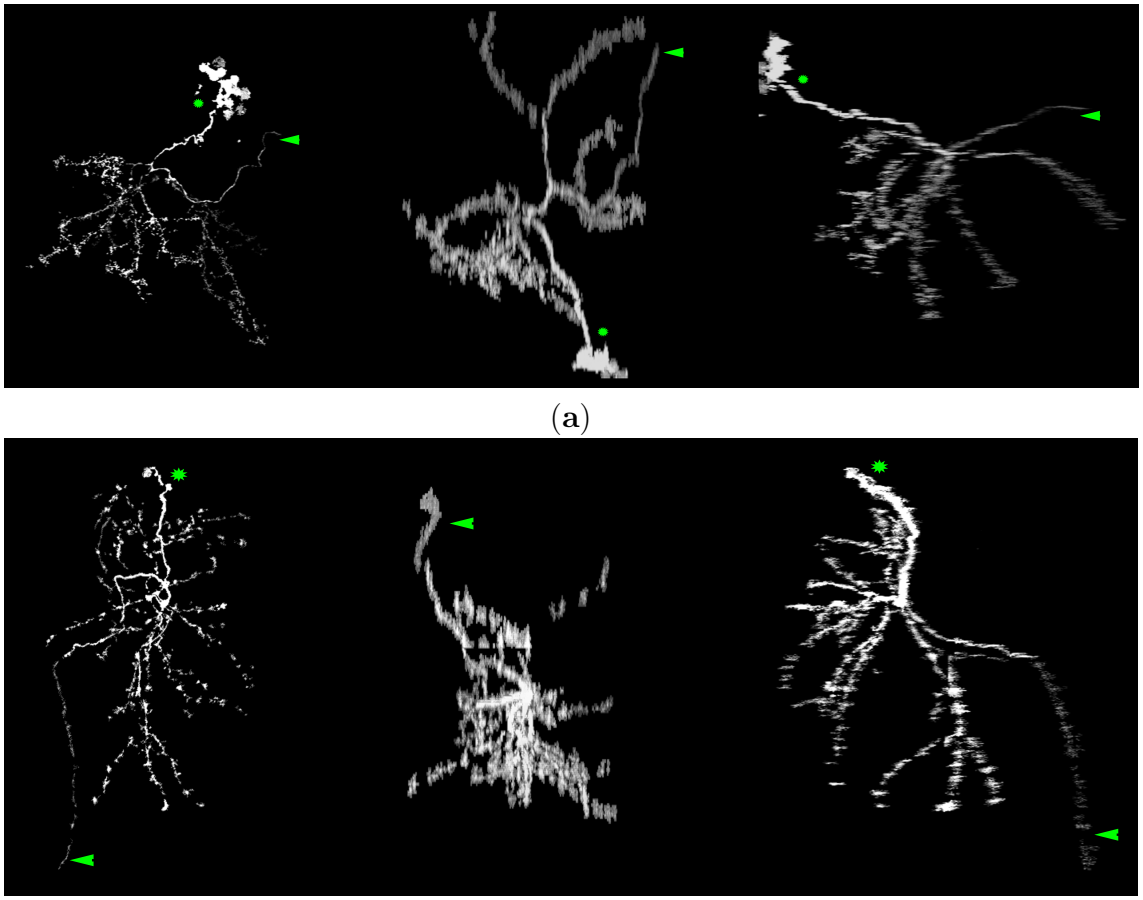
Single-tree cells are another set of frequently encountered cells. These cells are distinguished by their pyramidal-shaped dendritic trees. Single-tree cells were seen to occur in all parts of the OC (medial, lateral or central areas). The general span of these cells is smaller than the other two types considered here. Figures 4.10 to 4.12 show six single-tree cells.



(**a**)

(**b**)

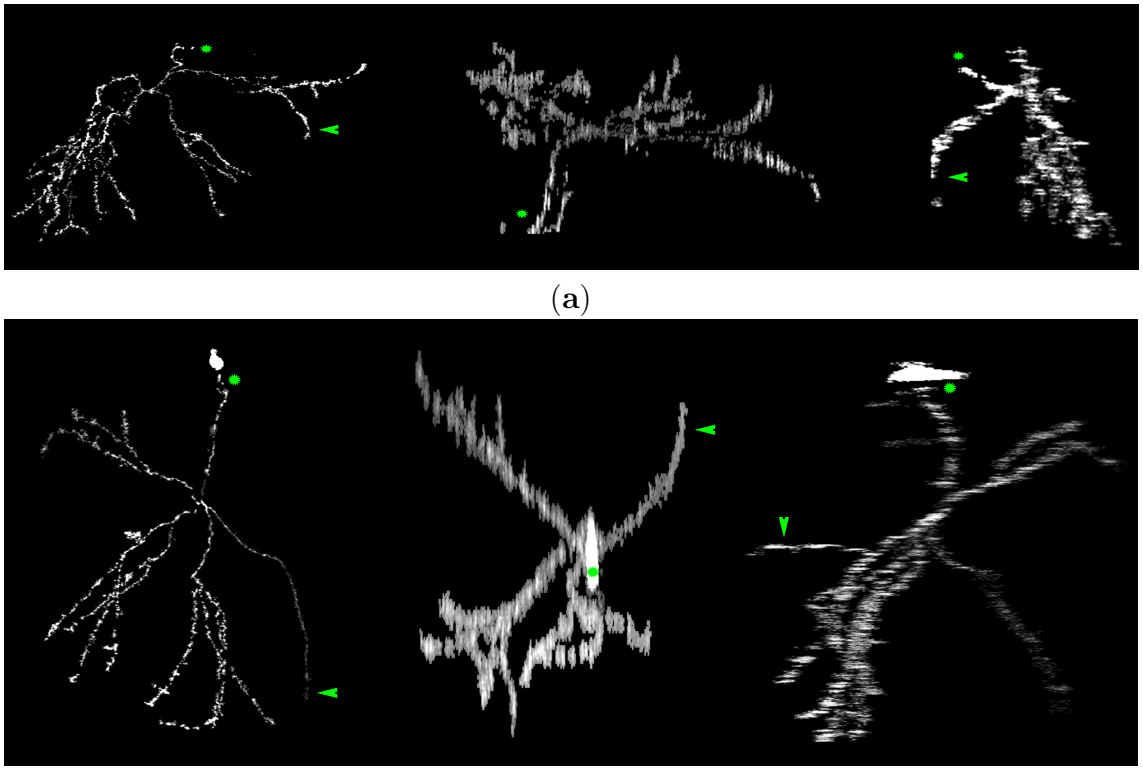
Figure 4.2: (**a**) Imaris reconstructions of an OC-exclusive, fan-shaped cell from the front, top and side views, from left to right, respectively. (**b**) Ray-traced reconstruction of the cell in part (a) in the same three views. Green stars indicate the primary neurite from the soma (soma not shown) and green arrows indicate the axon.



(**a**)

(**b**)

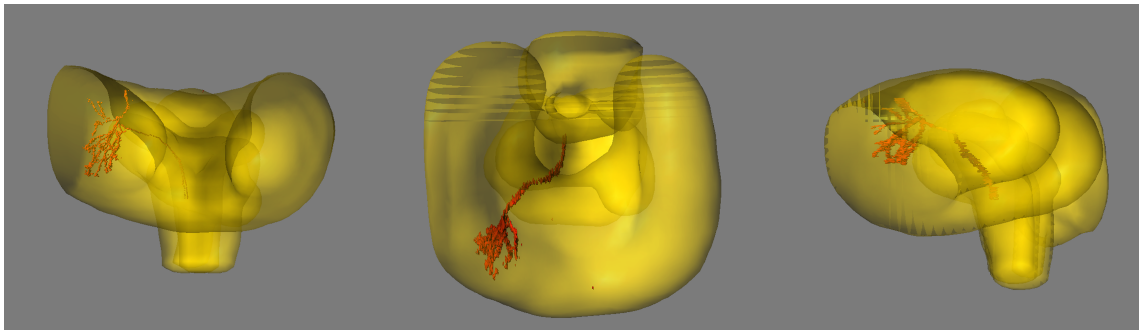
Figure 4.3: Ray-traced reconstructions of OC-exclusive fan-shaped cells in three views (as before). (**a**) Green arrows indicate axon. Note the cluster of somata indicated by green stars. (**b**) Green stars indicate primary neurite from soma (soma not shown) and green arrows indicate the cell's axon.



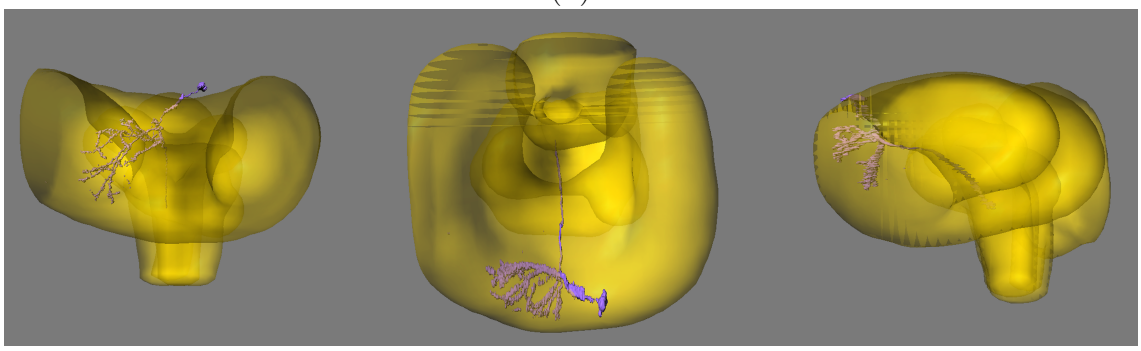
(**a**)

(**b**)

Figure 4.4: Ray-traced reconstructions of OC-exclusive fan-shaped cells in three views (as before). (**a**) Green stars indicated primary neurite from soma (soma not shown) and green arrows indicate the cell's axon. (**b**) Green stars indicate the soma and green arrows indicate the cell's axon.



(**a**)



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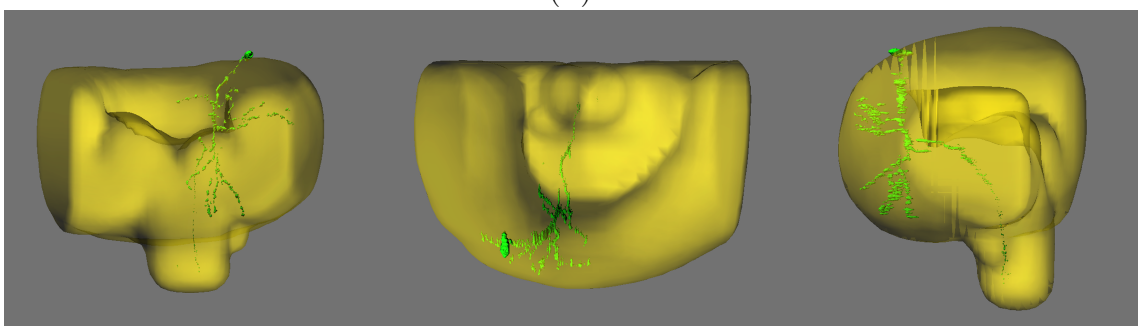
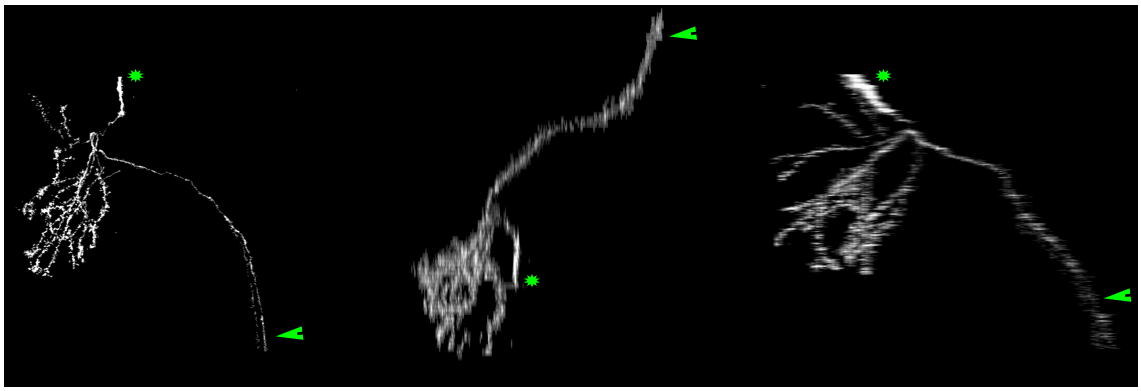
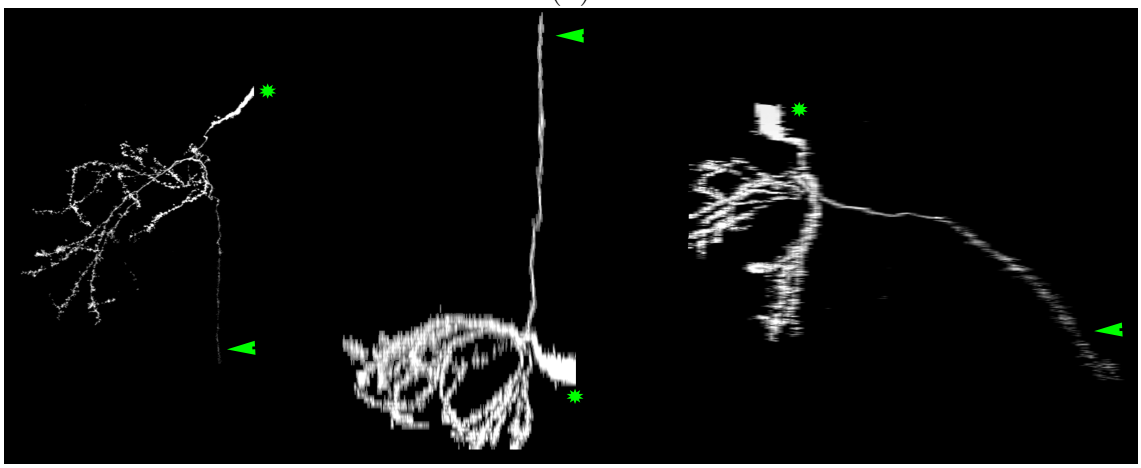


(**c**)

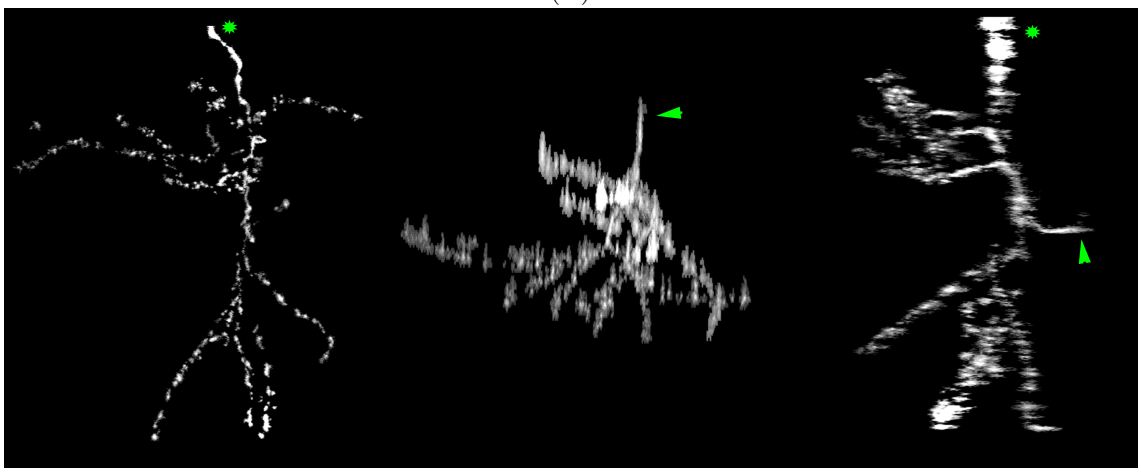
Figure 4.5: (**a-c**) Imaris reconstructions of OC-exclusive bipartite cells from front, top and side views (left to right). Ray-tracings of these three cells are shown in the following figure.



(**a**)



(**b**)



(**c**)

Figure 4.6: (**a-c**) Ray-traced reconstructions of the OC-exclusive cells depicted in figure 4.5 in same three views as before. Green stars indicate the primary neurite from the soma (somata not shown), and green arrows indicate the axon.

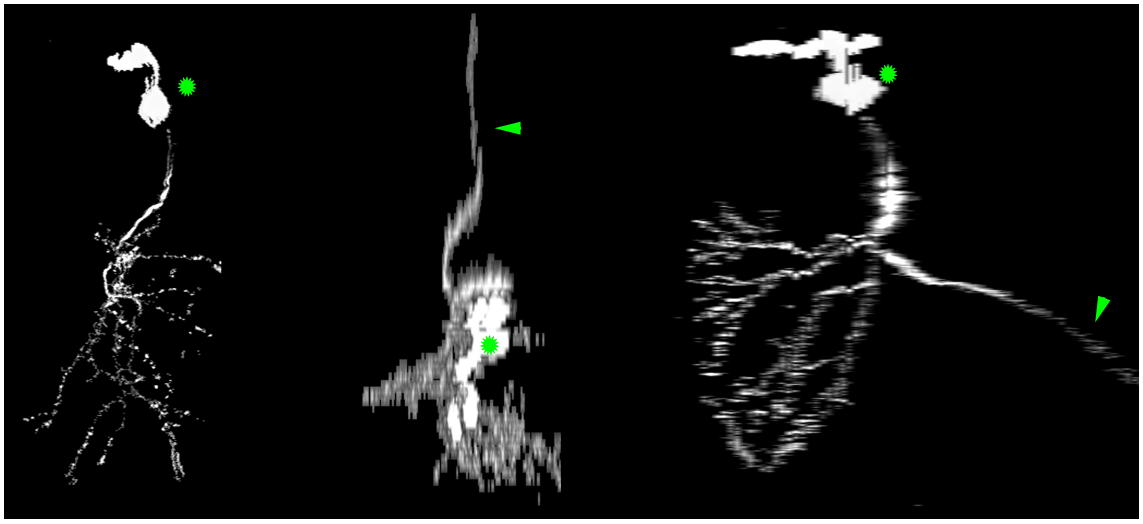
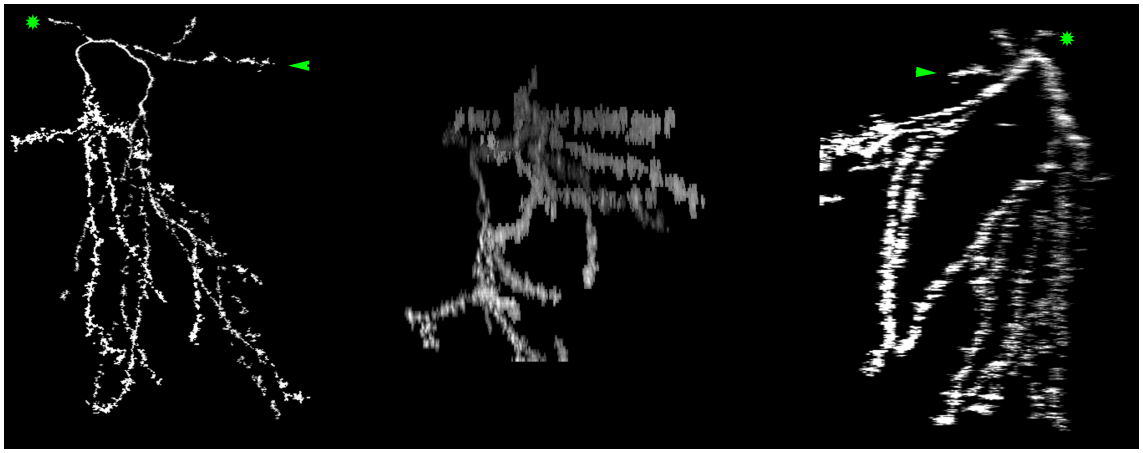
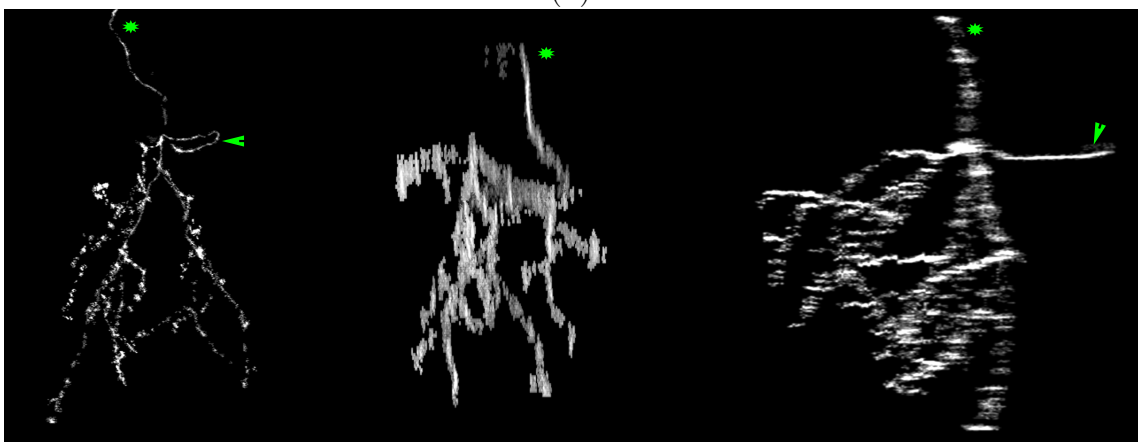


Figure 4.7: Ray-traced projections of an OC-exclusive bipartite cell, from three views. Green stars indicate the soma (a cluster of somata were stained, though only one cell's processes are visible). Green arrows indicate axonal projections.



(**a**)



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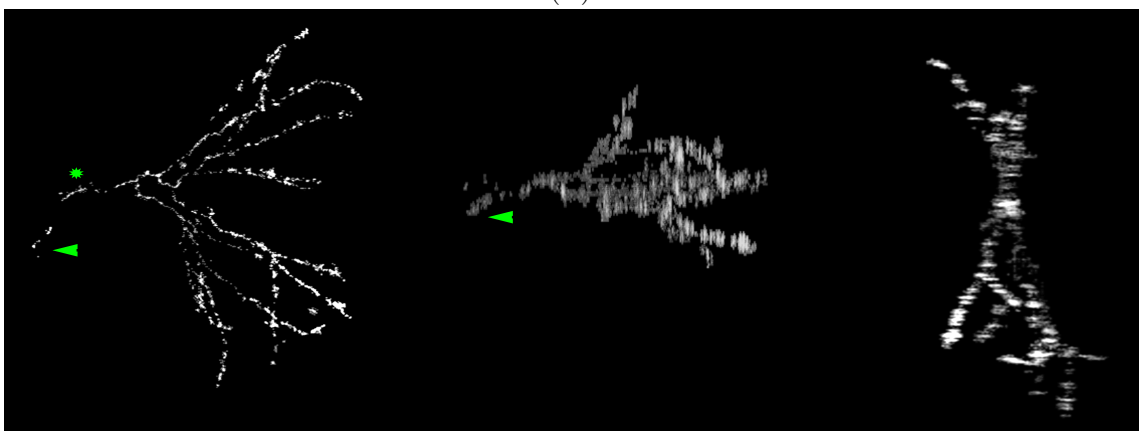
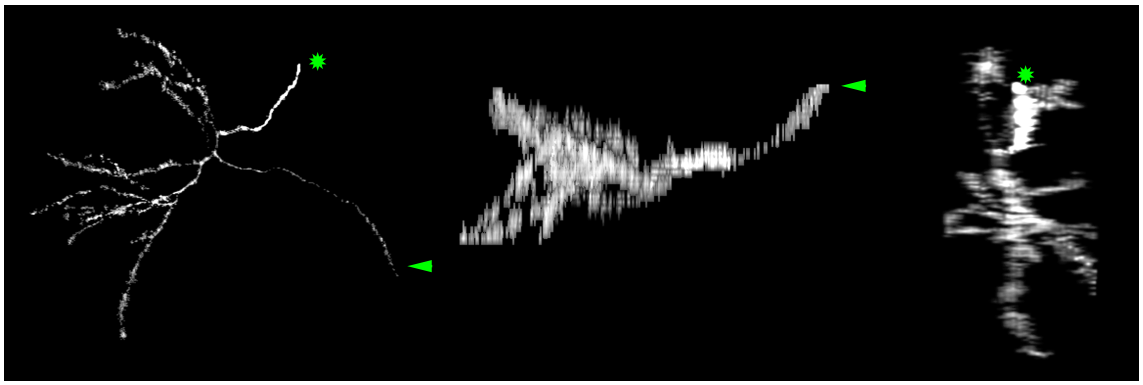
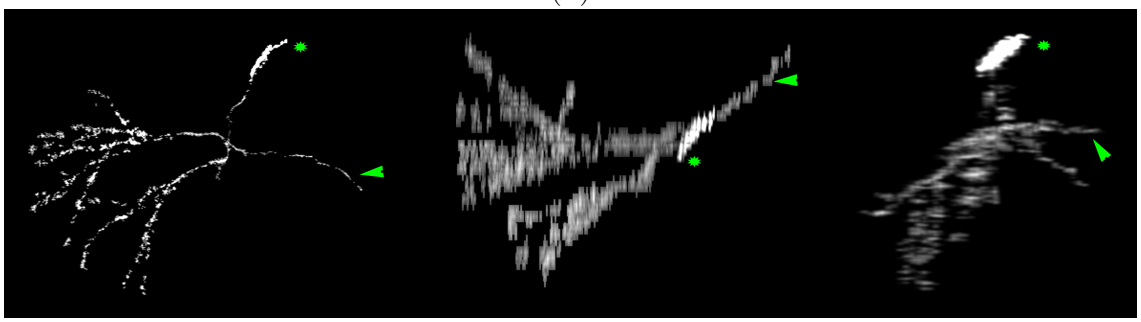


(**c**)

Figure 4.8: (**a-c**) Ray-traced reconstructions of OC-exclusive bipartite cells in three views. Green stars indicate the primary neurite to soma (somata not shown) and green arrows indicate a cell's axonal projection.



(**a**)



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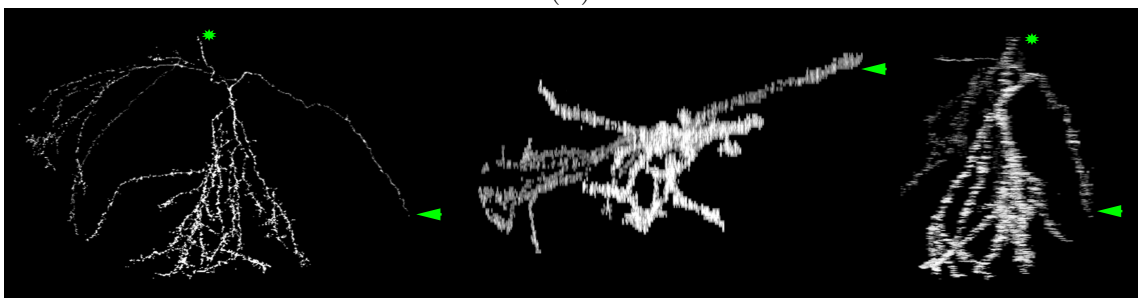

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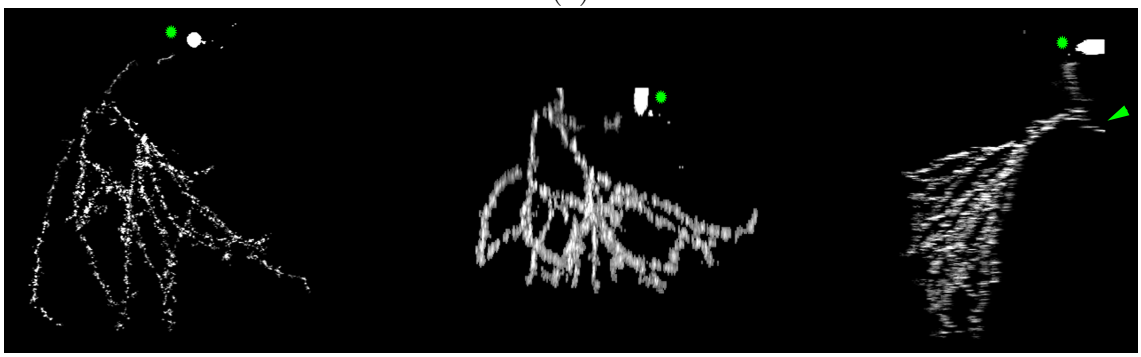
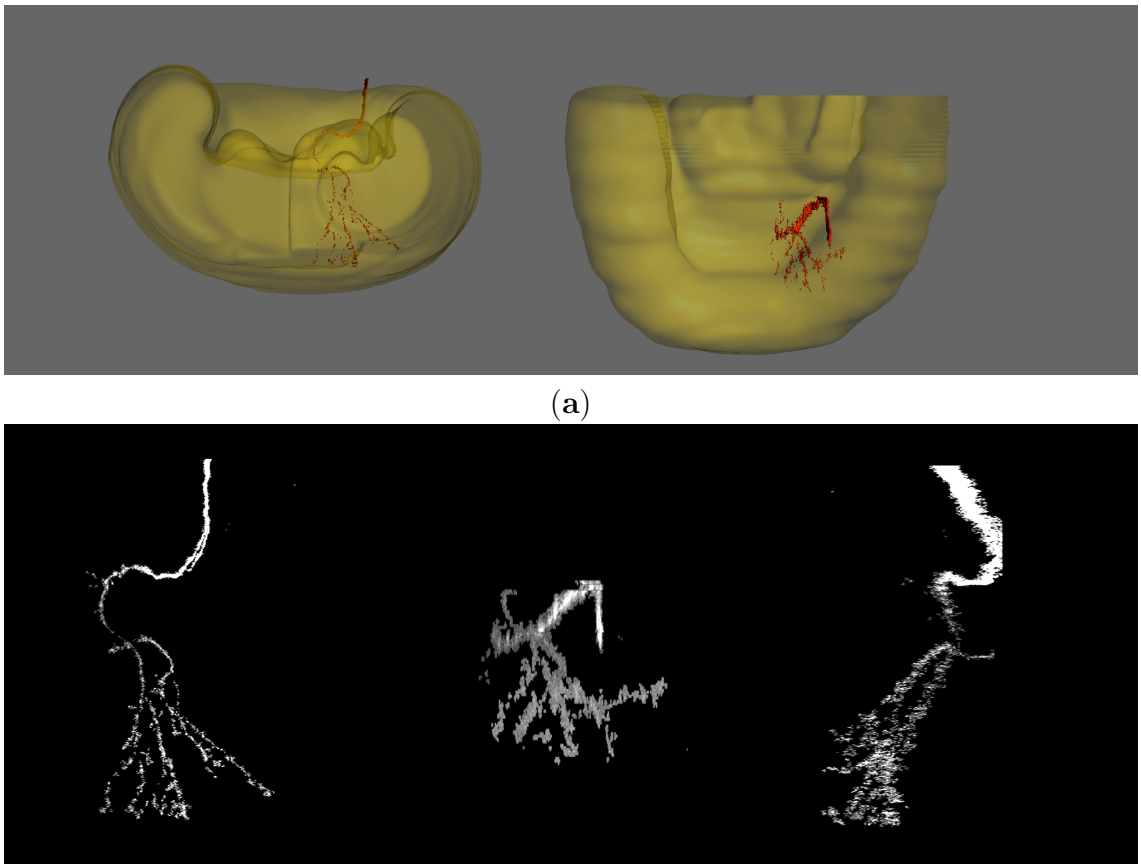


(**d**)

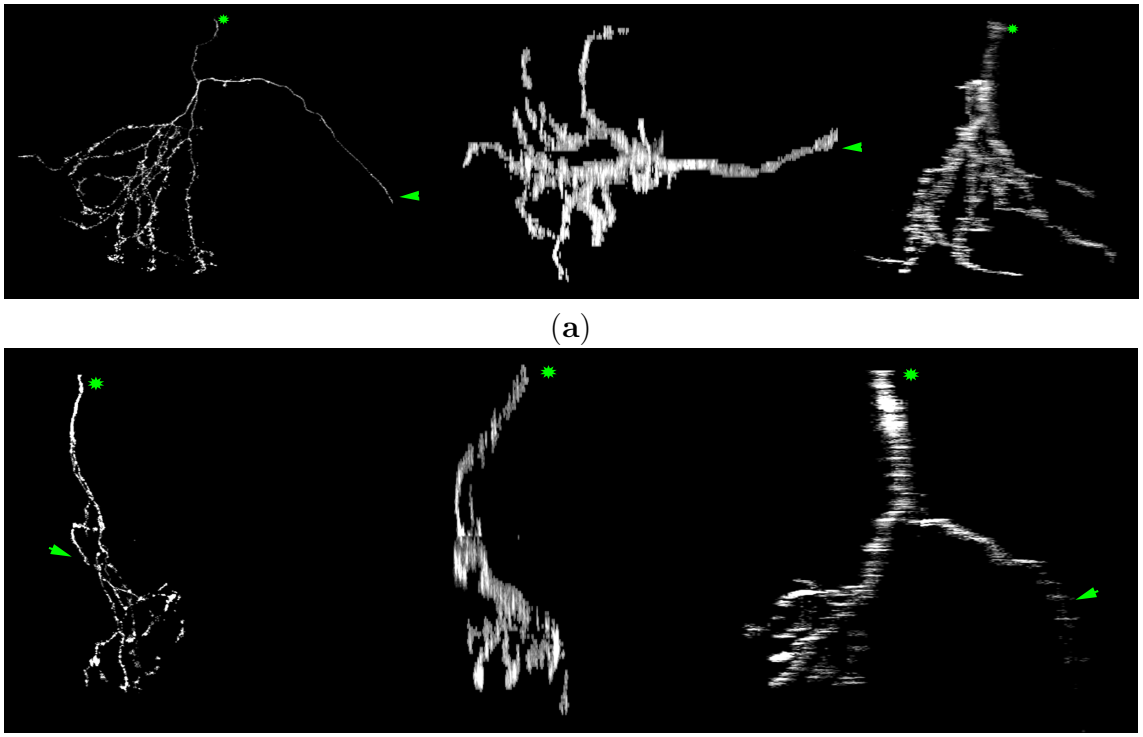
Figure 4.9: (**a-d**) Ray-traced reconstructions of OC-exclusive bipartite cells in three views. Green stars indicate the primary neurite to soma (soma is shown for only the cell in figure (d)) and green arrows indicate a cell's axonal projection.



(**a**)

(**b**)

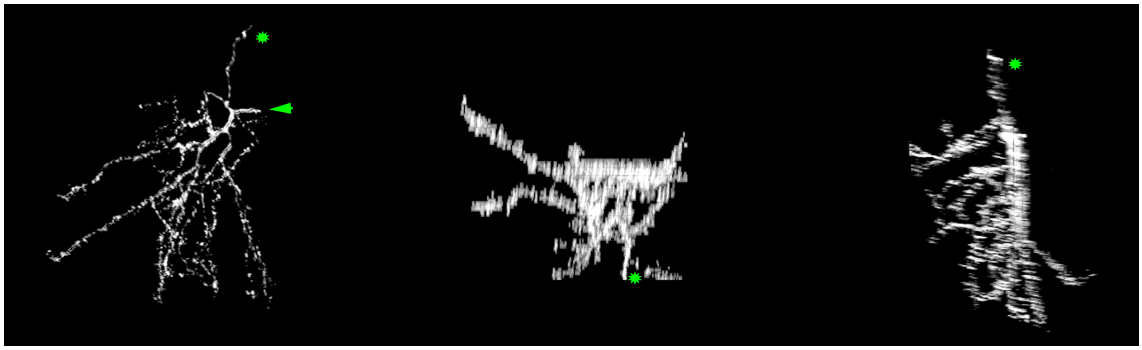
Figure 4.10: (**a**) Imaris reconstruction of an OC-exclusive single-tree cell in two views. (**b**) Ray-traced projection of a single-tree cell.



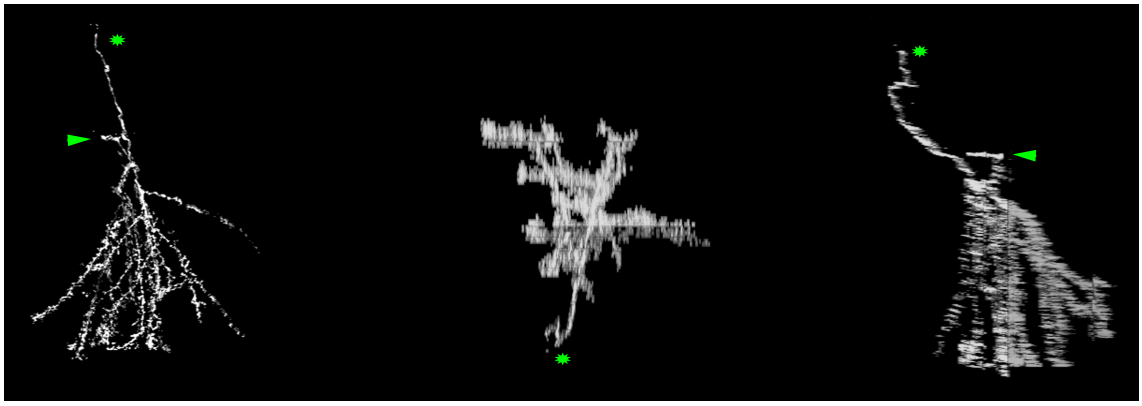
(**a**)

(**b**)

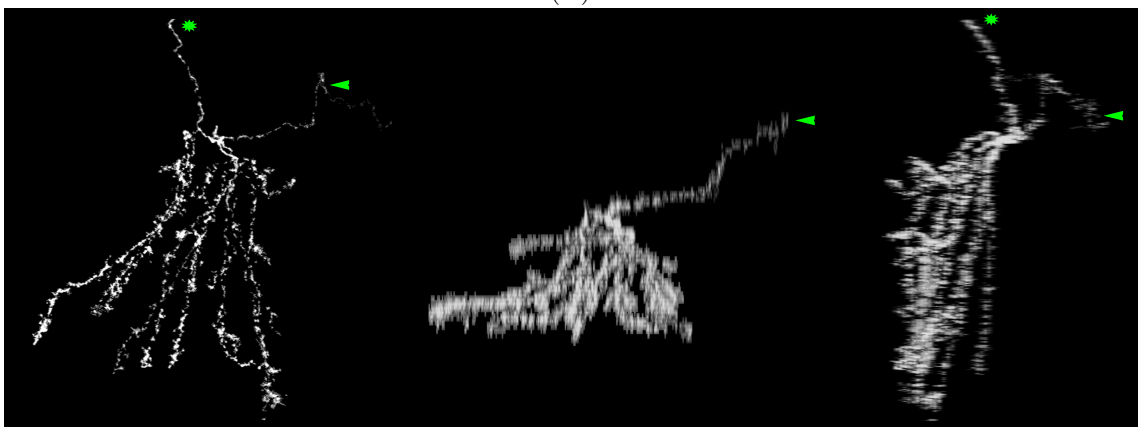
Figure 4.11: Ray-traced reconstructions of OC-exclusive single-tree cells in three views. Green stars indicate the primary neurite to soma (somata not shown) and green arrows indicate a cell's axonal projection.



(**a**)



(**b**)



(**c**)

Figure 4.12: (**a-c**) Ray-traced reconstructions of OC-exclusive single-tree cells in three views. Green stars indicate the primary neurite to soma (somata not shown) and green arrows indicate a cell's axonal projection.

4.2.2 Kenyon cells of the Mid- and Outer-Calyces

The OC/MC KCs are a class of cells having main branches in the outer-calyx, that send one set of relatively small, short dendrites within the disc-shaped MC. Figure 4.13 shows the relationship of the MC (orange) to the OC (yellow) in two views. Each OC/MC cell's MC branches comprise a relatively small area in the MC. In large part, the MC itself is comprised of decorationless axons from OC-exclusive cells as they pass through to the pedunculus. Only a few cells contain the spines and varicosities associated with input regions, and these cells are defined here as the OC/MC cells.

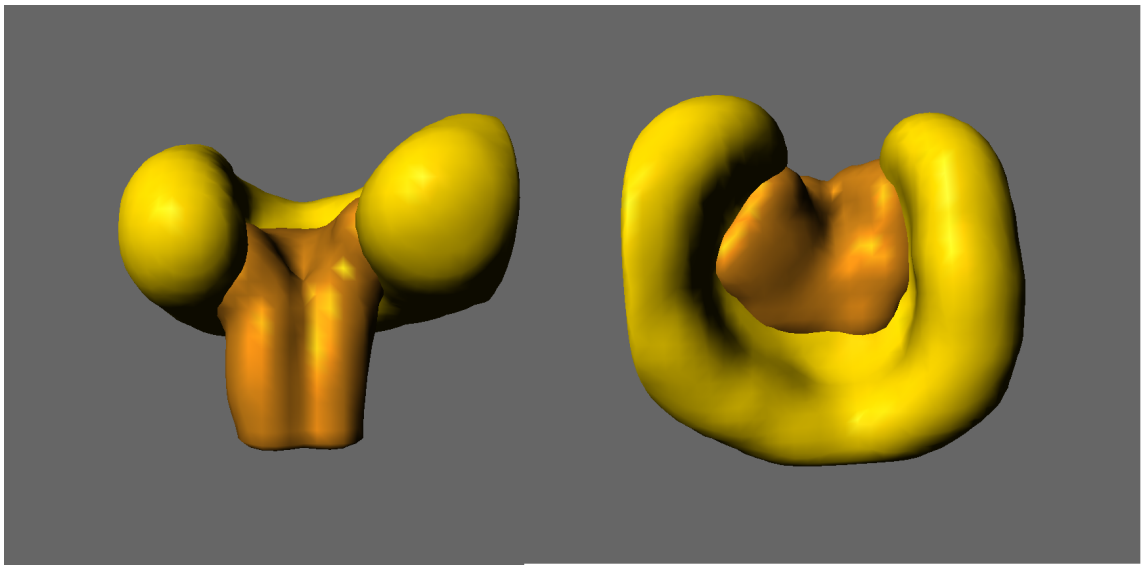
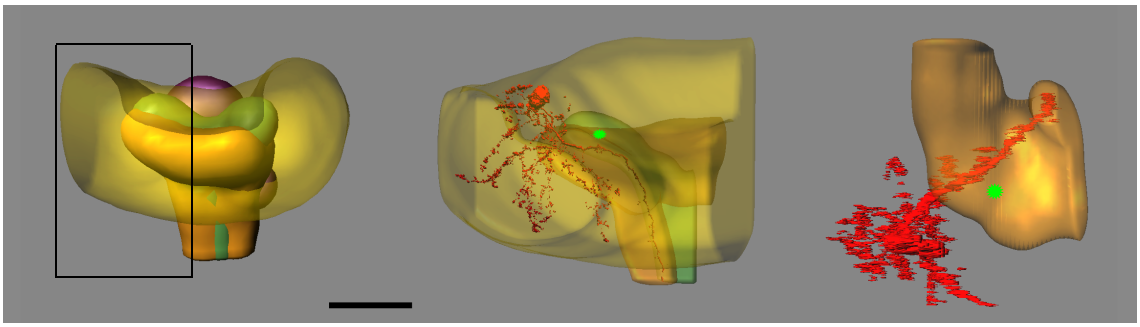


Figure 4.13: The mid-calyx in relation to the outer-calyx (yellow). The MC (orange) is shown connected to the parts of the pedunculus to which it projects. Left hand figure is viewed from the dorsal or back aspect; right hand figure is viewed from the posterior or top aspect. Note that the IC and accessory calyx are not depicted.

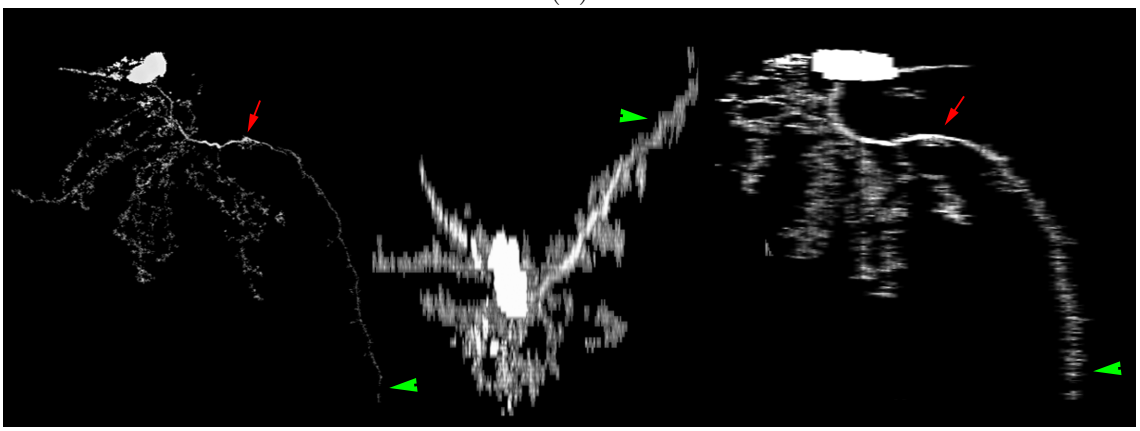
Probably due to the fact that so few of these cells actually exist, only four were successfully filled in the present study and all are shown here. The OC projections of these cells are reminiscent of OC-exclusive cells. There is merely a modification that adds branches in the MC. Figure 4.14 shows an OC/MC KC whose OC innervation is fan-shaped. As the primary neurite continues through the MC to the pedunculus, varicosities and spines occur (red arrows). Figure 4.15 shows an OC/MC reminiscent of single-tree OC-exclusive cells. The secondary neurite, again on its way to the

pedunculus, branches within the MC (red arrows).

The total number of OC branches of each of the OC/MC cells ranges from five to 10. The MC branches are thin, short and diffuse, and a count was not feasible with the current data. In some cases, MC 'branches' are not actual branches, but merely spines and varicosities on the neurite that passes though the MC to the pedunculus and becomes the axon (figure 4.14). MC processes are most likely dendritic, however, because according to the electron microscopy study conducted by Leitch and Laurent [44], all KC processes within the calyx contain only post-synaptic specializations.

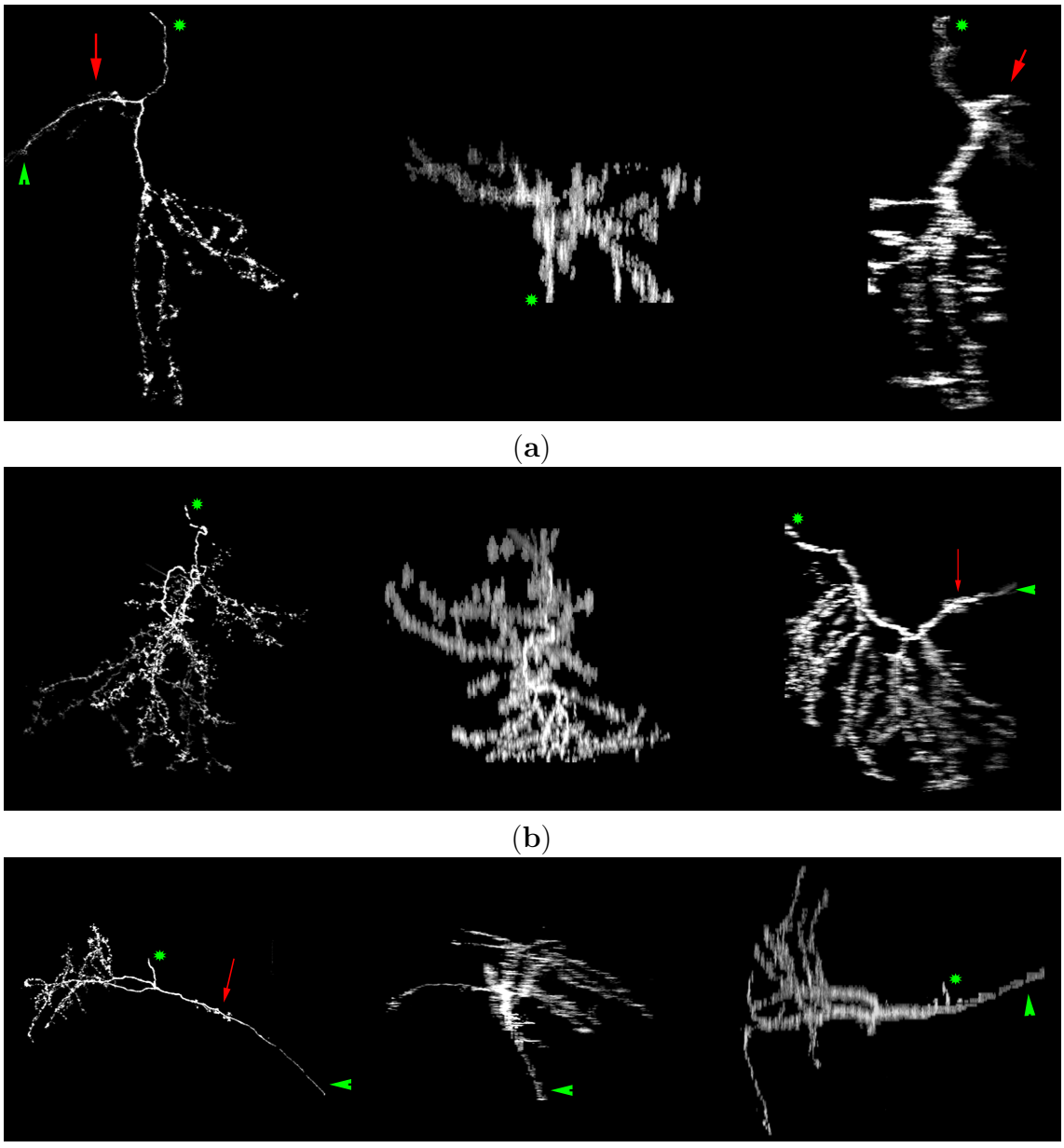


(**a**)



(**b**)

Figure 4.14: (**a**) Imaris reconstructions of an OC/MC cell, reconstructed with outer-calyx (yellow), mid-calyx (orange) and inner-calyx (green). Green star indicates MC processes of the cell. (**b**) Ray-traced projection of the cell in part (a) in three views. Red arrow indicates mid-calyx processes, and green arrows indicate the cell's axon.



(**a**)

(**b**)

(**c**)

Figure 4.15: Ray-traced projection of three OC/MC cells. Green stars indicate the primary neurite from the soma (somata not shown), green arrows indicate the cell's axon and red arrows indicates MC projections/varicosities.

4.2.3 Kenyon Cells of the Inner- and Outer-Calyces

The inner-calyx is a structure that is composed of two hump-like neuropils on either side of the calyx's mediolateral midline (figure 4.16, shown in green). The cells defined as the OC/IC cells have one set of branches within the IC and one (larger) set in the OC. Although few such KCs were filled (n=6), all that were found were situated on either the medial or the lateral side of the calyx. They were never found in the central regions. OC/IC cells have the same OC dendritic characteristics as OC-exclusive cells. The IC branches tend to be densely covered with varicosities and spines.

Figures 4.17 to 4.19 exhibit three OC/IC cells, where the IC branches are indicated by red arrows. The cell in figure 4.17 is reminiscent of fan-shaped OC-exclusive cells, with one extra branch in the IC. The neuron in figure 4.19(b) is similar to a bipartite OC-exclusive cell, again with one extra branch in the IC. Like the OC/MC cells, these KCs seem to be a variation on the OC-exclusive cells. All ten OC/IC cells filled send their axons down the central peduncular fiber. As shown in figure 4.16, the green IC region flows into this center peduncular region.

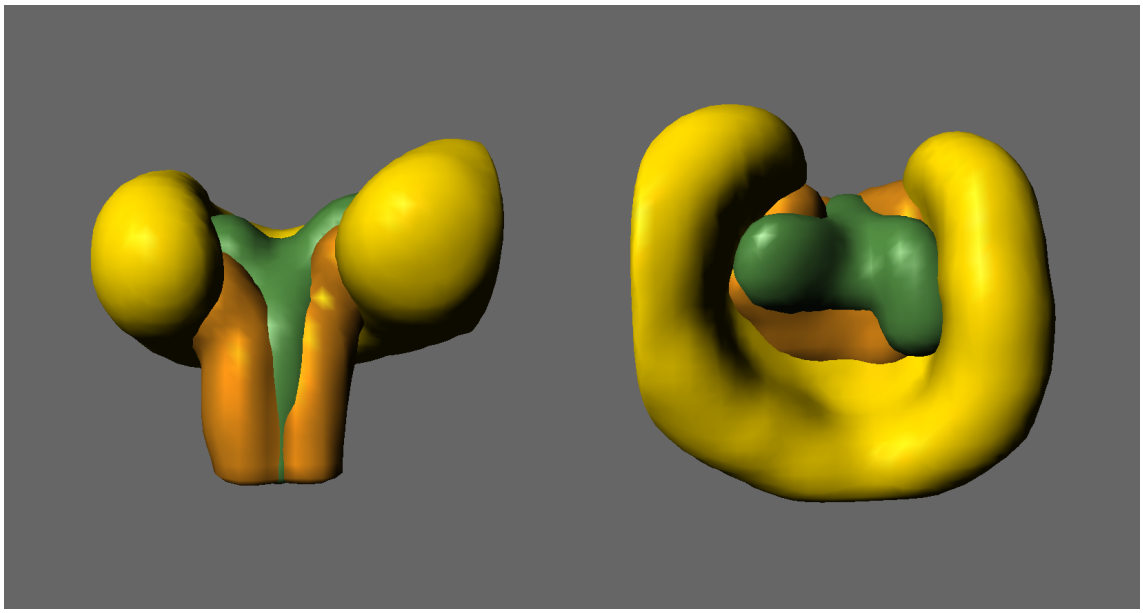
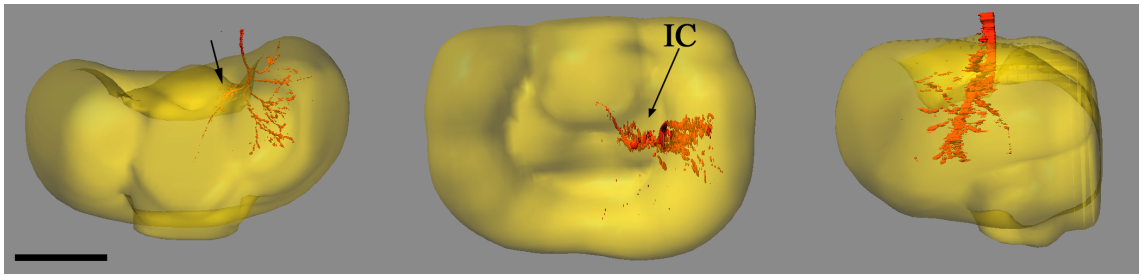
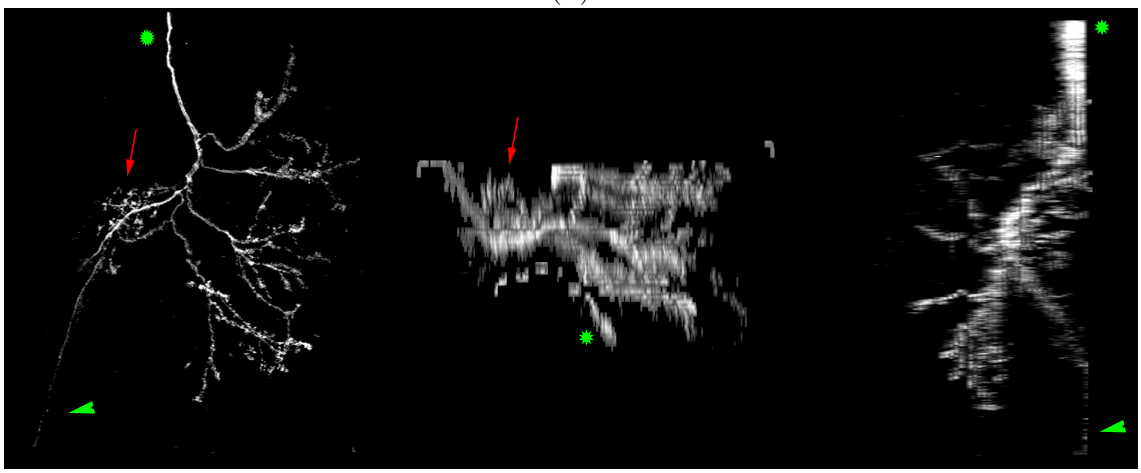


Figure 4.16: Imaris reconstruction showing the divisions of the calyx; IC is green, MC is orange and OC is yellow. Left hand figure is a view from the dorsal or back aspect of the brain and right hand figure is a view from the posterior or top aspect.

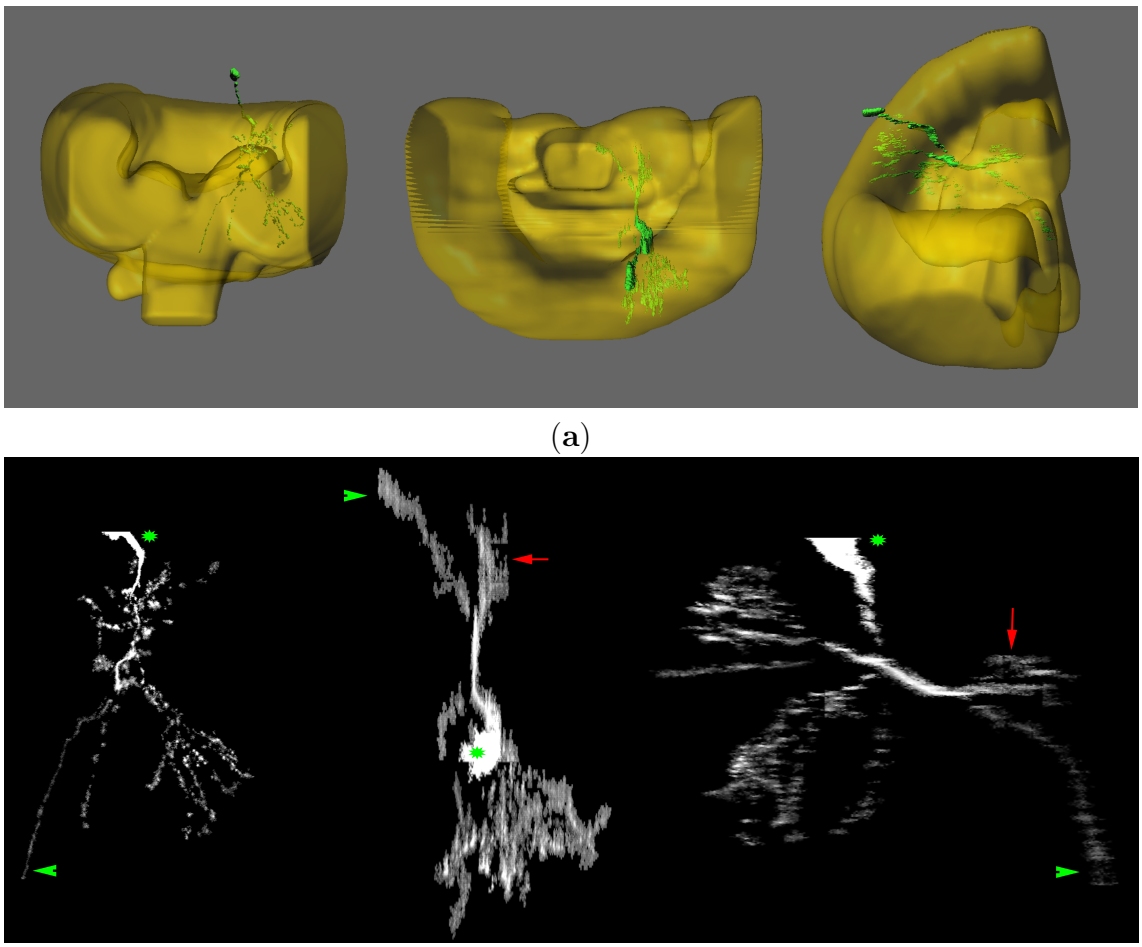


(**a**)



(**b**)

Figure 4.17: (**a**) Imaris reconstructions of an OC/IC cell in three views. Black arrows indicate the cell's IC processes. (**b**) Ray-traced projection of the cell depicted in (a). Green stars indicate primary neurite from the soma, green arrows indicate the axon, and red arrows indicate the cell's IC processes.



(**a**)

(**b**)

Figure 4.18: (**a**) Imaris reconstruction of an OC/IC cell in three views. (**b**) Ray-traced projections of the cell in part (a) in three views. Green stars indicate the primary neurite to the soma (soma not shown here, but can be seen in part [a]). Green arrows indicate the axon and red arrows indicate the cell's IC processes.

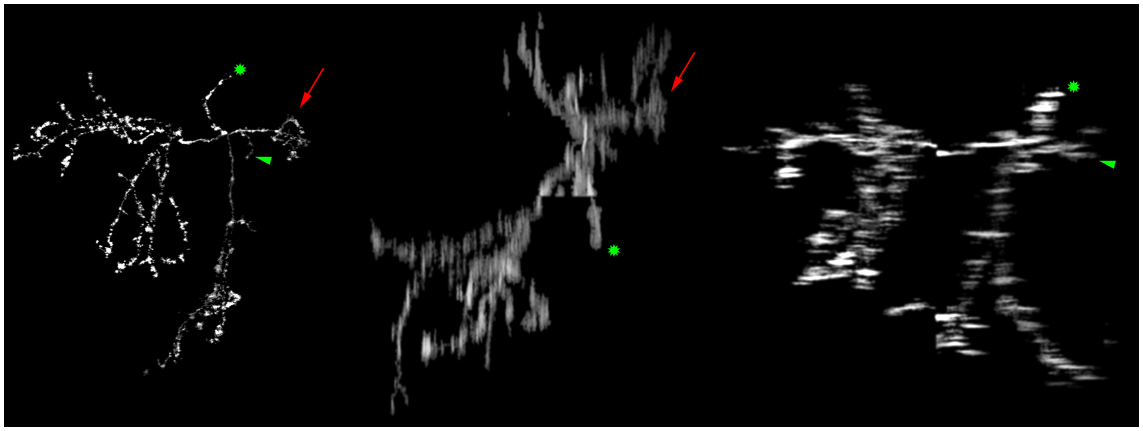


Figure 4.19: Ray-traced projection of another OC/IC cell in three views. Green stars indicate the primary neurite (soma not shown), green arrows indicate the axon, and red arrows indicate the cell's IC processes.

4.2.4 Dendritic Complementation

In some individuals, an attempt was made to fill several KCs. In some other cases, an attempt at a single fill yielded multiple fills of nearby cells (presumably because the electrode damaged or penetrated several neurites or somata). This section presents reconstructions of these multiple fills for comparison of dendritic fields and relative positions of primary neurite, dendrites, and axons within the pedunculus.

Figures 4.20 shows a set of three KCs stained in one brain. All are OC-exclusive cells, stained in different areas of the OC. The details of the individual cells are presented in figures 4.5(a), 4.6 and 4.2.

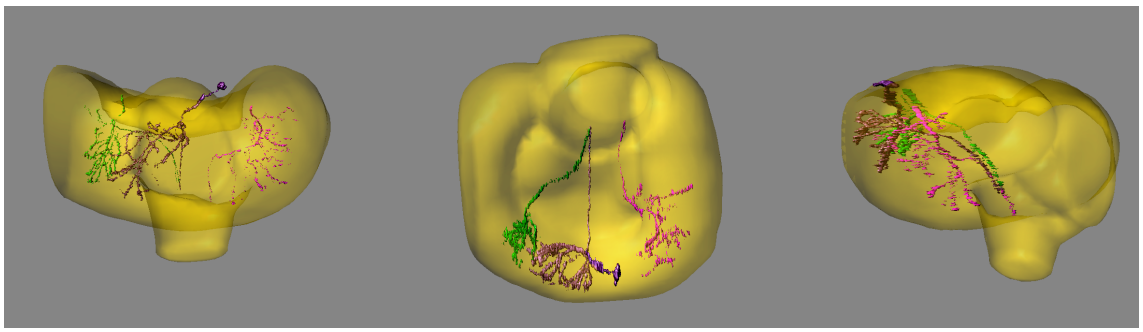


Figure 4.20: Imaris projection of three KCs stained concurrently in one brain, from the front, top and side views. All three cells depicted here are OC-exclusive cells.

Figure 4.20 shows three cells in one brain. In each case, the set of cells were stained concurrently. The cells in figure 4.22 are shown individually in figure 4.17. The red KC is an OC/IC cell, while the green cell is an OC-exclusive cell. In the ventral and lateral views, the dendrites show complementary innervation. The dendrites do not overlap, but the separate branch groups of the two cells are intercalated. Figure 4.22 shows three KCs (two OC-exclusive cells and one OC/MC cell) whose dendritic fields also show clear complementation. The green bipartite OC-exclusive cell's two dendritic clusters surround the red cells' single dendritic tree.

Figure 4.23 shows two sets of cells stained concurrently, but whose dendritic trees are so similar that they are not separable. Following a common theme in the locust olfactory system (see discussion), each set is a morphologically near-identical pair

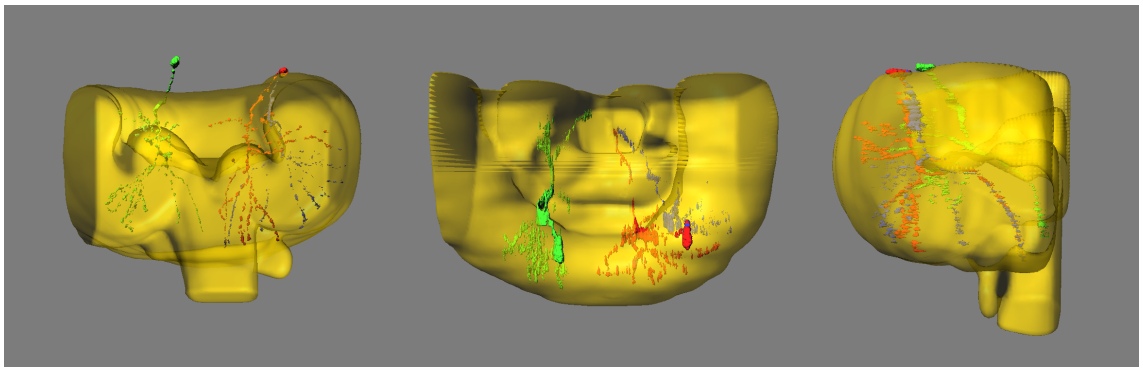


Figure 4.21: Imaris reconstruction of three cells filled in one brain. Green cell (left-most cell in front and top views) is an OC/IC cell, while the other two cells are OC-exclusive cells.

whose members are offset slightly from each other.

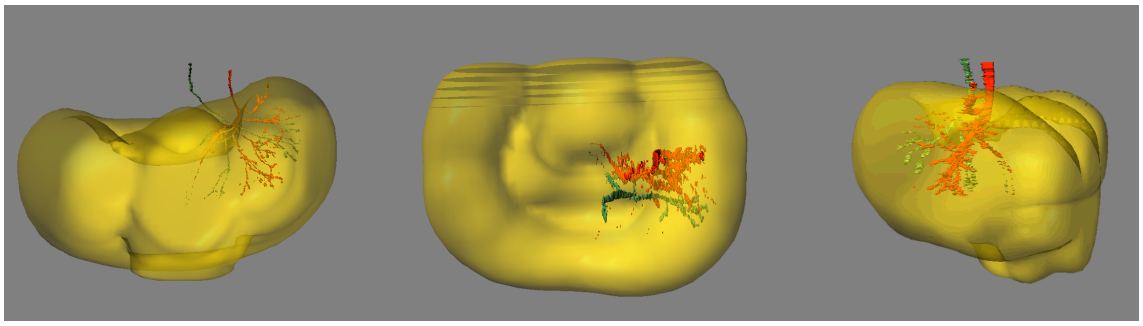
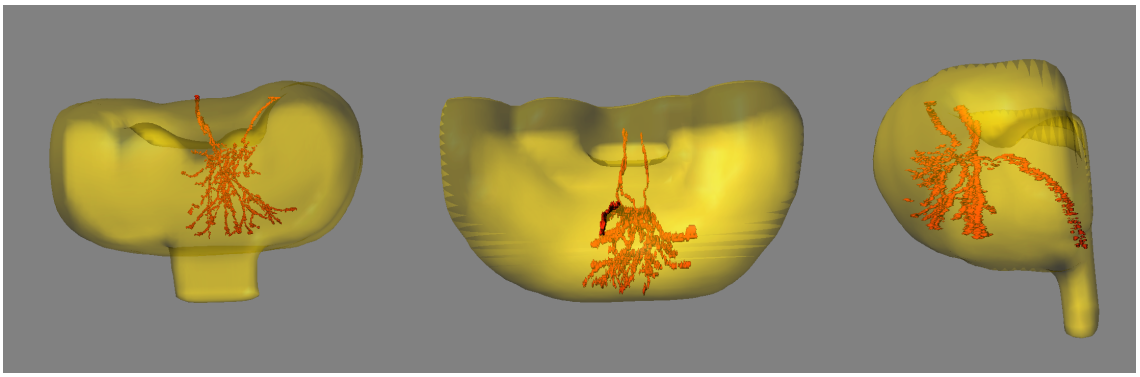
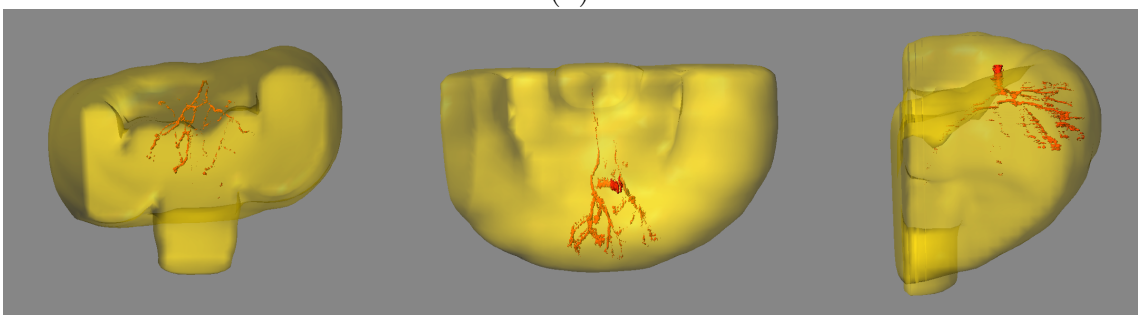


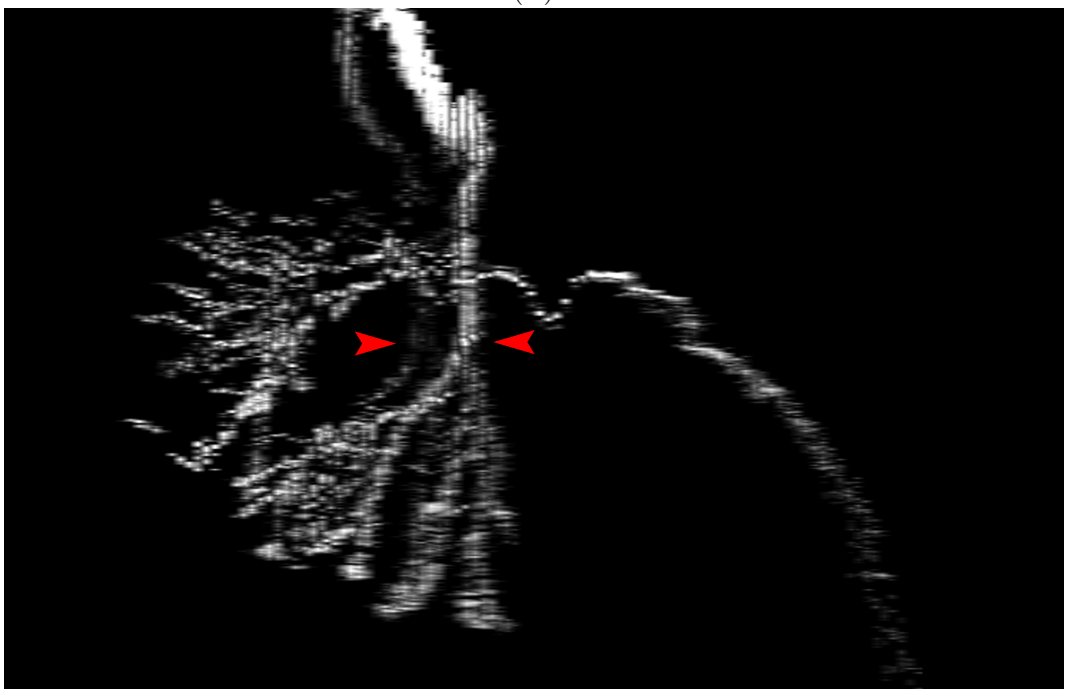
Figure 4.22: Imaris reconstruction of two KCs filled in one brain. Red cell is an OC/IC cell, and green cell is an OC-exclusive bipartite cell.



(**a**)



(**b**)



(**c**)

Figure 4.23: (**a-b**) Imaris reconstructions of two pairs of OC-exclusive cells. Due to their close resemblance, the two cells in each pair were not separable. (**c**) Ray-traced reconstruction of the side view of the cells shown in part (a). Red arrows indicate the two different cells.

4.2.5 Innervation of the Pedunculus and Lobes

In several intracellular fills of KCs, the dye diffused out to stain axonal processes within the MB pedunculus and lobes. As discussed in chapter 3, OC/IC cells send processes down the central fiber bundle while the pattern of OC/MC and OC-exclusive cells innervation of the pedunculus was not clear. These cells innervated all three fiber bundles of the pedunculus. For discussion of lobe innervation, refer to chapter 3.

4.3 Extrinsic Cells

This section presents a sampling of MB extrinsic cells. An extrinsic cell is defined as a neuron whose soma lies outside the MB and whose processes either partially or completely innervate some aspect of the MB neuropils. Their function is presumed to be largely output from (efferent) or modulatory output to the MB (pedunculus and lobes). This group of cells does not include the projection neurons from the antennal lobe, who are more properly referred to as afferent cells. The extrinsic cells presented here are all odor responsive cells, where a response consists of either a significant increase or decrease in the cell's spiking activity or membrane potential upon odor presentation.

This is by no means an exhaustive atlas. Due to the difficulty in impaling these neurons, relatively few complete fills were obtained. The neurons were penetrated in a process within the β lobe, and dye injection is needed to be maintained for at least 20 minutes for reasonable fills.

Another caveat is that these neurons were selected for filling only if they were odor responsive. The protocol was such that one particular odor — either hexanol or cherry — was puffed onto the antenna any time a cell was penetrated stably with an electrode. These odors were chosen because of observations that the LFP power in the calyx was greatest in response to these odors across individuals. If the neuron exhibited a response to the odor, then an attempt was made to inject it with the dye, Lucifer Yellow. This increased the likelihood that any of the cells filled was

promiscuous in its odor response. If a cell was responsive to only a few odors not including hexanol or cherry, it was missed by this process. For those cells shown here whose odor tuning was tested with additional odors, I observed a lack of selectivity. All extrinsic cells filled and tested exhibited a response to all the odors with which they were challenged, although their responses differed in length or patterning.

Under these conditions, five different cell types were found. A cell is defined as a type based on its morphology. Of these, the two classes (I and II) as defined in chapter 3 were filled several times across individuals. There are four examples of class I (CI) cells and five examples of class II (CII) cells. Three other cells shown were filled only once.

4.3.1 Class I Extrinsic Cells

Figure 4.24 shows an example of a CI cell. This cell's soma lies in the superficial pars intercerebralis. A primary neurite extends posteriorly and bifurcates at the brain's midline. One neurite extends toward the α lobe where it branches diffusely sending other processes into non-MB regions in the medial protocerebrum along the way. The α lobe neurite branches and extends into the pedunculus from the heel. At the heel, six separate branches split off of the primary neurite at distinct divisions in the heel [figure 4.25]. These six processes climb anteriorly in the pedunculus' central fiber bundle, branching profusely throughout. In all stains (n=4), these peduncular processes terminated midway along the length the pedunculus.

From the primary neurite's original bifurcation at the brain's midline, the second neurite crosses diagonally towards the contralateral β lobe contralateral. This process traces a path on the medial perimeter of the contralateral β lobe, never entering it, then crosses back over to the ipsilateral side in a tract just posterior to the central body. From here, the process continues into the ipsilateral β lobe where it branches into the spiny-axon division of the β lobe (see chapter 3). Finally, this process projects to the lateral horn lobe where it branches sparsely and terminates [figure 4.26].

One striking observation about this cell is the co-localization of its MB processes

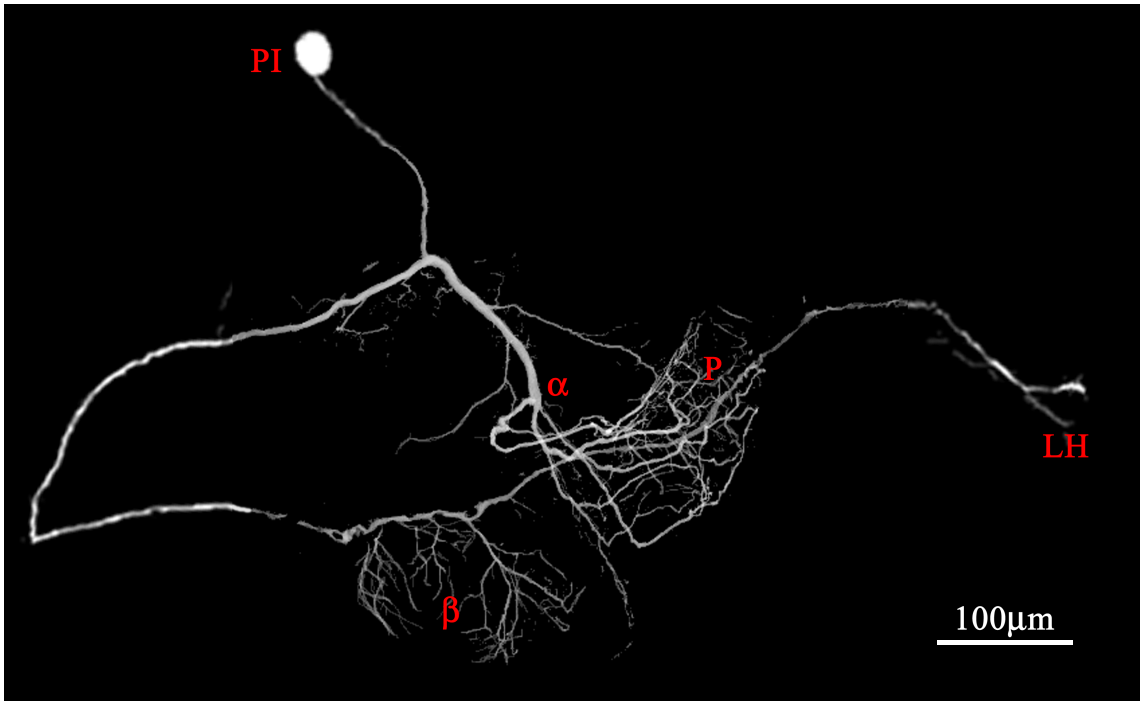


Figure 4.24: A ray-traced projection of a class I extrinsic cell.

with previously defined regions of NADPH diaphorase immunoreactivity [67]. In the heel of the MB, six such clear immunopositive zones are visible. In figure 4.27 two of these zones are indicated by the red arrows. Immunoreactivity has also been described in the core of the pedunculus, some of which also co-localizes with CI processes.

CI cells exhibit promiscuous odor responses. Responses to four odors are displayed in figure 4.28, for the cell shown in figure 4.24. Odor trials were performed prior to injecting the cell with dye. For each odorant, the cell was challenged with a one-second puff of odor (as described in chapter 2) every 10 seconds, 10 times in a row. The cell was allowed to 'recover' for approximately 2 minutes between each set of odor trials. This cell's responses to all four odors tried were similar although not identical, and similar to those recorded from the three other examples of CI cells in three other animals.

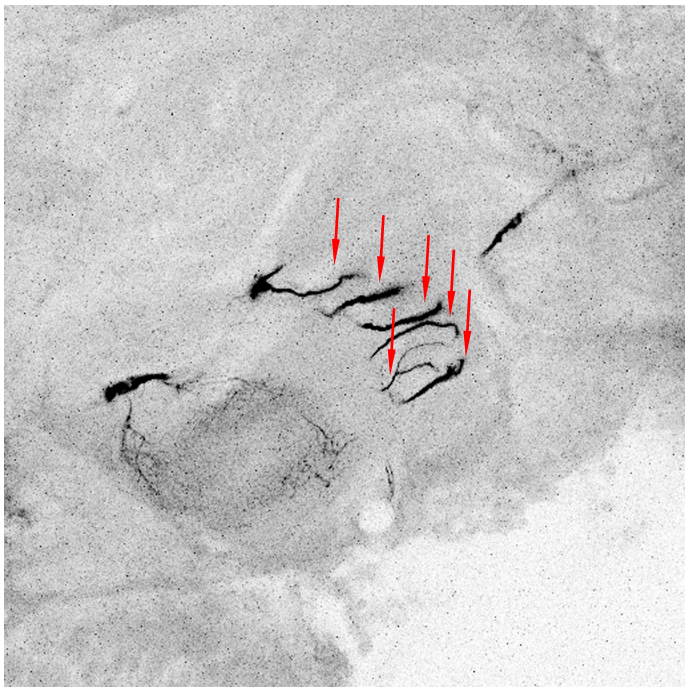


Figure 4.25: Ray-traced projection of the heel region of the MB, showing six branches (red arrows) of a CI extrinsic cell, corresponding to the NADPH diaphorase positive zones shown in figure 4.27.

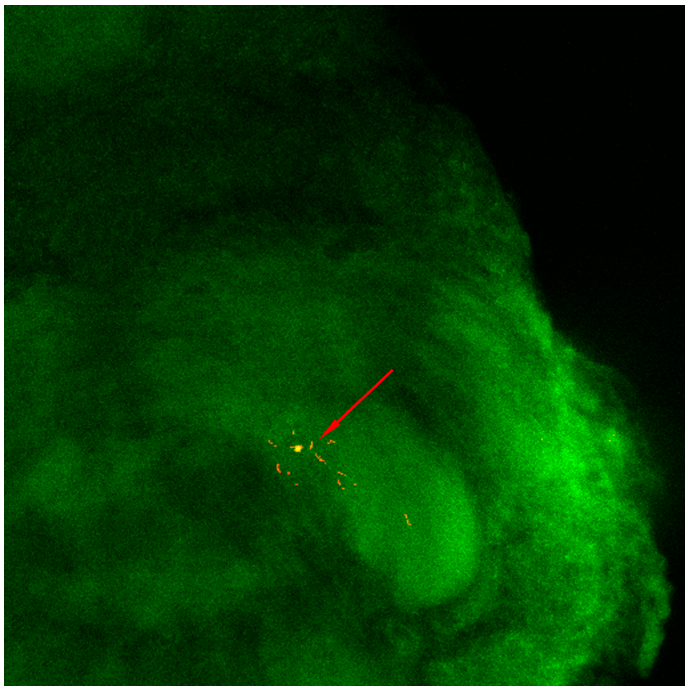


Figure 4.26: Ray-traced projection of the LH; CI terminals in the LH lobe are orange and indicated by the red arrow.

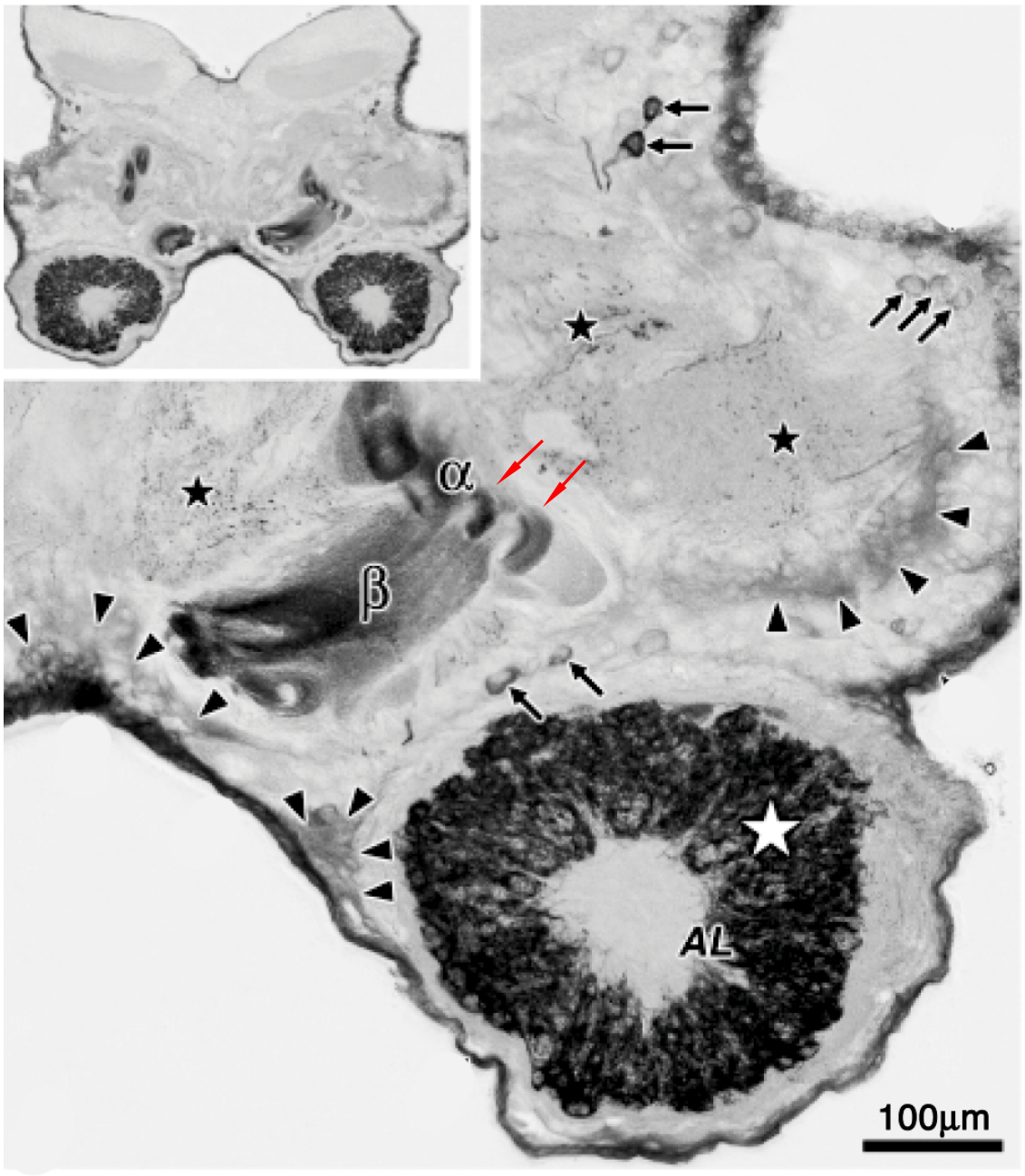
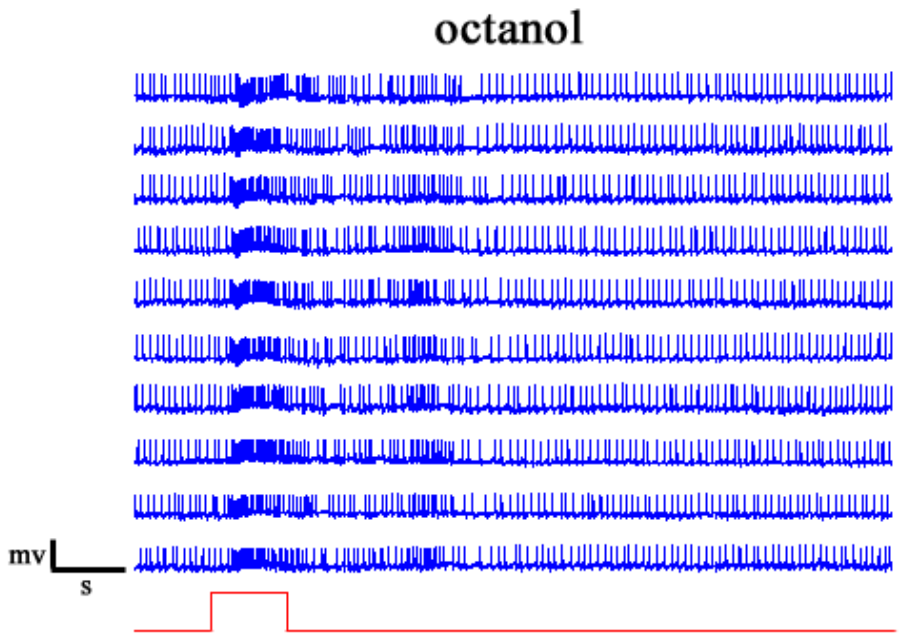
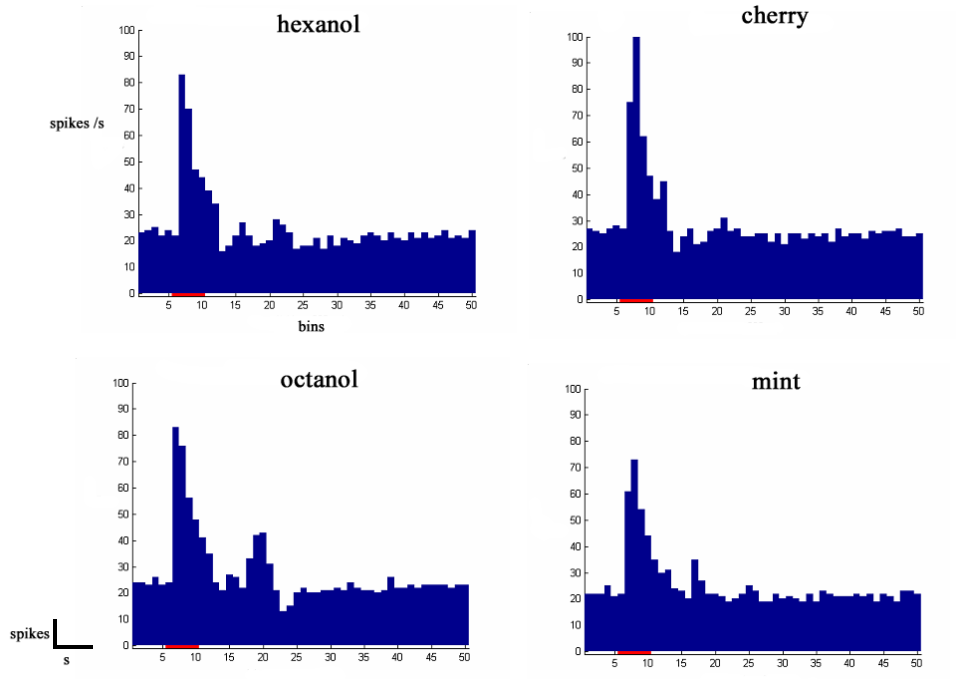


Figure 4.27: NADPH diaphorase staining in the brain of the locust. Red arrows indicate two of six immunoreactive lamina that begin in the peduncular heel and continue up into the α lobe. Adapted from [67].



(**a**)



(**b**)

Figure 4.28: Odor responses of a class I extrinsic cell. (**a**) Ten trials with one odor (octanol). Each line (in blue) is one 10 second trial, where the odor was presented from between seconds 2 and 3 (as indicated by red line). Horizontal scale bar is equal to 1 second, vertical scale bar is equal to 20 mV. (**b**) Histograms of spike counts over ten trials with each of four odors: hexanol, mint, octanol and cherry. Each bin is the sum of 200 mS bins, odors presented during bins 5-10. Horizontal scale bar is equal to 1 second, vertical scale bar is equal to 10 spikes.

4.3.2 Class II Extrinsic Cells

Figures 4.29 show three examples of CII cells. Part (a) shows a ray tracing of a single CII cell. Figure 4.30 shows a pair of cells filled concurrently and reconstructed with their underlying brain structure. These cells' somata lie in the lateral protocerebrum just anterior to the optic stalk, 200 μm from the ventral surface of the brain.

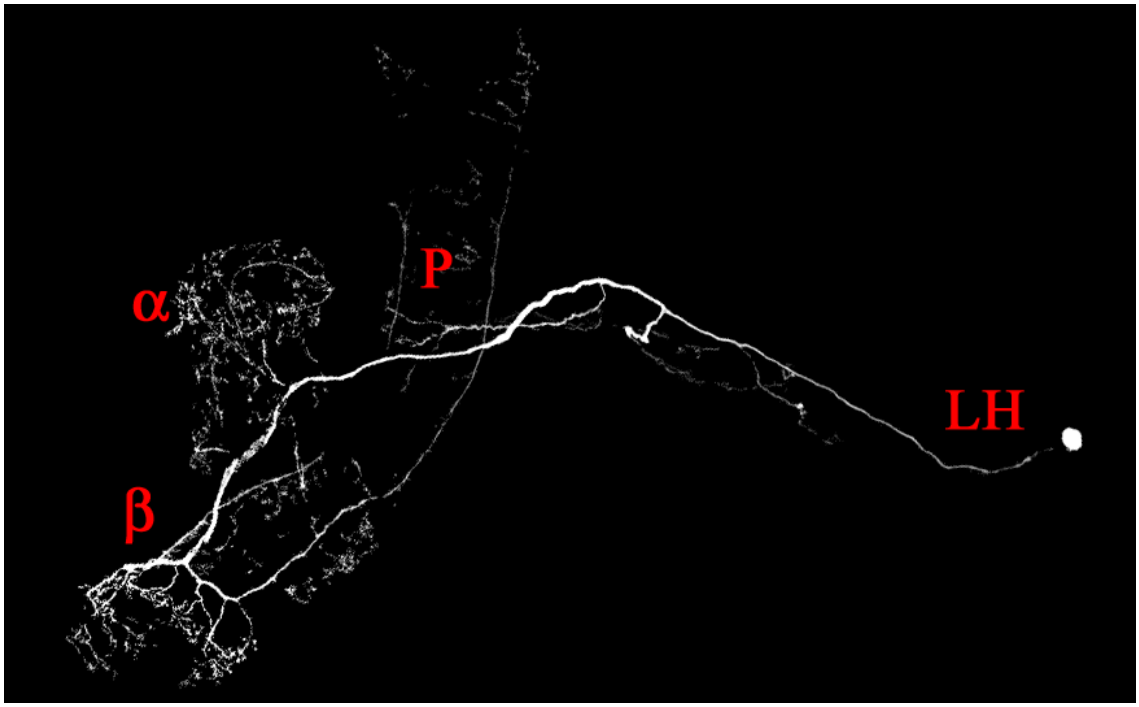
The primary neurite of CII cells extends through the lateral horn lobe (as described in Chapter 3), branching sparsely there, and on to the ipsilateral MB. A few other diffuse processes extend into unidentified areas of the protocerebrum between the lateral horn lobe and the MB neuropils. The primary neurite crosses over the ventral surface of the β lobe; one set of fibers separates and branches profusely in the α lobe. The primary neurite continues into the β lobe medially, branching into processes which curve to form a 'C' (perpendicular to the β lobe's long axis) around the spiny axon division of the β lobe. From this 'C', neurites branch at regular intervals, perpendicular to the long axis of the β lobe (figure 4.31). From either end of the C-shaped neurite, two other branches turn into the heel of the MB and progress posteriorly up the pedunculus (as explained in Chapter 3), sending fine fibers into the pedunculus. These processes continue up and into the calyces. In the case of the paired fill, there is innervation of the IC and MC (figure 4.32).

As discussed in chapter 3, Bodian stains show a set of 9-12 processes at the ventral surface of the α lobe that correspond in shape and lobe innervation to CII cells (figure 4.33). In Bodian stains, the somata can be found adjacent to the optic stalk, as seen in fills (figure 4.34).

In GABA stains (figure 4.35) a corresponding group of GABA-positive cell bodies can be found in the same protocerebral region. GABA studies by Leitch and Laurent [44] also confirm GABA processes both in the lobes and pedunculus in areas where CII cells are seen to innervate.

As discussed in chapter 3, double fills of CII cells indicate that these cells are a morphologically identical group who are positioned at different offsets within the lobes, pedunculus and calyx, and which may each sample different subsets of KC

axons.



(**a**)

(**b**)

Figure 4.29: CII extrinsic cells. (**a**) Ray-traced projection of a single CII extrinsic cell, showing its soma in the lateral horn (LH) and its innervation of the MB lobes and pedunculus. (**b**) Ray-traced projection of a pair of CII cells filled concurrently.

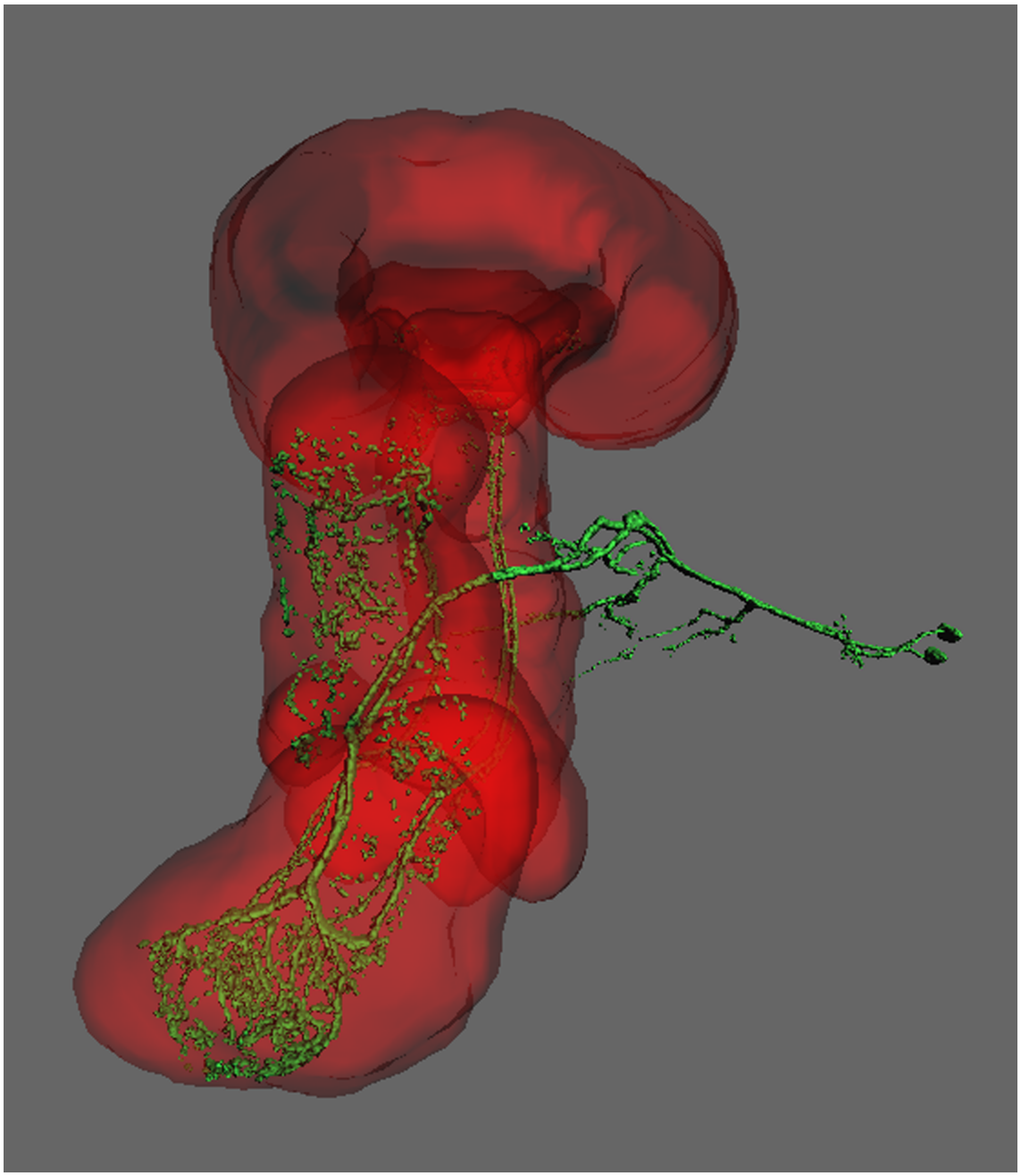


Figure 4.30: Imaris reconstruction of the pair of CII extrinsic cells shown in figure 4.29(b). The MB neuropil (α and β lobes and calyx) is reconstructed in red. Note that this is a ventral view, but the brain is tilted back slightly.

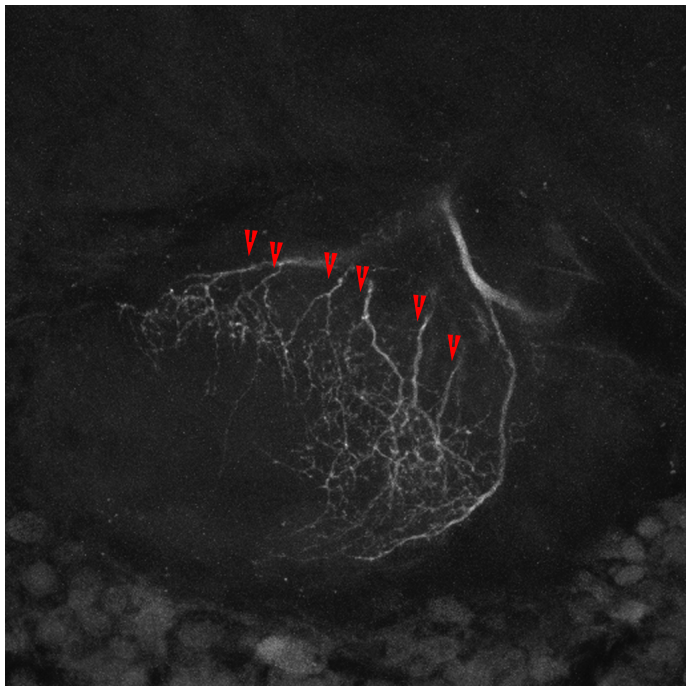
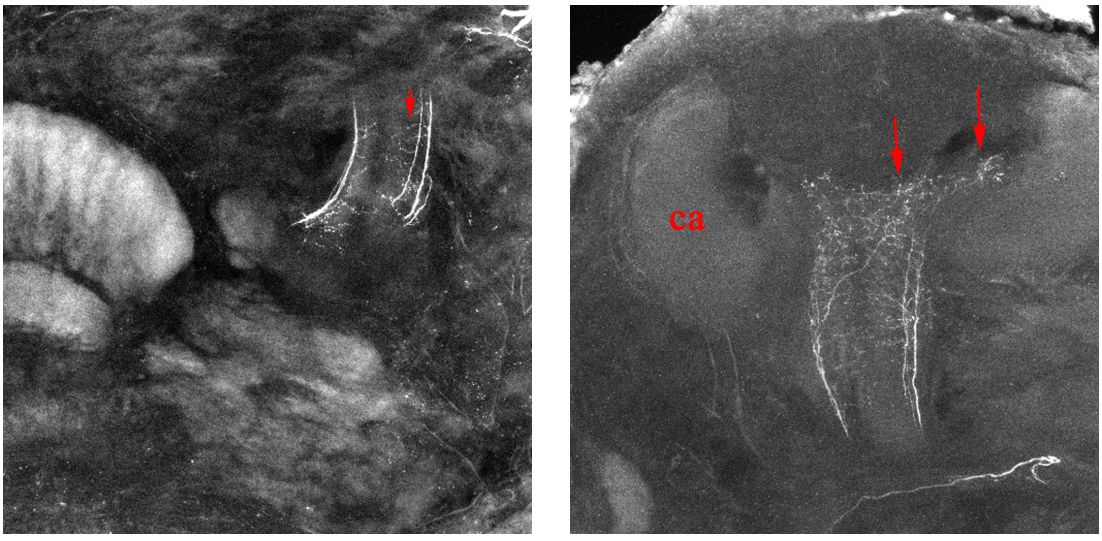
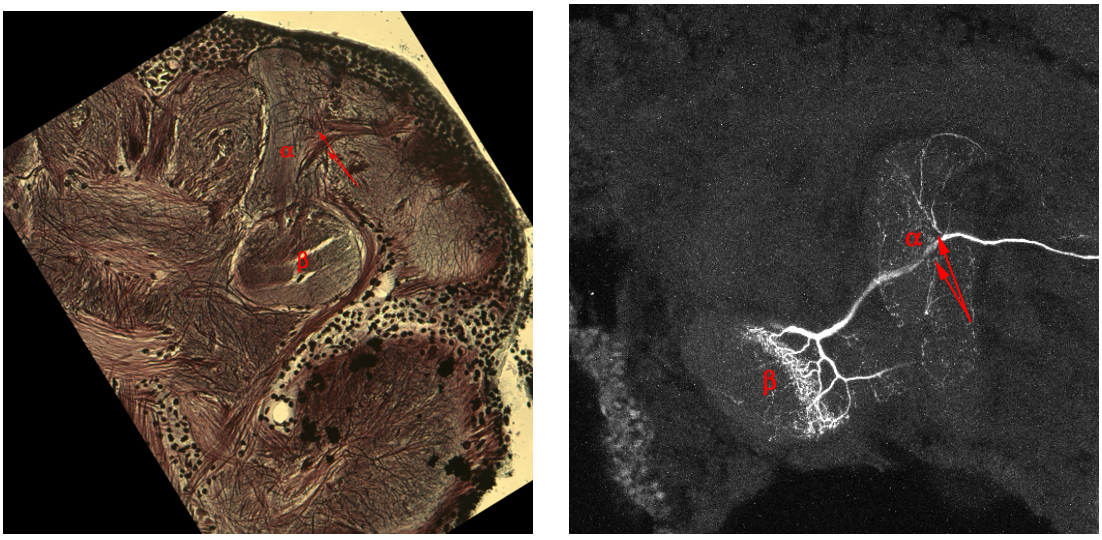


Figure 4.31: 6 μm thick ray-traced projection of the β lobe processes of the pair of CII extrinsic cells shown in figures 4.29(b) and 4.30. Red arrows indicate the processes that branch off at regular intervals into the lobe.



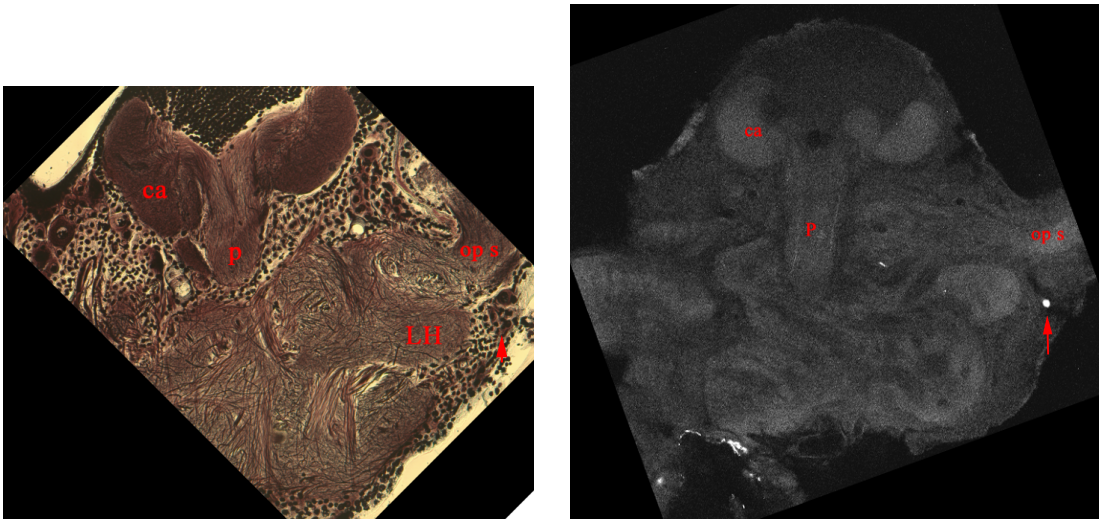
(**a**) (**b**)

Figure 4.32: Peduncular and calycal innervation of CII extrinsic cells. (**a**) Red arrow indicates the fine fibers innervating the pedunculus. (**b**) Peduncular and calyx innervation of the same cells as in part (a). Red arrows indicated the MC and IC divisions.



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

Figure 4.33: Comparison of CII extrinsic cell fill with Bodian stained processes. (**a**) A 10 μm Bodian slice showing a bundle of processes entering the MB lobes that correspond to the CII cell processes. (**b**) A high magnification 10 μm ray-projection of the primary neurite of the CII cell innervating the MB lobes.



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

Figure 4.34: Comparison of CII extrinsic cell fill with Bodian stained somata. (**a**) A 10 μm Bodian slice showing possible candidates for CII somata in the lateral horn. (**b**) A 10 μm ray-projection exhibiting the soma of a CII extrinsic cell in the lateral horn.

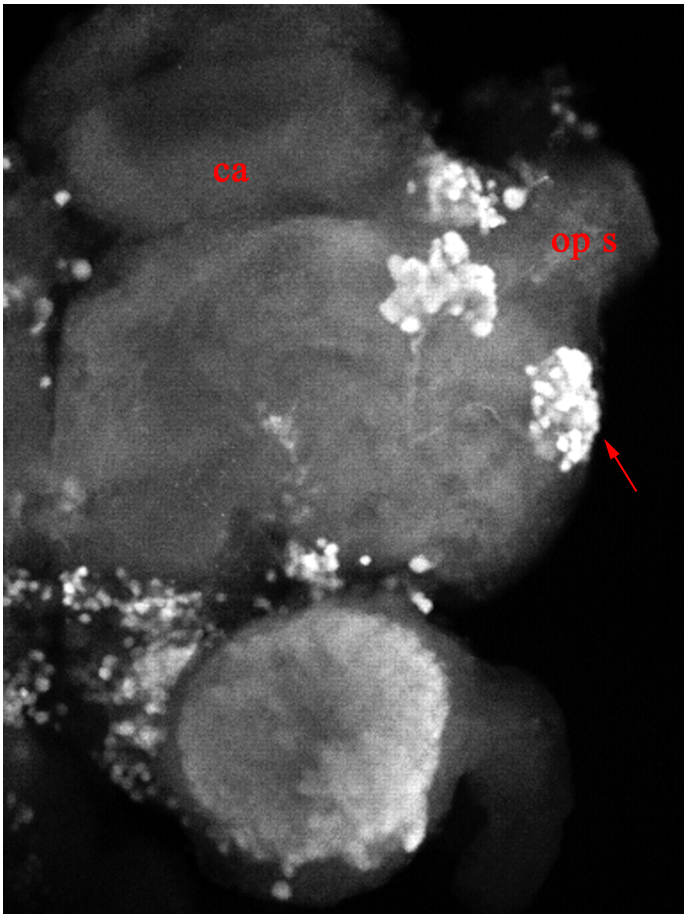


Figure 4.35: Ray-traced projection of a brain stained for GABA immunoreactivity. Red arrow indicates a cluster of GABA immunoreactive cells that correspond in position to CII somata position.

4.4 Projection Neurons

This section presents an anatomical look at the projection neurons in ray-traced and Imaris projections. A total of 14 examples from 65 filled cells are shown. First, I show a variety of individual PNs reconstructed within the AL, demonstrating that PNs are more similar to each other than any other class of cells. This also presents the most striking example of repeated morphological units situated to sample slightly offset areas of a structure. A second section presents a comparison of multiple PNs within the same AL, showing their relationships to one another. Finally, PN axonal innervation of the calyx and lateral horn lobe (as defined in chapter 3) are shown in Imaris and ray-traced reconstructions.

4.4.1 Projection Neurons and the Antennal Lobe

Each of the paired bilaterally symmetric antennal lobes is a half dome structure, situated with the flat side facing dorsally in the brain. The AL contains a central tube-like core that contains the axonal fibers of the PNs and feeds into the antennocerebral tract to the calyx and LH. Around this core of fibers within the AL are the numerous glomeruli arranged in concentric circles in planes roughly 25 μm thick throughout the AL. PN somata occur in a cluster on the posterior half of the ventral surface of the AL (figure 4.36). In general, PNs send one primary neurite from the soma dorsally toward the center of the AL. At some depth — which varies for each PN — the neurite forms a planar horse-shoe shape around the AL core bundle tube of axons, sending an axon into the core to continue into the ACT. From the horseshoe primary neurite, 5-7 secondary neurites branch off at regular intervals and from each of these 2-3 tertiary fibers branch off, terminating in glomeruli. Each PN thus innervates between 10 and 20 glomeruli. PN neurites are symmetrical; each PN's glomeruli occur in one 25 μm plane and at a fixed distance (referred to here as the PN's radius) from the AL core bundle, so that a circle is formed by tracing a line through adjacent glomeruli of one PN.

In a 25 μm plane of the AL, several PNs with different radii are visible. Figures

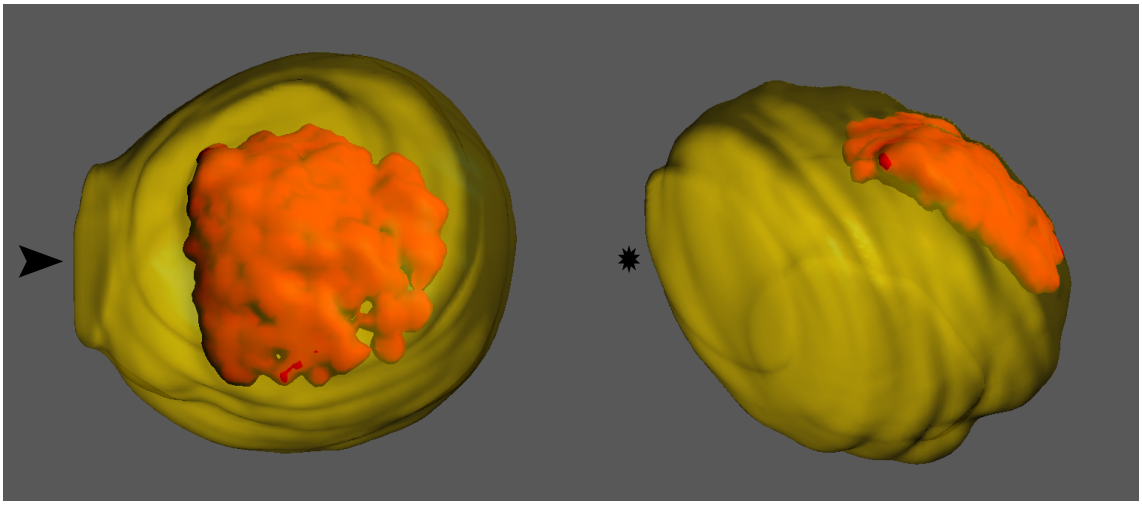
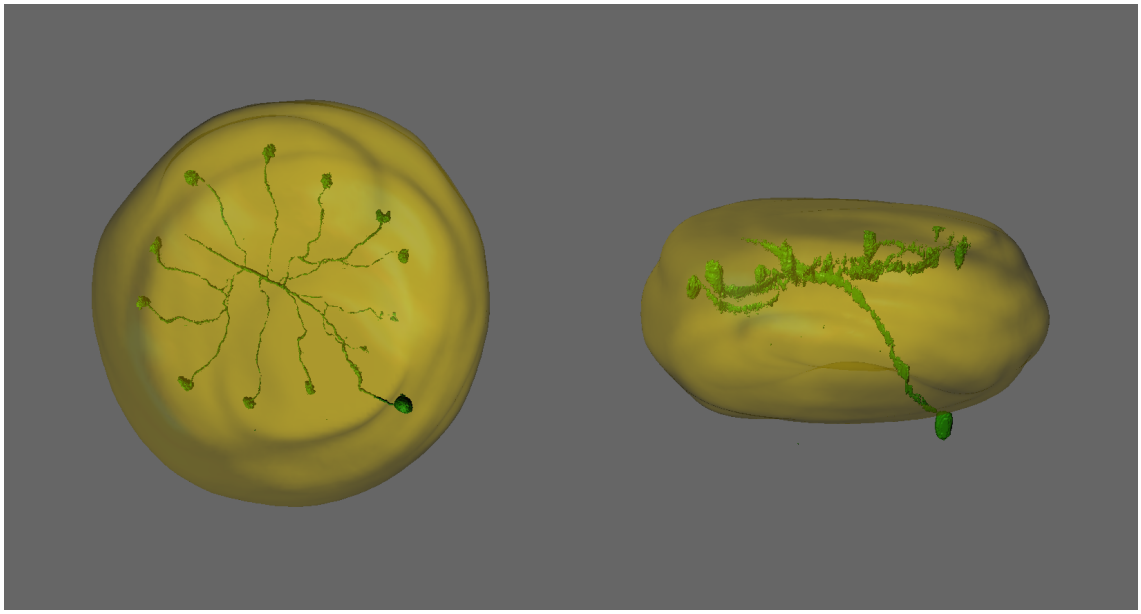


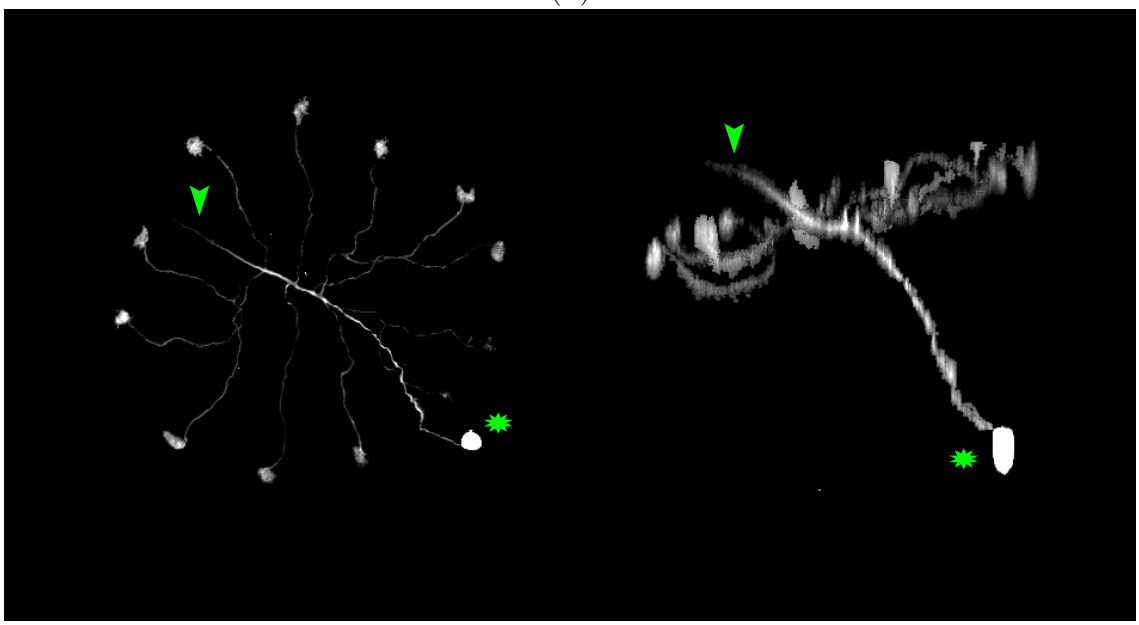
Figure 4.36: Imaris reconstruction of the antennal lobe (yellow) showing the position of the projection neuron somata (red). Left image shows the frontal view, with the position of the antennal nerve indicated by the black arrow. Right image shows a side view in which the antennal nerve is in back. The black star indicates the position of the antennocerebral tract.

4.37 to 4.41 show medium and long-radius PNs while figures 4.42 to 4.46 show PNs with shorter radii. Multiple fills of pairs of PNs within a single AL show that PNs are arranged in planes, and that the planes lie parallel to each other as shown in figures 4.47 to 4.49. These figures also display the relative sizes of PN radii as each pair contains one large-radius PN and one small-radius PN.

Figure 4.50(b) shows three planar cells that were not separable. However, this is a good example showing one small radius and two large radius cells within one plane. Figure 4.50(c) also shows a pair of coplanar cells, with different radii. Within a plane, cells with the same radii are also visible with adjacent and overlapping glomeruli. In figure 4.51, three cells are displayed: two unseparable planar cells in green and one cell in a different plane in red. Overlapping glomeruli are visible between the two planar cells. Whether individual glomeruli form larger glomerular complexes is not known.

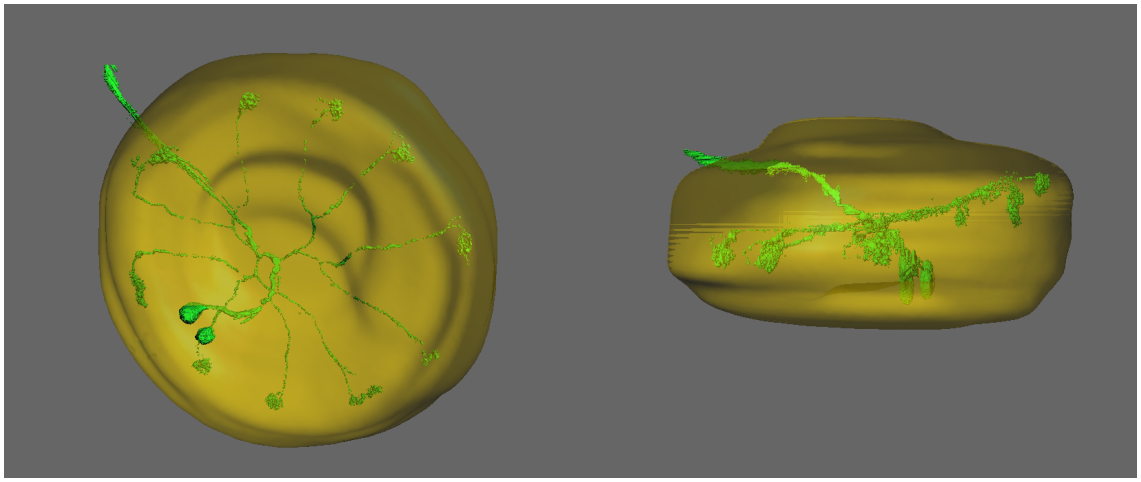


(**a**)

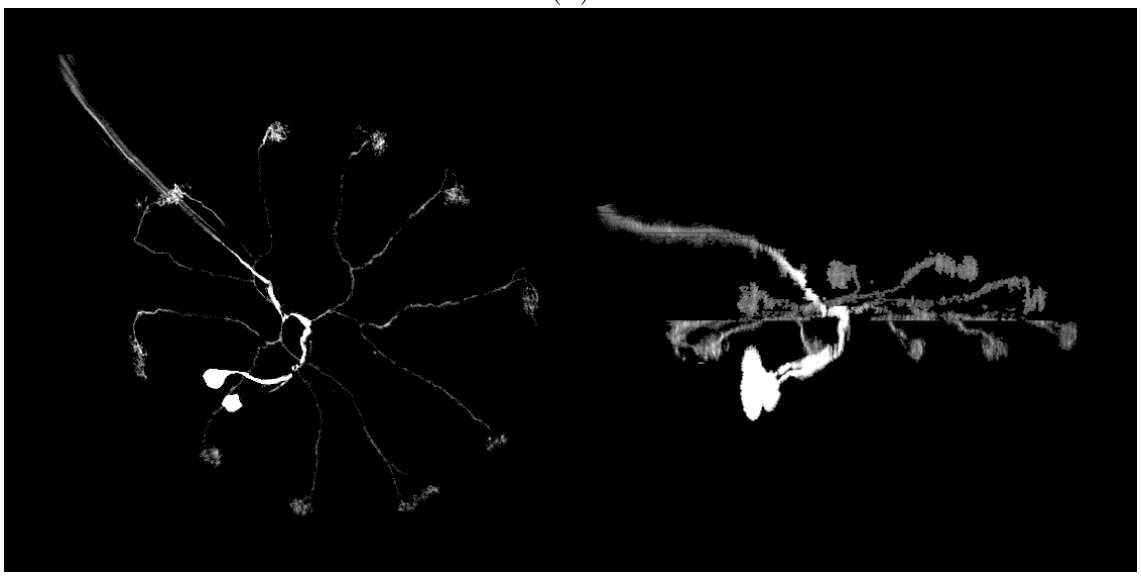


(**b**)

Figure 4.37: (**a**) Imaris reconstruction of a single PN within the AL from the frontal (left image) and posterior (right image) views. (**b**) Ray-traced projection of the cell in part (a), from the same two views. Green stars indicate the cell's soma and green arrows indicate the axon.

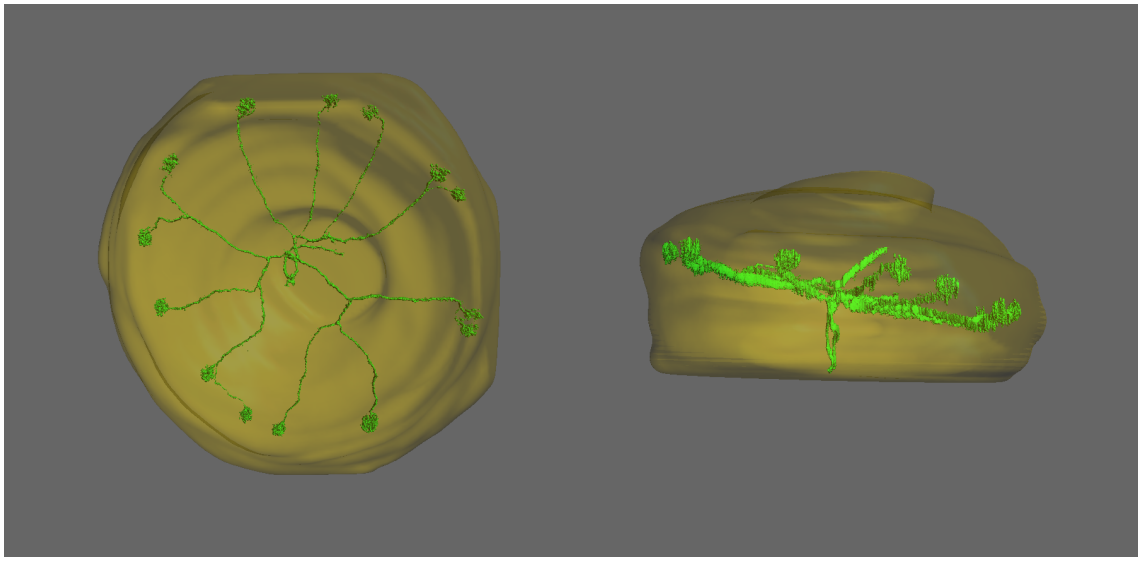


(**a**)

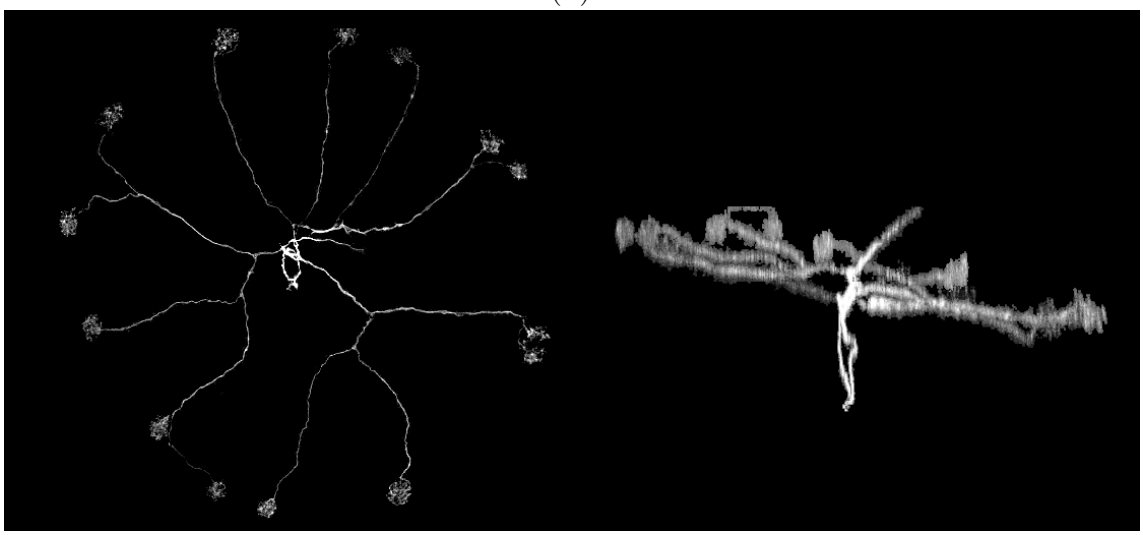


(**b**)

Figure 4.38: (**a**) Imaris reconstruction of a single PN within the AL from the frontal (left image) and posterior (right image) views. Note that there are two somata filled, but only one cell's processes are visible. (**b**) Ray-traced projection of the cell in part (a), from the same two views.

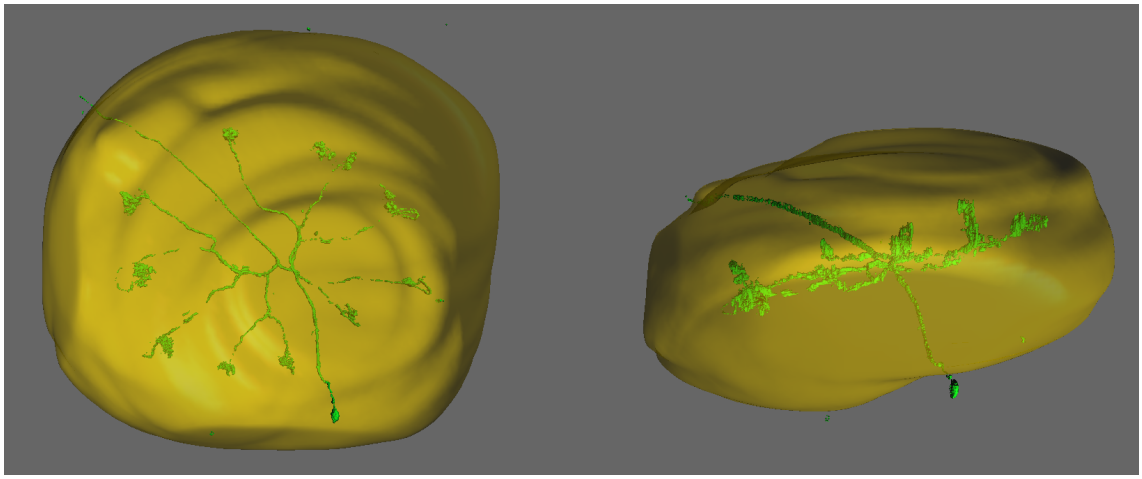


(**a**)

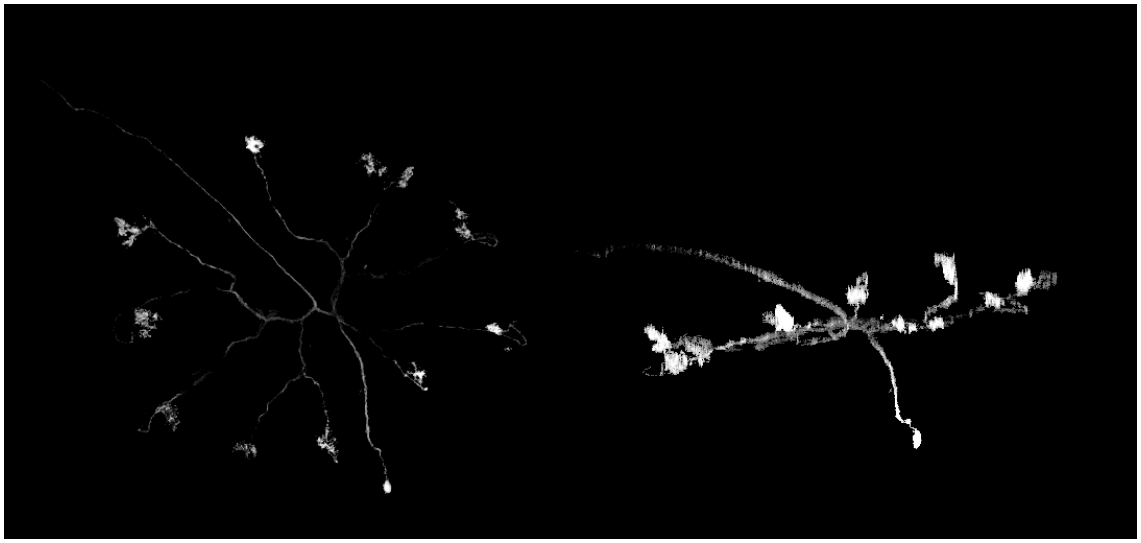


(**b**)

Figure 4.39: (**a**) Imaris reconstruction of a single PN within the AL from the frontal (left image) and posterior (right image) views. (**b**) Ray-traced projection of the cell in part (a), from the same two views. Note that the soma is missing.

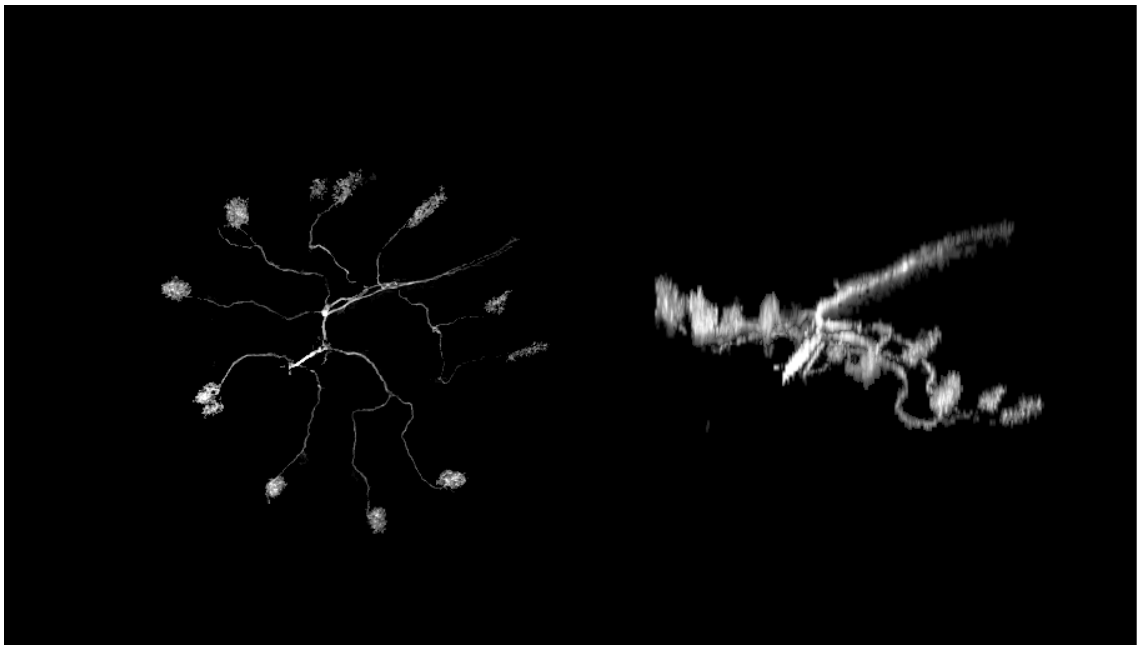


(**a**)

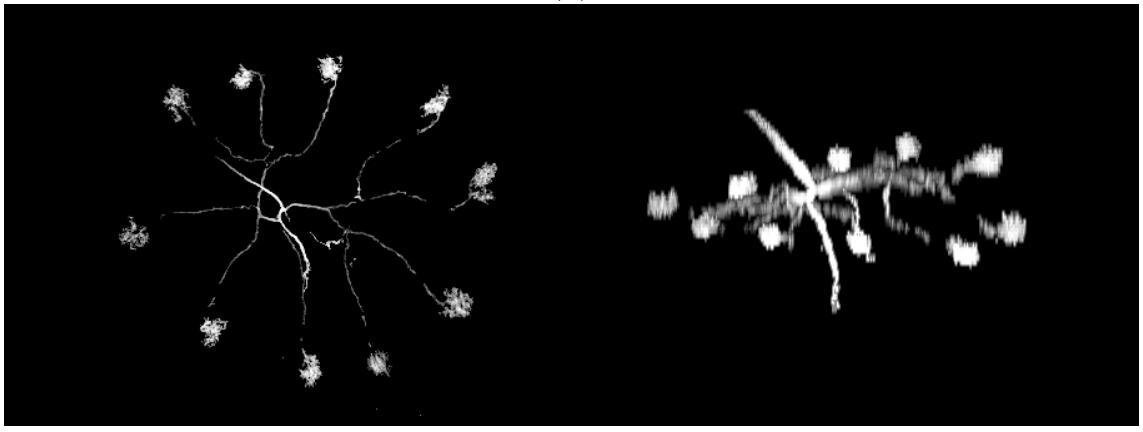


(**b**)

Figure 4.40: (**a**) Imaris reconstruction of a single PN within the AL from the frontal (left image) and posterior (right image) views. (**b**) Ray-traced projection of the cell in part (a), from the same two views.

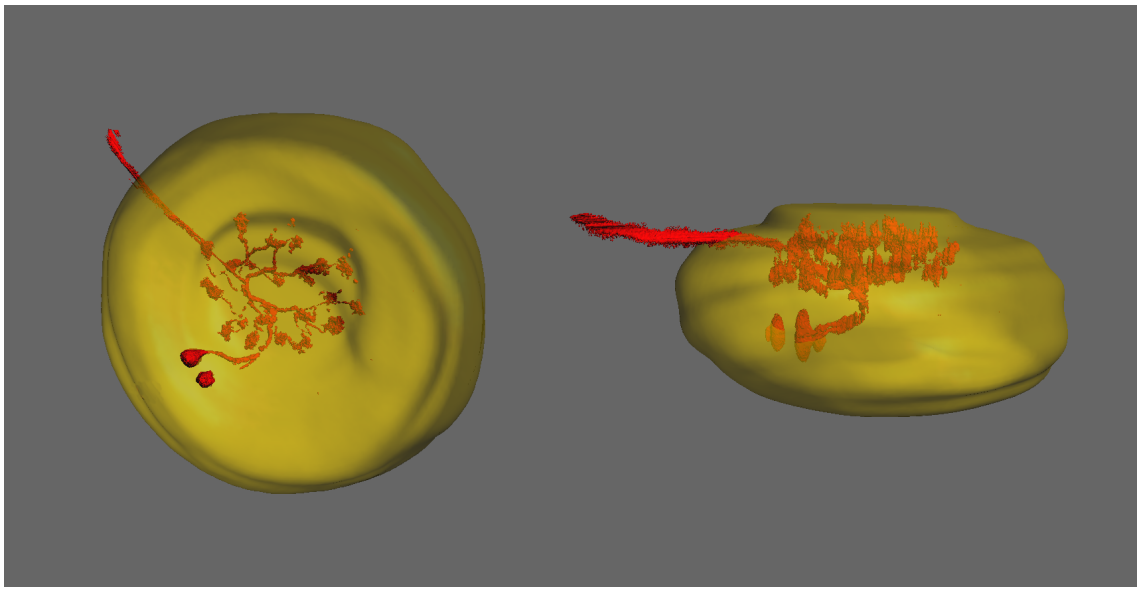


(**a**)

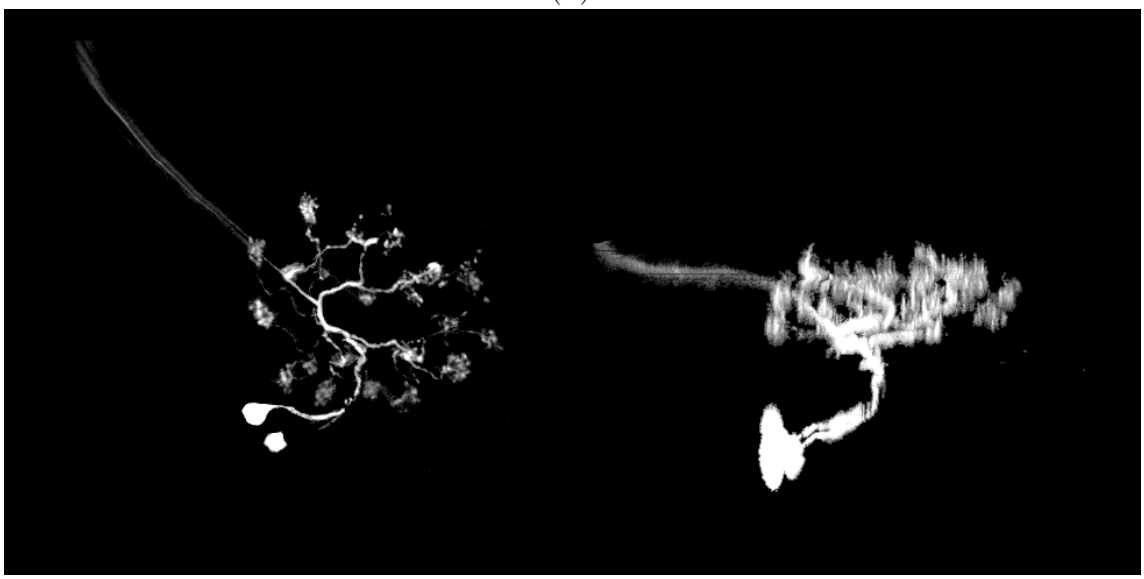


(**b**)

Figure 4.41: Ray-traced projections of two different PNs from the same two views as before. Note that both cells are missing their somata.

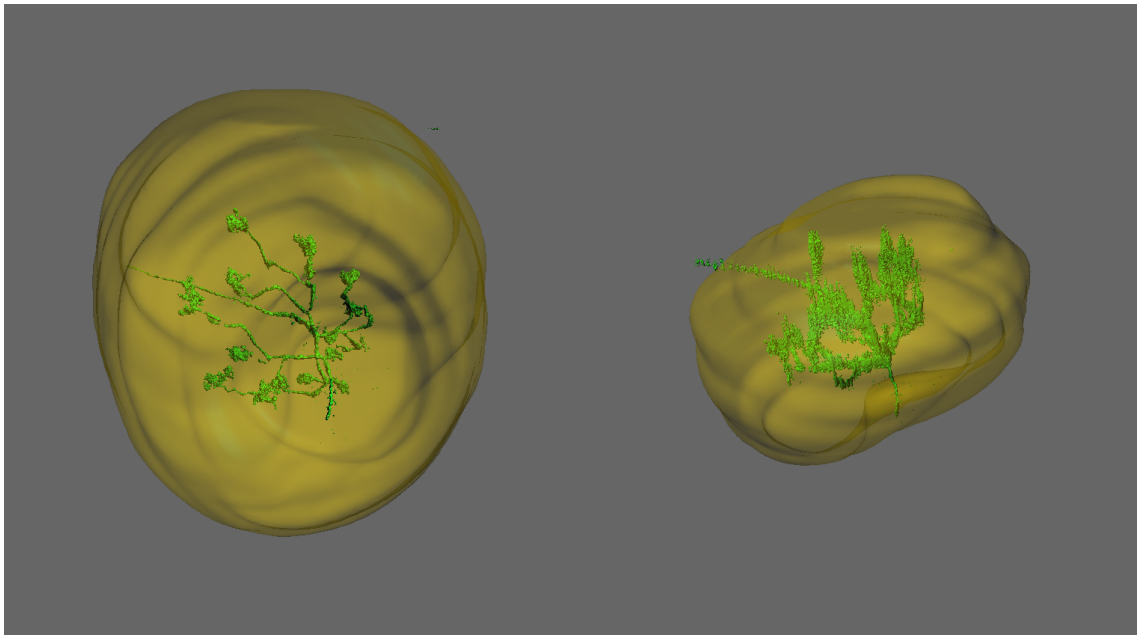


(**a**)

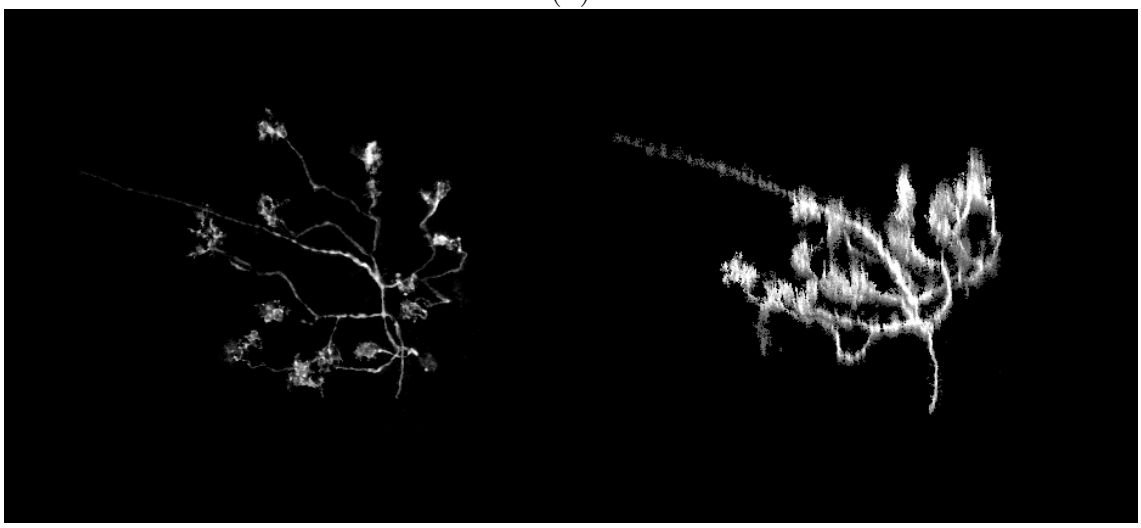


(**b**)

Figure 4.42: (**a**) Imaris reconstruction of a single PN within the AL from the frontal (left image) and posterior (right image) views. (**b**) Ray-traced projection of the cell in part (a), from the same two views. Note that there are two somata filled, but only one cell's processes are visible.

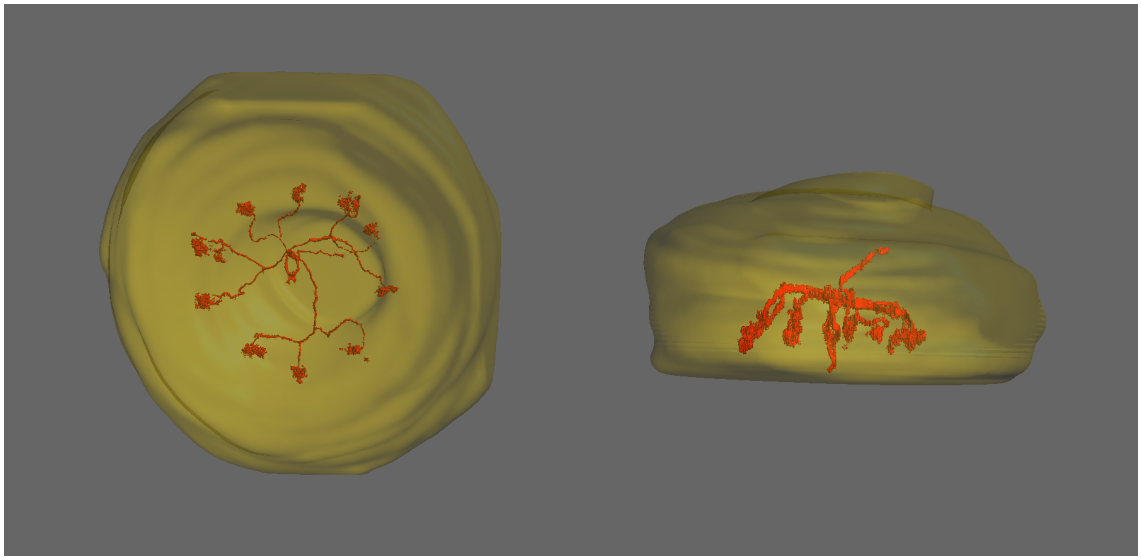


(**a**)

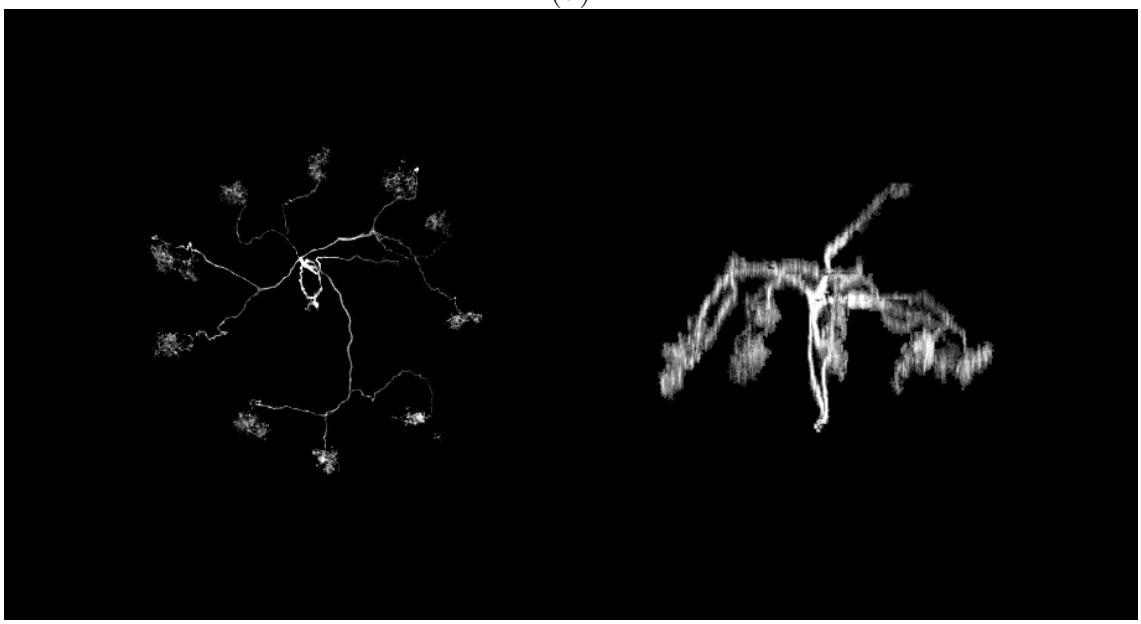


(**b**)

Figure 4.43: (**a**) Imaris reconstruction of a single PN within the AL from a slightly oblique frontal view (left image) and the posterior (right image) view. (**b**) Ray-traced projection of the cell in part (a), from the same two views. Note that the soma is missing.

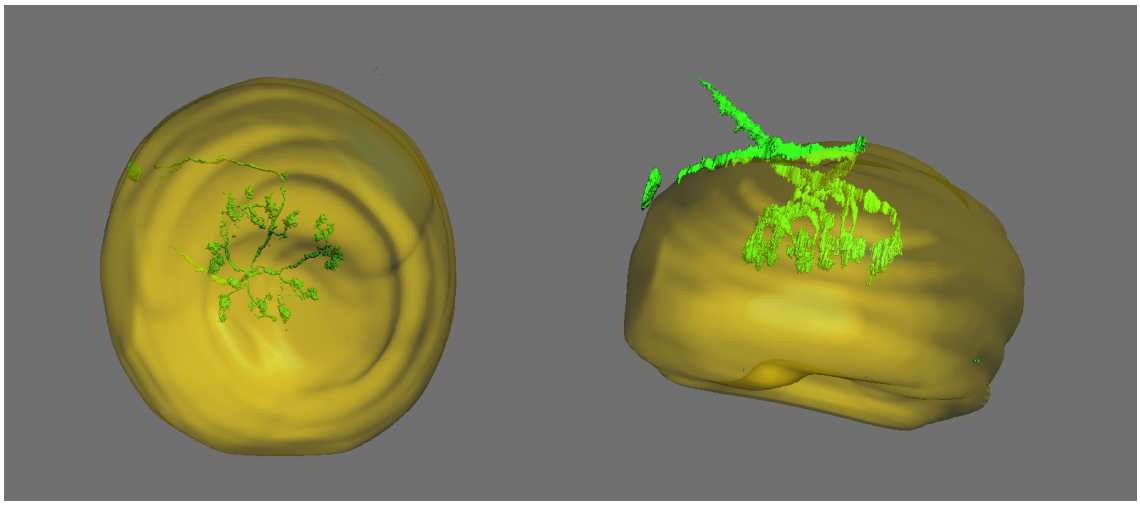


(**a**)

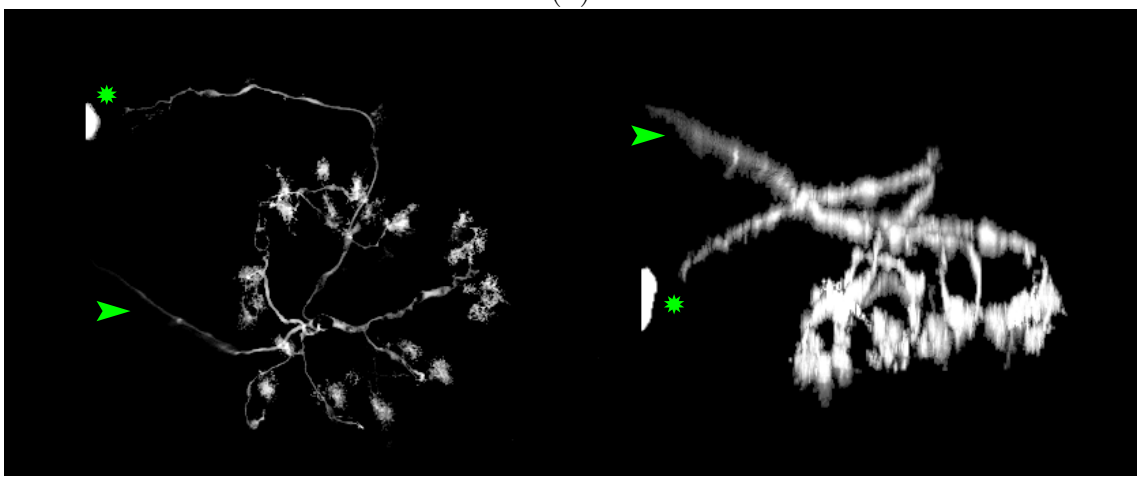


(**b**)

Figure 4.44: (**a**) Imaris reconstruction of a single PN within the AL from the frontal (left image) and posterior (right image) views. (**b**) Ray-traced projection of the cell in part (a), from the same two views. Note that the soma is missing.



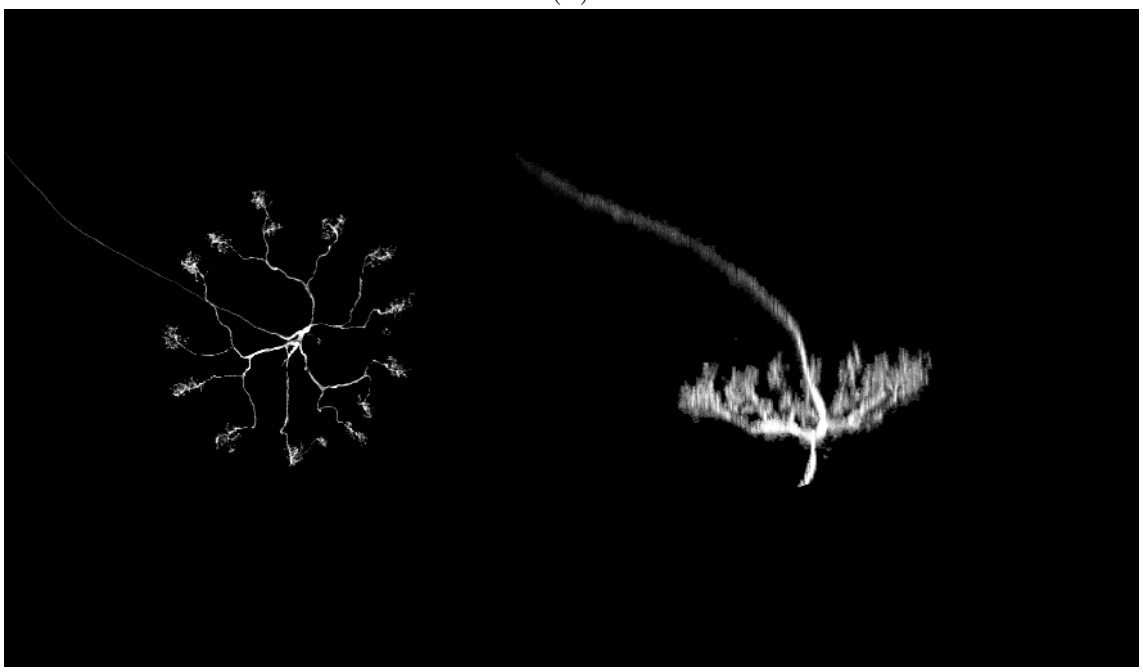
(**a**)



(**b**)

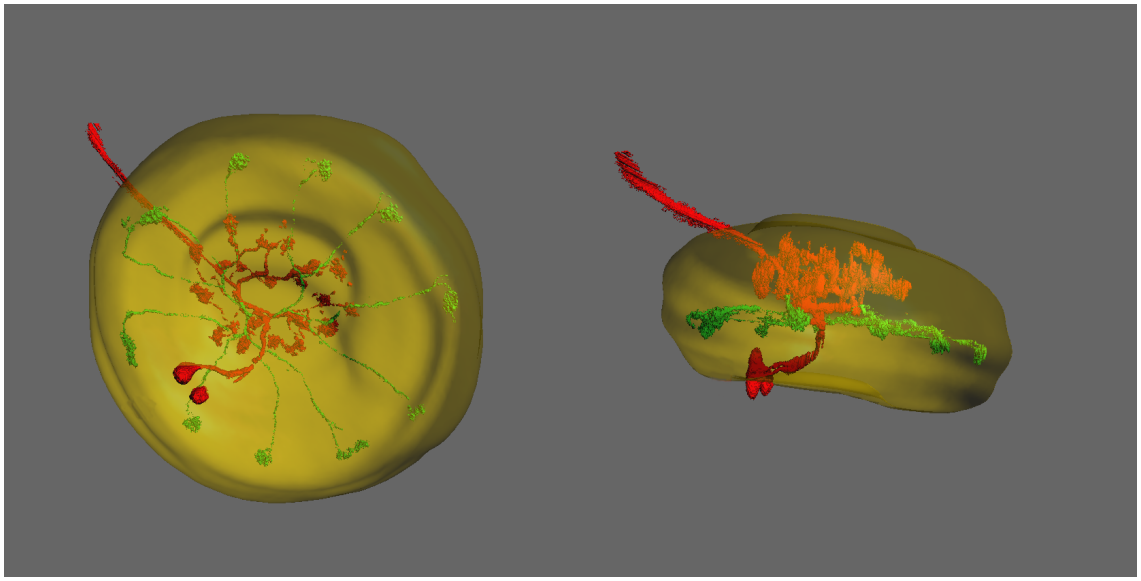
Figure 4.45: (**a**) Imaris reconstruction of a single PN within the AL from the frontal (left image) and posterior (right image) views. (**b**) Ray-traced projection of the cell in part (a), from the same two views. Green arrows indicate the axon, and the green stars indicate the soma. Note that this PN's soma is in a different location that the other PNs shown above.

(**a**)

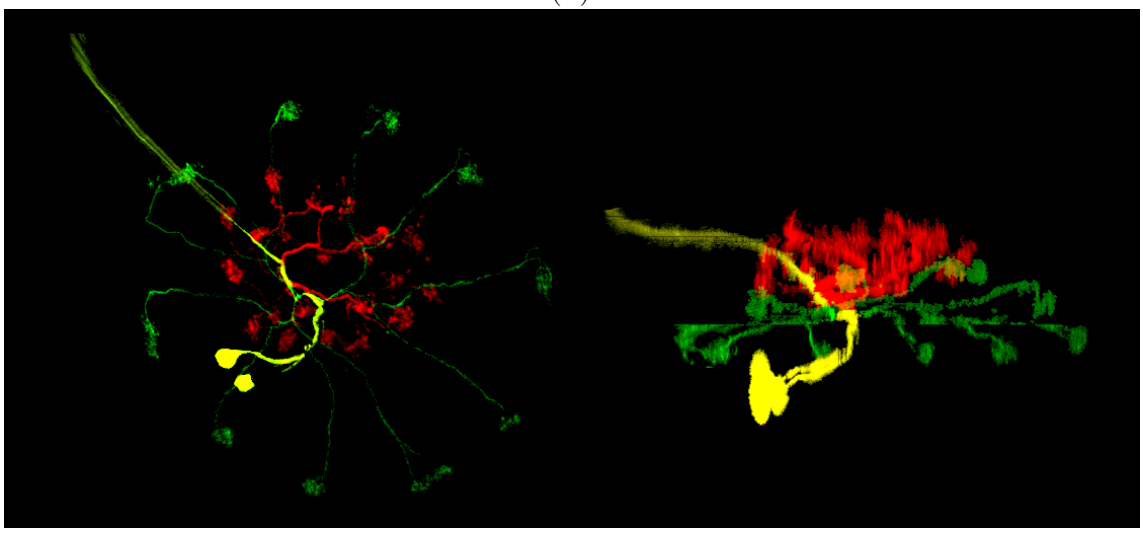


(**b**)

Figure 4.46: Ray-traced projections of two different PNs from the same two views as before. Note that the cell in part (b) is missing its soma.

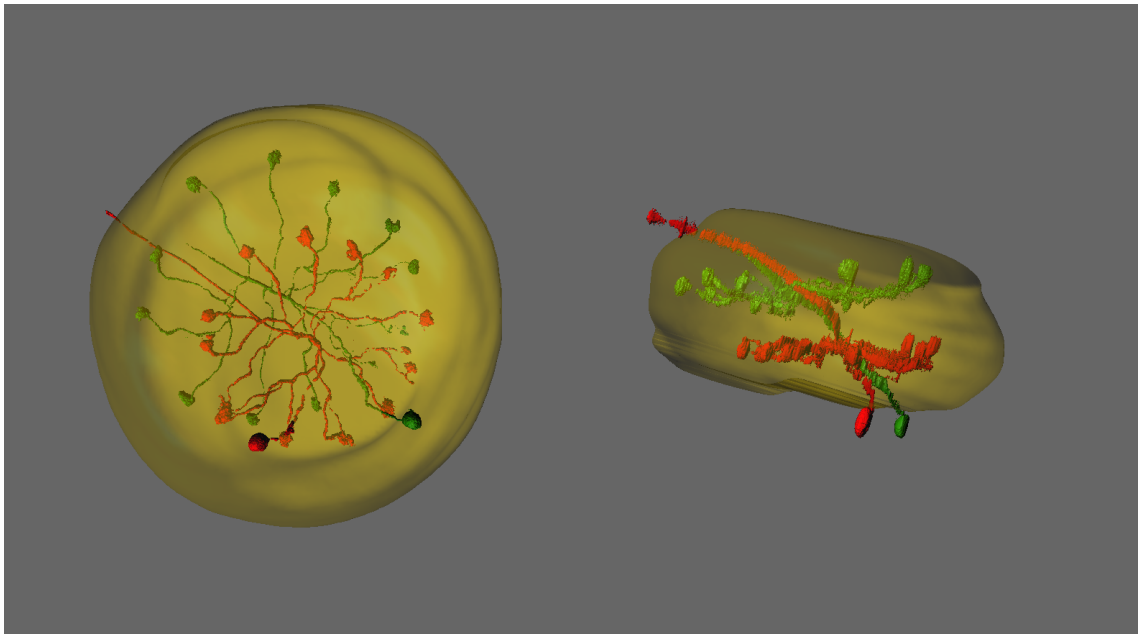


(**a**)

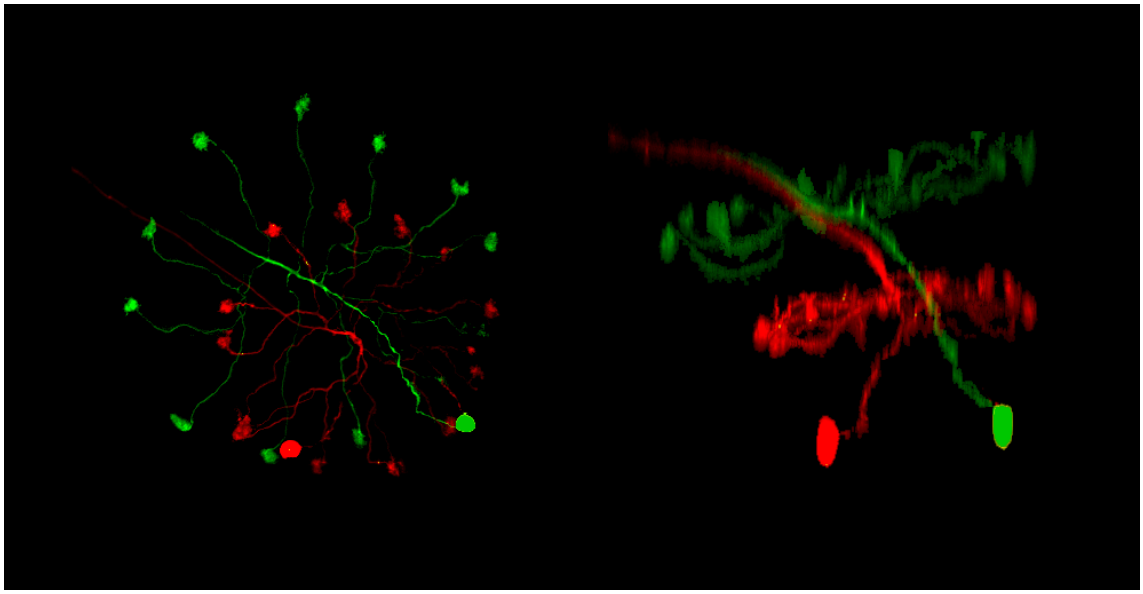


(**b**)

Figure 4.47: A pair of PNs filled concurrently, showing different radii in the same AL. These are the PNs shown in figures 4.38 and 4.42. (**a**) The same pair shown in an Imaris reconstruction from the frontal and posterior views. (**b**) Ray-traced projections of the cells shown in (a), in the same two views. Overlaps or areas where cells could not be separated are shown in yellow.

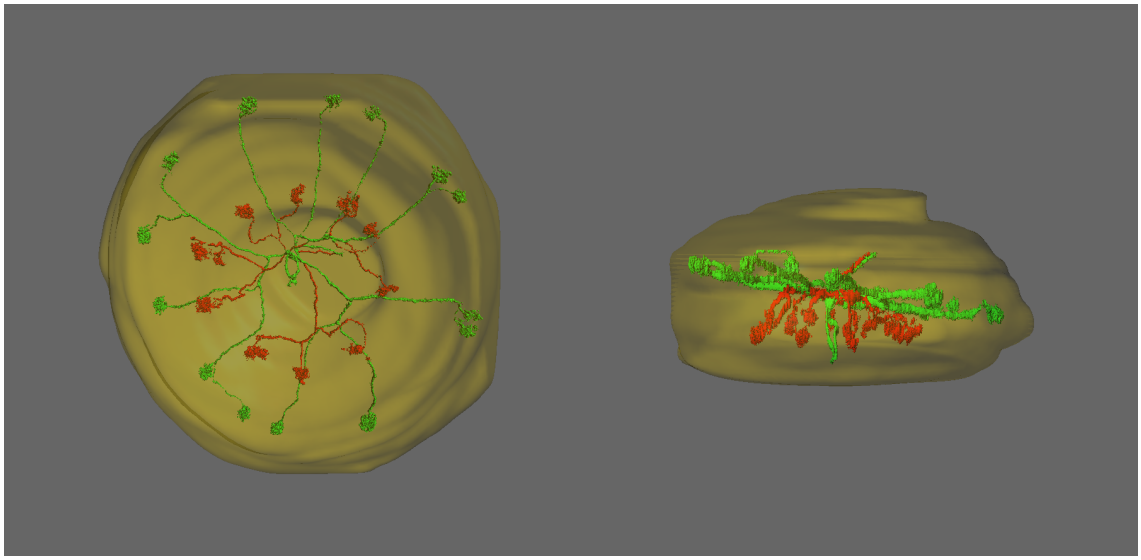


(**a**)

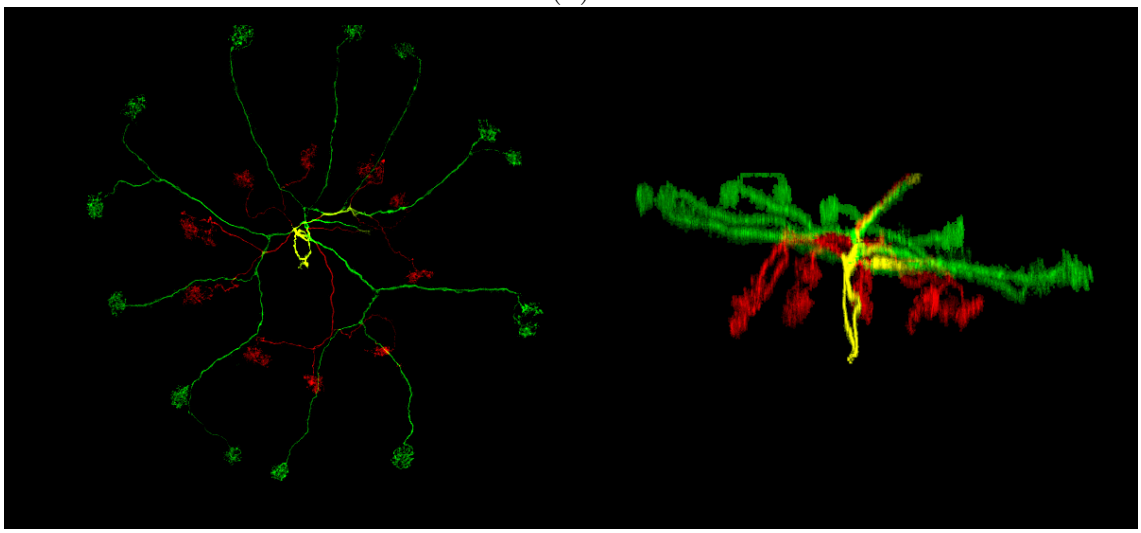


(**b**)

Figure 4.48: A pair of PNs filled concurrently, showing different radii in the same AL. Green PN is also shown in figure 4.37. (**a**) The pair of PNs shown in an Imaris reconstruction, from the frontal and posterior views. (**b**) Ray-traced projections of the cells shown in (a), in the same two views, but note that the posterior image is slightly turned about a vertical axis.

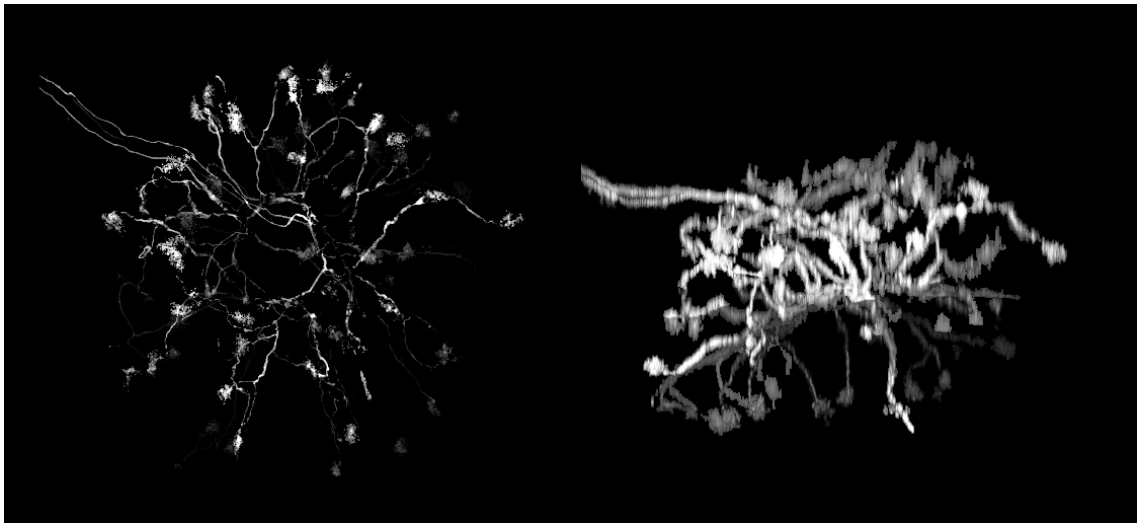


(**a**)

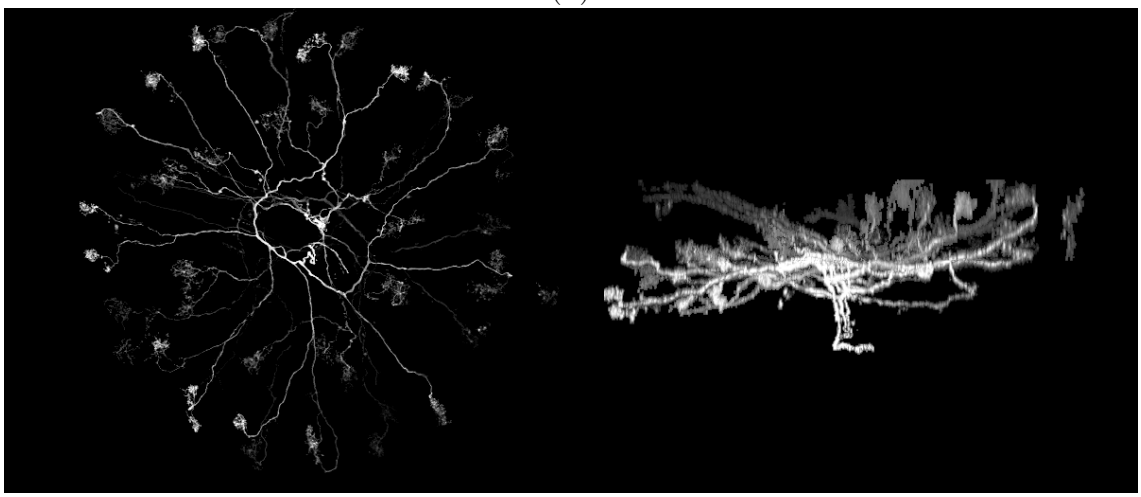


(**b**)

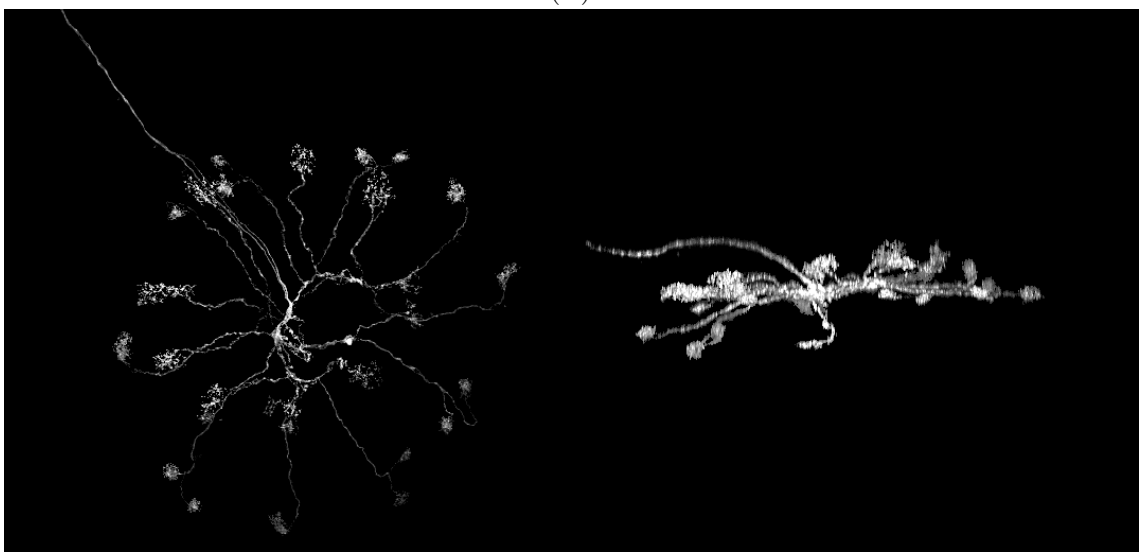
Figure 4.49: A pair of PNs filled concurrently, showing different radii in the same AL. These are the PNs shown in figures 4.39 and 4.44. (**a**) The same pair shown in an Imaris reconstruction from the frontal and posterior views. (**b**) Ray-traced projections of the cells shown in (a), in the same two views. Overlaps or areas where cells could not be separated are shown in yellow. Note that cell bodies are missing.



(**a**)

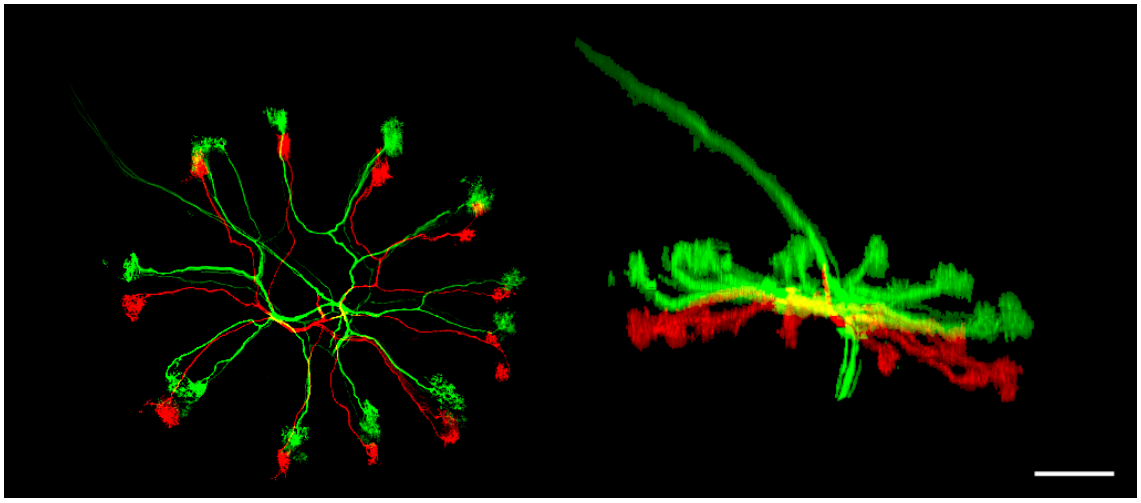


(**b**)

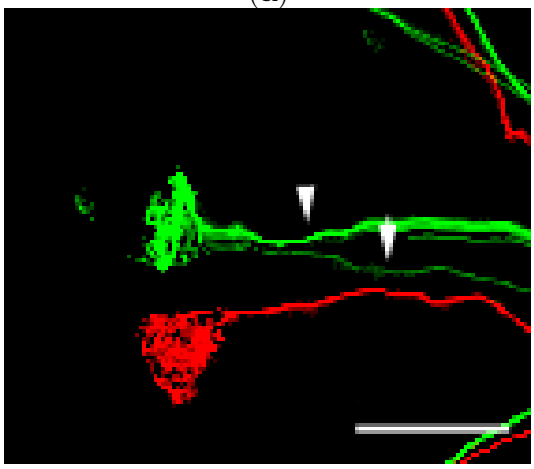


(**b**)

Figure 4.50: (**a**) Ray-traced projection of three PNs in one AL. (**b**) Ray-traced projection of three PNs in one AL. (**c**) Ray-traced projection of two PNs in one AL.



(**a**)



(**b**)

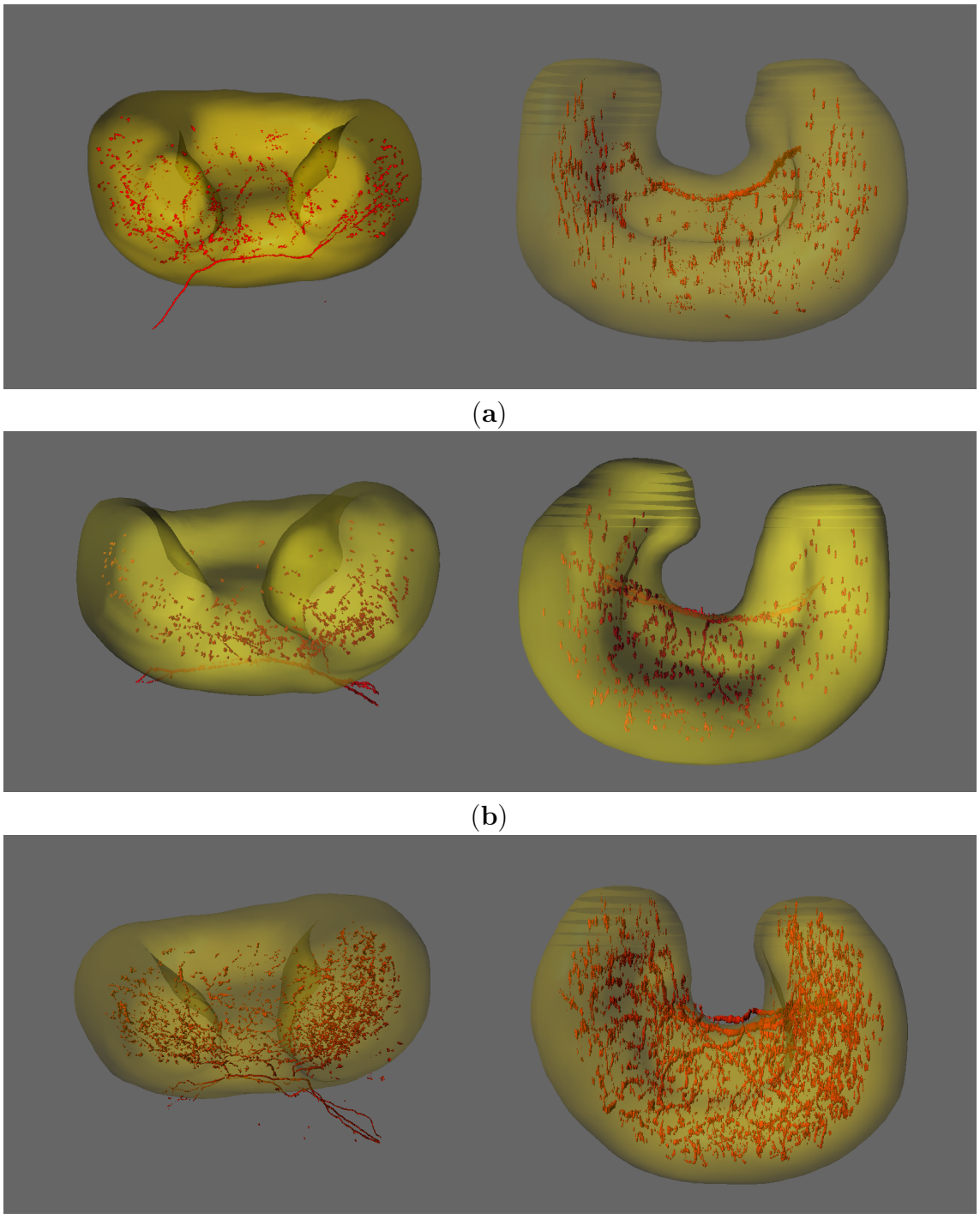
Figure 4.51: Three cells with the same radius size. (**a**) Ray-projection of three PNs stained concurrently within one AL. Two are shown in green and one is shown in red. Scale bar is equal to 70 μm. (**b**) Close up of the area around one set of glomeruli for cells in part (a). The two green cells are visible as different cells because one is less bright than the other (indicated by white arrows). The two cells innervate the same glomerulus. Scale bar is equal to 35 μm.

4.4.2 Projection Neuron Axonal Output

As discussed in chapter 3, PN axon collaterals innervate only the outer-calyx. PN axons course through the ACT and branch at the medioposterior edge of the calyx, at the depth of the pedunculus, and send several collaterals into the medial side of the outer-calyx. The axon continues to the lateral side of the calyx (across the pedunculus-calyx boundary) and branches again, sending several collaterals to the lateral outer-calyx. The main branch of the axon continues to the lateral horn where it branches and finally terminates in the lateral horn lobe.

Figure 4.52 shows a single, a double, and a triple fill of PNs within the calyx. The axon of any one PN branches extensively and exclusively within the OC and covers this entire calyx division in all three dimensions. Figure 4.53 shows the relationship between a single KC and the axon terminals of a PN. In this particular example, four possible sites of contact are visible. Overall, in 15 such concurrent fills of a PN and KC within the calyx, between one and four possible sites of contact (where the branches of these cells come within 1 μm of each other) were found.

Figure 4.54 shows the position of the lateral horn lobe with relation to the calyx and PN innervation. This lobe is also visible in Bodian stains (figure 4.55). In these stains, the bundle of PN axons can be followed out and seen to end in the LH lobe. This is the site of extrinsic cell processes as well, as described in the previous section.



(**a**)

(**b**)

(**c**)

Figure 4.52: Calyx innervation of PN axons. Imaris reconstructions of PNs within the OC, viewed from the ventral and posterior aspects. (**a**) Single PN in the OC. (**b**) Two PNs in the OC. (**c**) Three PNs in the OC.

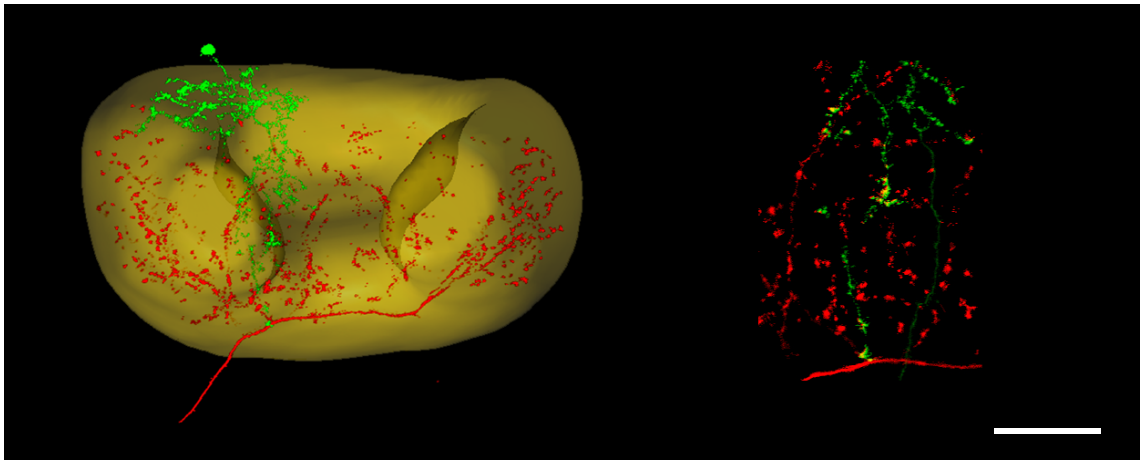
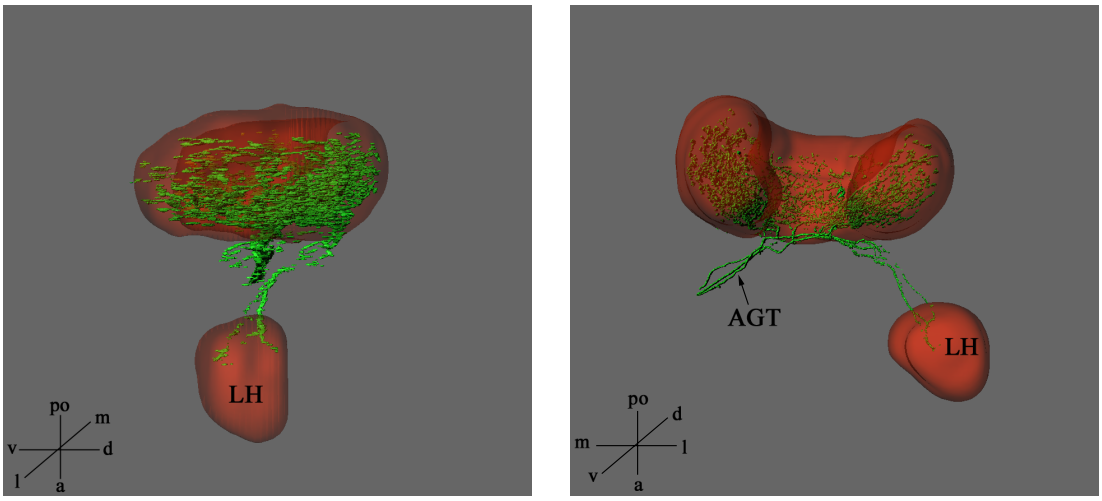


Figure 4.53: Relationship of PN axons and KC dendrites within the calyx. Left hand figure shows an Imaris reconstruction of both cells within the calyx. Right hand figure shows a high magnification ray-projected image of the same set of cells; KC is shown in green, PN is shown in red, and areas of over lap appear in yellow.



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

Figure 4.54: Relationship of the lateral horn lobe to the calyx. (**a**) Lateral view of Imaris reconstructed of a PN (as shown in figure 4.52[c]) within the calyx and LH lobe. (**b**) Dorsal view of the same reconstruction.

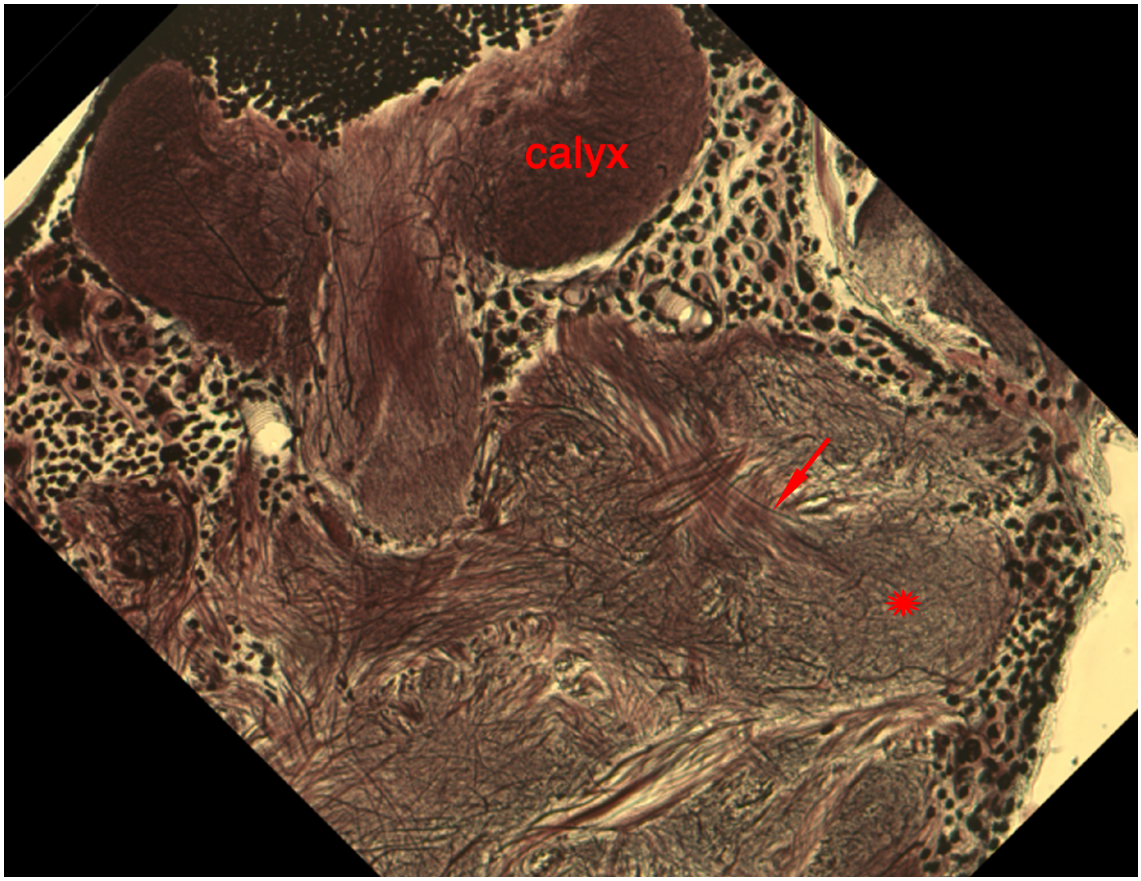


Figure 4.55: 10 μm thick Bodian-stained section showing the bundle of PN fibers (red arrow) as they innervate the LH lobe (red star).

4.5 Discussion

This chapter outlines the anatomy of a large part of the olfactory system of the locust, *Schistocerca americana*, from AL PNs to MB KCs to a subset of MB extrinsic cells. One broad principle emerges out of this consideration of the neurons of the locust olfactory system: at each level, morphologically similar neurons occur at slightly offset positions such that each particular structure (AL, MB calyx, MB β lobe) is built up from a mass of similar cells. The cell neurites gradually transform in shape and/or size so that in many cases, different 'types' of cells are actually just gradations from one extreme to another.

The PNs occur with different radius sizes, but their general organizing principles within the AL are the same. The number of glomeruli innervated by each cell is similar as is the pattern of glomeruli innervated. Glomeruli are in one plane and at a fixed distance from the AL core.

The concept of the repeated unit is apparent in the KC population as well. KCs are presented as falling into three broad morphological classes, defined by the shape of their dendritic tree: fan-shaped, bipartite and single-tree. All these 'types' are seen to occur as repeated and offset units. This finding coupled with the existence of KCs with dendritic trees that are hybrids of two types, makes it likely that KCs are in this way repeated and offset units that gradually change shape, conforming to the calyx structure. Thus, it is unlikely that dendritic tree shape reflects anything other than the physical constraints of the brain.

KCs do, however, form what are likely functional classes. Three parts of the primary calyx are defined here based on physical separation, KC innervation and efferent and afferent cell innervation. All KCs shown sample the outer-calyx — the site of PN output — and thus, all probably receive olfactory inputs. Some KCs, however, innervate calycal zones (inner- and mid-calyces) that do not receive PN input, but possibly other inputs from extrinsic cells (class II cells) that innervate the region.

Finally, the CII extrinsic cells themselves are seen to form repeated offset units.

A group of 9 to 12, possibly GABA-ergic, CII cells are postulated to exist per brain hemisphere, each sampling a slightly different position within the β lobe.

Olfactory brain regions also contain the neurites of cells that are unique. Class I extrinsic cells may be unique to each hemisphere as evidence for the existence of more than one per side was not found. It is possible that other extrinsic cells that were only encountered once also fit this category. Other extrinsic cells shown previous to this study are also unique, such as the giant GABA-ergic cell [44].

Thus, the olfactory system is comprised of groups of similar neurons doing the bulk of the processing, e.g., PNs in the AL and KCs in the MB, while other cells, unique and occuring as groups seem to primarily act as modulators that fine-tune this bulk processing.