



# Extended Connectivity Analysis of DCN (LC14a and b) neurons in *Drosophila* *melanogaster*

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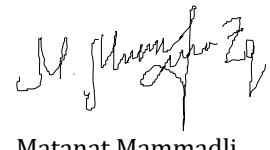
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December 12, 2023

## **Declaration on oath**

I declare that this thesis titled "Extended connectivity analysis of the DCN (LC14a and b) neurons in *Drosophila melanogaster*" has been composed solely by myself and not by anyone other than myself. It has not been submitted, in whole or in part, in any previous application for a degree. All resources used, publications, articles, books, websites or similar, has been noted as such in the bibliography.

Berlin, December 12, 2023



Matanat Mammadli

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# Abstract

To survive, navigate the environment, and acquire information about surrounding objects, animals heavily depend on visual feedback. Vision plays a crucial role for numerous animal species, and the functioning and development of visual systems are generally alike across different species. In the fruit fly *Drosophila melanogaster*, the optic lobe and central brain are responsible for processing visual information. The fly's brain uses visual information and stimuli to guide specific behaviors. To understand the underlying mechanisms governing these behavioral patterns in *Drosophila melanogaster*, it is useful to explore and analyze its whole brain connectome.

For this research, I used the online community and software called Flywire, containing the full brain connectome of the Drosophila female brain, with AI-segmented and human-proofread neurons, including their interconnections, synapses, neurotransmitters, and labels. Additionally, this software provides 3D images of the *Drosophila* brain and its sections through which neurons traverse. I was also working on the Flywire database called Codex, a search engine for *Drosophila* brain neurons. It contains proofread static snapshots of the FlyWire full-brain connectome-wiring diagram of the adult fly brain.

This thesis explores the Lobula columnar neurons LC14a and b (also called Dorsal cluster neurons) in a visual circuit of *Drosophila melanogaster*. They are commissural neurons, connecting the two opposite brain hemispheres, left and right. These neurons are responsible for the object orientation in flies. LC14a and b neurons' axonal terminals display variable wiring asymmetry, dictating the object responses of the fly. The dependency or correlation between these two events is linear, meaning that higher asymmetry results in a better orientation toward an object. We conducted an extended analysis of the most important input and output partner neurons of LC14a and b cells in this thesis, as well as attempted to visualize them and obtain their summary statistics. Essentially, our goal was to identify the input partner cells through which LC14a and b neurons receive stimuli or signals and to determine the output partner cells through which they transmit their responses. Partner cells that build the highest number of synapses to DCNs give us hints about additional functions these neurons may have, of which we may not be aware, and the essential roles they play in the visual system of the fly's brain.

# Introduction

*Drosophila melanogaster* is very popular as a model organism and often used in neuroscience because it has a highly tractable nervous system and also offers endless genetic resources for research purposes. The brain of the fruit fly *Drosophila melanogaster* is a complex organ containing thousands of neurons connected, as well as multiple neural circuits [Lin et al., 2023].

One of the most efficient ways for studying the brain of *Drosophila* and mapping connections between fly brain neurons are through Electron microscopic (EM) brain images [Dorkenwald et al., 2023]. Understanding the network characteristics of these neurons brings us closer to comprehending the mechanisms of connectomes and information flow in the fly brain. Thanks to the Flywire project, which also contains EM images of the fly brain, as well as the 3D visualizations of the neuropil fly brain regions (see Figure 2.2), it's now possible to view and have access to the full-brain connectome of the *Drosophila melanogaster*. Electron microscopy-based connectomes can also reveal how the visual circuitry of *Drosophila* works and affects various visual features, as well as the behavioral and physiological programs of this fly.

As stated before, *Drosophila melanogaster* has been extensively used as a model organism in neuroscience and biomedical science for investigating the cellular and molecular mechanisms in the brain. *Drosophila* brain contains just under 200,000 neurons and millions of neuronal connections [Raji and Potter, 2021]. Each of these neurons have different functions and play different yet very significant roles in the connectome analysis of the fly brain and the behavioral patterns of the fly itself.

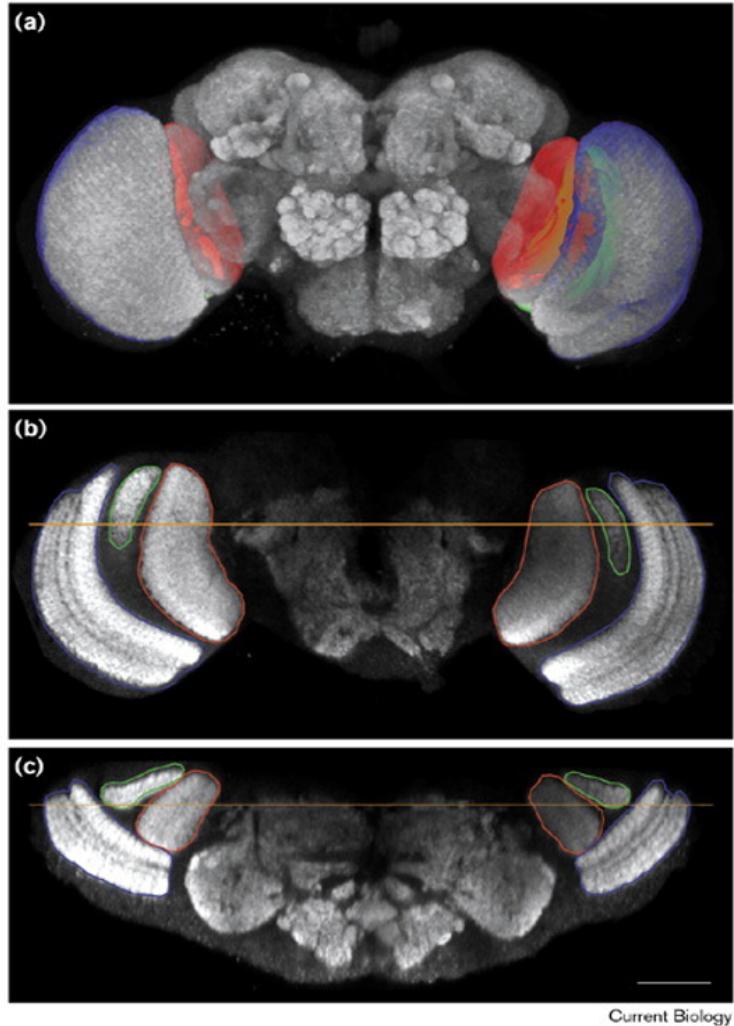
The optic lobes of *Drosophila* consisting of visual neuropils (Lamina, Lobula, Lobula Plate, and Medulla) are the major centers of the visual system of the fly where neuronal computations connect the outer visual world with the central brain [Nérée and Desplan, 2016], and the place where visual processing occurs 2.1. The visual neuropils are formed by more than 100 different cell types, distributed and interconnected in an invariant highly regular pattern.

Out of all of these visual neuropils, The Lobula Plate and the Lobula (the highest order visual neuropils), creating the Lobula-complex, are the main motion computation centres of the optic lobe controlling the optomotor responses of the fly [Hausen, 1984].

The Lobula Plate contains selective motion-sensitive interneurons. There is indirect evidence that the Lobula is responsible for visual signals initiating escape behavior [Hausen, 1984].

There is a large variety of neuronal cell types in the optic lobes of *Drosophila melanogaster*. These neurons can be classified as either columnar or tangential. Columnar neurons cover the retinotopic parts of the Lobula-complex, Lamina, and Medulla neuropiles, while tangential neurons fill different parts of the optic neuropiles (with their dendrites potentially occupying the retinotopic field either completely or partially). Tangential neurons are oriented perpendicular to the columnar cells. Columnar neurons construct multiple parallel information processing networks that are retinotopically organized. This structural arrangement can explain the behavioral phenotypes of the optic lobe [Fischbach and Dittrich, 1989].

In this thesis, our focus will be on the Dorsal Cluster Neurons (DCNs) of the *Drosophila* brain, specifically on the Lobula Columnar neurons LC14a and b.

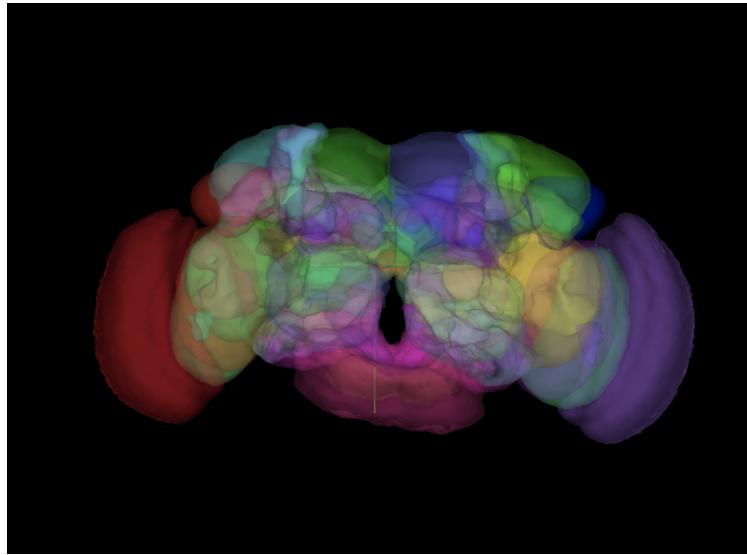


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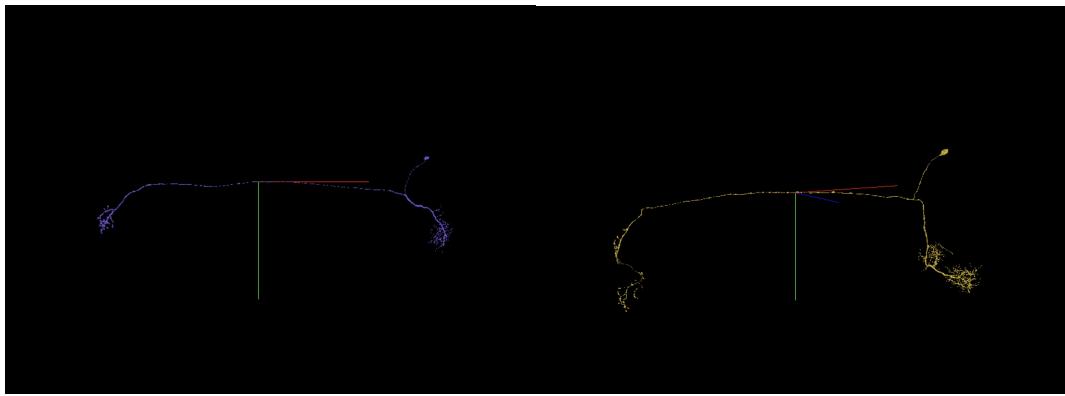
**Figure 2.1:** Optic lobe of *Drosophila melanogaster* containing Lobula (red), Lobula Plate (green) and Medulla (blue). Image was taken from [Rein et al., 1999].

We can observe behavioral patterns and differences between every species in the world (variation) and also between individuals in a genetically similar population (variability) [Mueller et al., 2021]. Behavioral individuality in the visual system of *Drosophila melanogaster* also has neurodevelopmental origins [Linneweber et al., 2020]. The sensory organs of this fly, with the help of multiple sensory inputs that they receive continuously, act as a gate between the inner organs and the outer environment. Sensory inputs through receptors are forwarded to the brain and processed there, resulting in a specific behavioral response. One of the primary driving forces behind these distinct behavioral programs in fly brains is their visual system, the optic lobe being the major backbone of it.

DCNs, playing a valuable role in the optic lobe, also contribute significantly to determining individual behavioral variability through non-deterministic mechanisms. As commissural neurons, DCNs connect the left and right hemispheres of the fly brain. The labels of these Lobula Columnar neurons reveal the fly brain region they traverse. The Lobula, a structure in the *Drosophila* brain, has an important role in detecting behaviorally relevant visual objects, such as other flies or predators [Tanaka () and Clark, 2022]. Understanding the role of Lobula Columnar cells in fly behaviors can answer many questions regarding how sensory circuits affect behaviors. However, it's mostly unknown how the input and output neurons of the Lobula are connected. Therefore, in this thesis, we analyze the most significant and remarkable input and output neurons of Lobula Columnar 14 a and b cells, using the Flywire



**Figure 2.2:** Neuropil 3D brain regions/segments of *Drosophila melanogaster*. The label and image was taken from flywire.ai



(a) LC14a

(b) LC14b

**Figure 2.3:** LC14a and LC14b cells of *Drosophila melanogaster*. The label and image was taken from flywire.ai

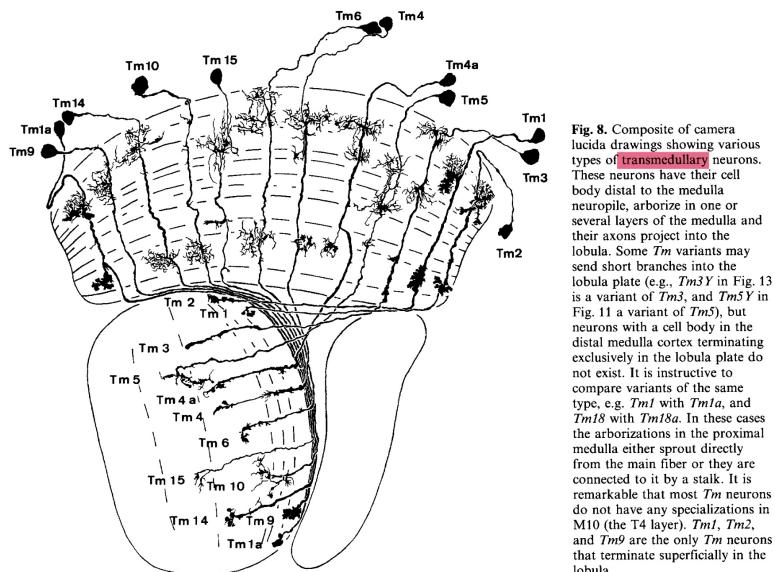
and Codex connectome databases. We categorized these neurons, based on their connectivity and morphology, into putative cell types. Through this analysis, we can shed light on previously less-known or researched connectivity between the input and output neurons of the Lobula, which can provide important information on how these neurons influence the selectivity of the Lobula for specific types of visual objects.

In Figure 2.3a and Figure 2.3b, we showcase the 3D representation of LC14a and b cells within the Flywire software. Similar to most fly neurons, LC14 cells are unipolar, which means that only one neurite extends from the cell body (soma). That "proximal neurite" (proximal means closer to the cell body) branches after a certain distance. The location where it joins the thicker neurite is often in between two arbors: a closer one that is more dendritic (more likely to receive synaptic input) and a farther arbor that is more axonal (with thick blobs of synaptic output terminals or boutons). LC14a and b cells are also no exceptions, as we can observe from images, they both have closer to the soma dendritic arbor and a farther away axonal arbor. Dendrites of LC14a are located in the Lobula part of the optic lobe and their axons are also situated in the Lobula of the opposite hemisphere. LC14b dendrites are also situated in the Lobula, but their axons can branch out further, be longer, and extend into both the Lobula and the Medulla parts of the optic lobe. LC14b

arbors are also located deeper and extend more downward than LC14a.

Our goal, during this thesis, was to find out and characterize the three most important input and output neurons of LC14 a and b neurons, along with the three most important input and output neurons of these first-layer neurons. To acquire this information we used the widely known R programming language, RStudio as our integrated development environment (IDE) and FAFB dataset (short for “full adult fly brain”). The FAFB dataset contains segmentations of an entire fly brain and is extensively used in neuroscience and image-processing research. To determine the major input and output neurons with the highest percentage of synapses to LC14a and b neurons, we analyzed six sets of LC14a and b neurons (Middle, Dorsal, and Ventral sets, two sets of each). Using the Flywire software, we obtained their neuron IDs and then added these IDs to the fafbseg library’s partner summary function in the RStudio terminal. This process provided us with information on their input and output partners, pre- and post-IDs, which we could then download and export as an Excel sheet. Subsequently, we analyzed and updated these Excel sheets using R functions, obtaining their summary statistics that highlighted specific neurons with the highest input or output synapses to LC14 neurons.

Throughout this research, one of my primary responsibilities, aside from proofreading neurons, was identifying neurons that were still unlabeled in the Flywire software. The Codex database, containing thousands of cell infos, stats, synapses, etc., proved to be highly useful for this task, along with the 1989 published Fischbach paper, which provided fly brain neuron cell classifications and images [Fischbach and Dittrich, 1989].



**Figure 2.4:** Various types of Transmedullary neurons in *Drosophila melanogaster*. The description and image was taken from Fischbach paper [Fischbach and Dittrich, 1989].

# Materials and Methods

## 3.1 Introduction to Flywire and proofreading fly brain neurons

Flywire is an all-in-one database, software, and online community designed through human-AI collaboration for constructing the whole-brain connectome of *Drosophila melanogaster* (<https://flywire.ai/about>). As an open community, Flywire continues to attract an increasing number of users who actively participate in proofreading and identifying neuron cells that have been proofread previously. It also requires members to share the acquired information, ensuring that the entire community contributes to its content and regularly updates the database. Flywire is built upon the FAFB dataset containing the full brain connectome of *Drosophila*.

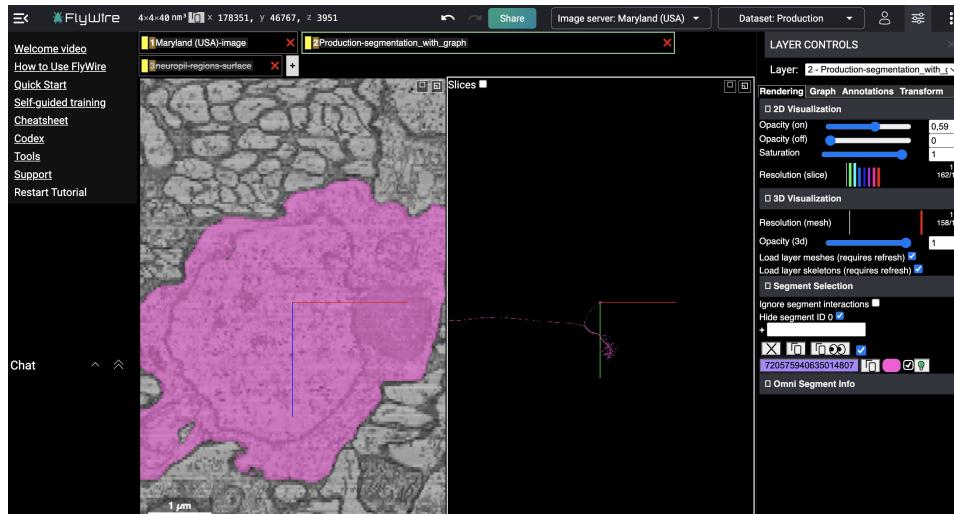
Before starting with my project, I was provided the "Flywire self-guided training" tutorial document to familiarize myself with the essentials and guidelines for using this software. The tutorial provided insights into the structure of the Flywire software, its purpose, goals, underlying motivation, and how it fosters a sense of community by bringing together neuroscientists and researchers in the biological field. This training included videos on proofreading fly brain neurons, navigating through neurons in EM image slides, identifying accidental mergers, and correcting mistakes made by Artificial Intelligence during the segmentation process. Additionally, the tutorial covered various aspects of using the software, such as adjusting the opacity of images, adding or removing neuropil (3D fly brain segments) to cells, merging cell parts that belong together, and performing cut or multi-cut options on cells that were incorrectly merged. The software also provides the capability to add annotations to the cells.

In the Flywire, you can either click on neurons in the EM view to obtain their IDs and view them in 3D, or you can add neuron IDs from Excel sheets obtained using Flywire partner summary functions from the fafbseg library (which will be explained in the next section) to access their EM and 3D view (see Figure 3.1).

By adding 3D brain segments to the neuron, one can observe the specific brain regions through which this neuron passes. This information is of utmost importance for categorizing them, such as identifying them as lobular neurons or transmedullar neurons. Additionally, it helps with labeling these neurons and helps to determine their orientation, whether they are in the left or right hemisphere of the fly brain.

By reviewing EM slides, it becomes easier to trace neurons and determine their starting or ending points, locate their soma, dendrites, and axons, identify areas where they are surrounded by glial cells, or find locations where they might merge with false neurons, etc. The software displays all of these locations along with their coordinates (x, y, z), allowing for easy saving or copying/pasting. In the EM view, we can see AI-segmented neurons and these segmentations consist of supervoxels.

Since Flywire has been an active community for some time with dedicated members, the proofreading part was mostly completed by the time I started acquainting myself with this software. My specific task was to identify neurons that lacked labels or names. Nevertheless, I engaged in extensive proofreading practice sessions with my supervisor for months, using



**Figure 3.1:** The view of the Flywire software. The image was taken from flywire.ai

the test/practice dataset called 'Sandbox,' before gaining access to the actual production dataset.

To access the production dataset and actively contribute to the software, I underwent thorough training and participated in an online meeting with representatives from Princeton University. During the meeting, my abilities in proofreading and cell identification were tested. After successfully passing the test meeting, I received an email granting me all access rights and providing login information for both Flywire and Codex databases.

### 3.2 R packages (Natverse, reticulate) and R interface to python

For my thesis, we selected RStudio as our primary Integrated Development Environment (IDE) and R as our programming language due to its widespread usage among data scientists, biologists, and neuroscientists. Additionally, R was chosen for its extensive collection of integrated packages specifically designed for neuroscience and fly brain connectome analysis.

I received a comprehensive R script for tracing the neurons that were the focus of my analysis, namely LC14a and b cells, along with their input and output neurons. Throughout my thesis, I worked on this R script, regularly modifying and updating it to ensure the most accurate and recent partner summary and statistics for my DCN cells.

We also opted for collaboration using two of the most popular programming languages in data science and biomedical science, R and Python. R is renowned for its powerful data visualization libraries, while Python is known for its machine-learning capabilities. To incorporate Python into R, I installed the 'reticulate' package, which provides a comprehensive set of tools for interoperability between Python and R, enabling an R interface with Python. Using the 'simple\_python()' function in R, we were able to install Python via an isolated miniconda environment, along with the recommended packages for 'fafbseg,' and tested the integration of Python in R.

```
install.packages('reticulate')
library(reticulate)
```

The most crucial step for my research, however, was discovering the Natverse package in R. This package is indispensable for neuroanatomical data analysis. Natverse is a comprehensive collection of interoperable R packages designed for importing, visualizing, analyzing, manipulating, and exporting 3D neuroanatomical data, encompassing neurons, brains, and

brain regions. It is widely used to investigate brain and circuit organization across various species such as flies, mice, and more (<https://natverse.org>).

To install the Natverse package, I initially installed the 'natmanager' package. Using this library, I then installed 'core' for the basic installation and use of Natverse, followed by the 'natverse' package for the complete Natverse installation. Subsequently, I could fully utilize the Natverse package with the command 'library(natverse)'.

```
install.packages('natmanager')
natmanager::install('core') # basic install
natmanager::install('natverse') # or full install

library(natverse)
```

As the final and most important step, we executed the 'fafbseg' package from the Natverse library. As the name implies, 'fafbseg' comprises a set of Python tools designed to handle various types of segmentation data in the 'FAFB' ('full adult fly brain') dataset. The primary aim of 'fafbseg' is to facilitate the analysis of segmented Electron Microscopy (EM) data. This encompasses support for working with neuroglancer mesh data for the complete adult female brain (FAFB) dataset. Notably, it provides support for the FlyWire and Google Brain automatic segmentations of FAFB data.

```
library(fafbseg)
dr_fafbseg()
```

After the successful execution of the diagnostic run using the 'fafbseg' package (dr\_fafbseg()), our environment was set up and ready for running my R tracing script focused on LC14a and b neurons, along with their crucial input and output neurons.

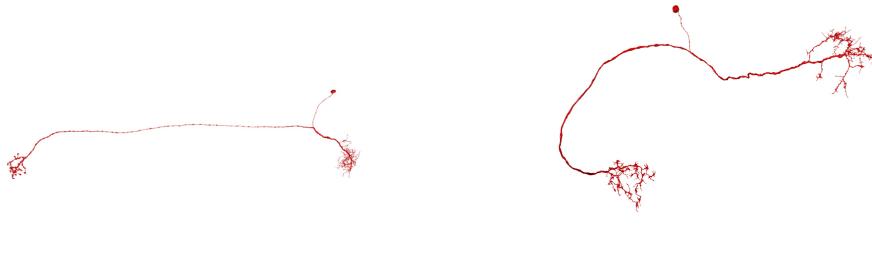
### 3.3 Getting partner summary and 3D plotting neurons (XQuartz)

To enable 3D plotting of neurons on my macOS-operated computer, I installed XQuartz software. The XQuartz is an open-source software as an effort to develop a version of the X.Org X Window System that runs on macOS.

After completing these installations and successfully running the 'fafbseg' diagnostic, I could add neuron IDs to 'flywire.partner\_summary()' and fetch supervoxel IDs along with synapse data. Using the 'read.cloudvolume.meshes()' function and providing the neuron ID as an input parameter, I used the 'plot3d()' function to visualize specific neurons in a 3D format.

For example, in Figure 3.2a and Figure 3.2b you can observe 3D plots of LC14a and LC9 neurons.

I was able to visualize neurons, along with their pre- and post-synapses, in a 3D brain sphere view using functions from the 'rgl' library, including 'plot3d()'. The 'RGL' package serves as a 3D visualization device system for R, employing OpenGL or WebGL as the rendering backend. It is specifically designed for generating interactive 3D plots, featuring high-level graphics commands that loosely follow classic R graphics but operate in three dimensions. The OpenGL RGL device is written in C++, providing an interactive viewpoint navigation facility with mouse and wheel support, along with an R programming interface. While WebGL is rendered in a web browser, 'RGL' produces the input file, and the browser displays the images (source: rdocumentation.org). The command 'rgl.snapshot()' is used to save the screenshot as a PNG file. The function 'rgl.viewpoint()' sets the viewpoint orientation, although it's worth noting that this function has been deprecated, and we now use 'view3d()' instead. This approach allowed me, for instance, to obtain images of pre- and post-synaptic connections, as well as neighboring neurons of the LC14-a neuron. The results of these plots can be found in the 'Results' section of this thesis.



(a) 3D plot of LC14-a neuron

(b) 3D plot of LC9 neuron

**Figure 3.2:** XQuartz 3D plots

```
plot3d("720575940635014807")    ## LC14-a cell ID
spheres3d()
view3d()
rgl.snapshot()
```

### 3.4 Codex software/database

One of my most frequently used tools during this research is Codex software ([codex.flywire.ai](http://codex.flywire.ai)), which stands for 'Connectome Data Explorer.' Codex provides access to proofread static snapshots of the FlyWire full-brain connectome-wiring diagram of the adult Drosophila brain. It serves as a snapshot and cell info database/dataset of Flywire, with the latest connectome snapshot being v630. This snapshot includes around 130,000 proofread cells, 100,000 labeled cells, more than 2,000,000 connections, and over 30,000,000 synapses ([codex.flywire.ai](http://codex.flywire.ai)).

I extensively utilized Codex to identify unlabeled cells in Flywire, gaining insights into the appearance of various cell types in the right, left, or both hemispheres of the fly brain. Codex provided valuable information on cell synapses, neurotransmitters, neighboring synaptic cells, as well as plots, diagrams, and other cell statistics. With the help of Codex and the information from the Fischbach paper [Fischbach and Dittrich, 1989], I successfully identified approximately 250 neurons, adding their names to our Excel database (which will be mentioned later) and the Flywire software.

In Figure 3.3, you can observe the interface of the Codex site's search engine.

### 3.5 Our Lab's Flywire Excel database

In addition to the widely-used Flywire and Codex databases, our lab has also developed a small database in the form of a Microsoft Excel sheet. This enables us to monitor all new changes and additions to the Flywire. The database includes information such as neuron names (either labeled from the Flywire database or identified by our team with custom labels), the latest neuron Flywire IDs, coordinates (x, y, z), lab names, and the names of the authors who entered this information.

I take pride in my results and humbly wish to share that I've inputted over 1000 neurons into our database, some of which were already labeled in the Flywire software. Additionally, I identified more than 250 previously unlabeled neurons and incorporated them into our database. The collaborative efforts of my lab colleagues, including students and my supervisor, Dr. Gerit Linneweber, have played a significant role in contributing to this database. It's important to note that the Flywire project is a collective effort involving all

CodeX Search Stats Cell Info Explore Connectivity Tools Info send feedback / question

LC14b

37 matches, further analysis:

Stats Network 3D view Copy IDs Pathways Synapse table CSV Cell IDs

Name/ID	NT	Size	Related Cells	Type [?]	Classification [?]	Community Labels [?]
<a href="#">LO.1093</a> 720575940630568960	ACH 0.8	1,766 µm 4,157 µm <sup>2</sup> 342 µm <sup>3</sup>	↑ 29 upstream ↓ 45 downstream ↔ mirror twin ⌚ 2 with similar shape ⌚ cells with similar connectivity more ▾	Side right Flow intrinsic Super Class optic Class bilateral Hemilineage VPNpt1_lateral	Lobula Columnar neuron 14b; <a href="#">LC14b</a> ; Lcn14b; FBbt_00111749 <a href="#">literature links</a>	
<a href="#">LO.627</a> 720575940630684672	ACH 0.76	2,014 µm 4,511 µm <sup>2</sup> 372 µm <sup>3</sup>	↑ 41 upstream ↓ 65 downstream ↔ mirror twin ⌚ 2 with similar shape ⌚ cells with similar connectivity more ▾	Side right Flow intrinsic Super Class optic Class bilateral	Lobula Columnar neuron 14b; <a href="#">LC14b</a> ; Lcn14b; FBbt_00111749 <a href="#">literature links</a>	

**Figure 3.3:** Search engine of Codex software

labs within the Institute of Biology at the Free University Berlin. Consequently, one can also find contributions from other students and participants in this database.

# Results

## 4.1 Cell identifying

As mentioned previously, when I started this project, proofreading was largely completed. Consequently, my primary responsibility involved identifying neurons that were unlabeled in the Flywire database.

To achieve this, I used Flywire software (flywire.ai) with its added "neuropil-regions-surface" feature to determine the specific fly brain regions through which certain neurons were passing. This approach enabled me to familiarize myself with fly brain regions and identify the segments where the dendritic or axonal parts of specific neurons were situated.

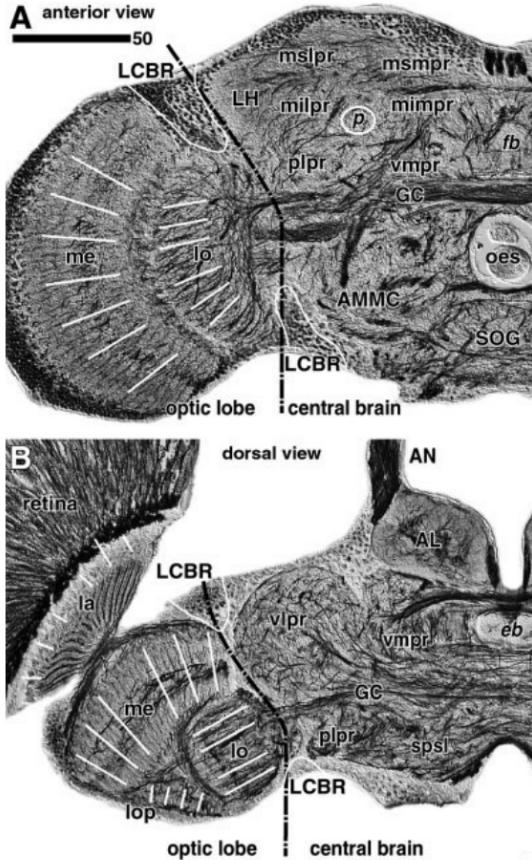
1 AME_R	16 MB_PED_R	31 PLP_R	46 LAL_L	61 SLP_L
2 LO_R	17 MB_VL_R	32 AOTU_R	47 CAN_L	62 SIP_L
3 NO	18 MB_ML_R	33 GOR_R	48 AMMC_L	63 SMP_L
4 BU_R	19 FLA_R	34 MB_CA_R	49 ICL_L	64 AVLP_L
5 PB	20 LOP_R	35 SPS_R	50 VES_L	65 PVLP_L
6 LH_R	21 EB	36 IPS_R	51 IB_L	66 WED_L
7 LAL_R	22 AL_R	37 SCL_R	52 ATL_L	67 PLP_L
8 SAD	23 ME_R	38 EPA_R	53 CRE_L	68 AOTU_L
9 CAN_R	24 FB	39 GNG	54 MB_PED_L	69 GOR_L
10 AMMC_R	25 SLP_R	40 PRW	55 MB_VL_L	70 MB_CA_L
11 ICL_R	26 SIP_R	41 GA_R	56 MB_ML_L	71 SPS_L
12 VES_R	27 SMP_R	42 AME_L	57 FLA_L	72 IPS_L
13 IB_R	28 AVLP_R	43 LO_L	58 LOP_L	73 SCL_L
14 ATL_R	29 PVLP_R	44 BU_L	59 AL_L	74 EPA_L
15 CRE_R	30 WED_R	45 LH_L	60 ME_L	75 GA_L

**Figure 4.1:** Neuropil brain segments of *Drosophila melanogaster* with numbers in Flywire

The brain of *Drosophila melanogaster* consists of 75 brain segments, the majority of which are present in both the left and right hemispheres, as illustrated in Figure 4.1. We will later use these regions for the identification and labeling of neurons.

The optic lobe is the primary visual center of the insect brain. Different than vertebrates, where their eye contains an intense neural network within its retina, in insects photoreceptor cells project directly to the optic lobe. The optic lobe consists of three or four neuropils. The Lamina, Medulla, and Lobula are detectable in all insect species. In flies the Lobula is further separated into two neuropils, which are called the Lobula and Lobula Plate, see Figure 4.2 [Otsuna and Ito, 2006]. Local neurons, interneurons, and the terminals of the photoreceptors that lie in identical directions build a columnar organization called the visual cartridge, whose spatial organization reflects that of the ommatidia (white lines in Figure 4.2). Lamina interneurons (second-order neurons) innervate the medulla and terminate in the cartridges covering the corresponding visual field. Columnar interneurons of the Medulla (second-, third-, or fourth-order neurons) later on project to the Lobula and Lobula Plate, in which the retinotopic arrangements of the visual cartridges are also maintained [Otsuna and Ito, 2006].

Neural circuits in the optic lobe are formed by four classes of neurons: photoreceptor axons, interneurons, intrinsic neurons (or local neurons), and visual projection neurons

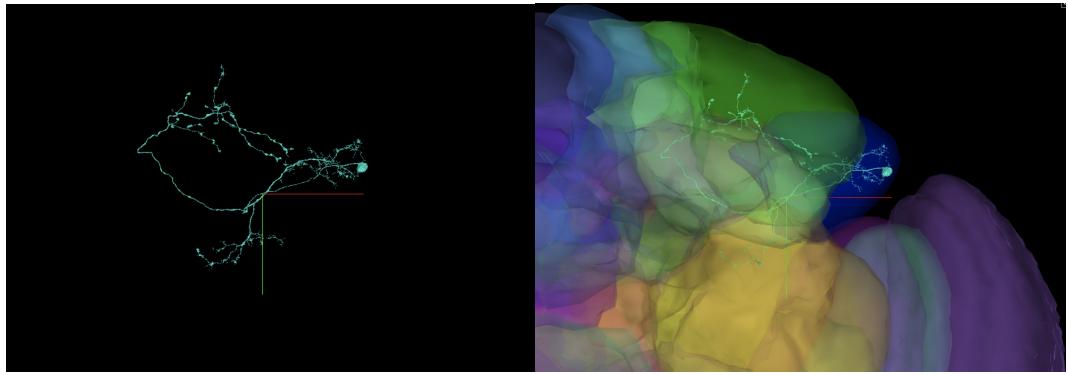


**Figure 4.2:** Visual system of *Drosophila melanogaster*

(VPNs). Intrinsic neurons have arborizations within a single optic neuropil, interneurons connect more than one neuropil within the optic lobe, and VPNs connect the optic lobe and the central brain, as mentioned before [Otsuna and Ito, 2006].

To name the unlabeled cells, I took inspiration from [Otsuna and Ito, 2006] and how they named VPNs in their paper. They suggested a two-letter naming system for the VPNs. The first character indicates the neuropil of the optic lobe it innervates: for ex, L (Lobula), P (lobula Plate), M (Medulla), and C (Complex of more than one neuropil). The second character shows the pattern of arborization in the optic lobe: C (Columnar) and T (Tangential or Tree-type). Neuron types that fall in the same category were distinguished by numbers. For the VPNs whose cell bodies lie in the lateral cell body region (LCBR, the area between the central brain and the optic lobe) to indicate the position of the cell bodies, ranges of numbers 1–30 were suggested, for those in the central brain, number range 31–60 was suggested and respectively for those whose cell bodies lie in the optic lobe, number range 61–90 was suggested. As an example, the neuron type “LT32” falls under the second category of the VPN that arborizes in the Lobula, has tangential or tree-type arborization, and has cell bodies in the central brain.

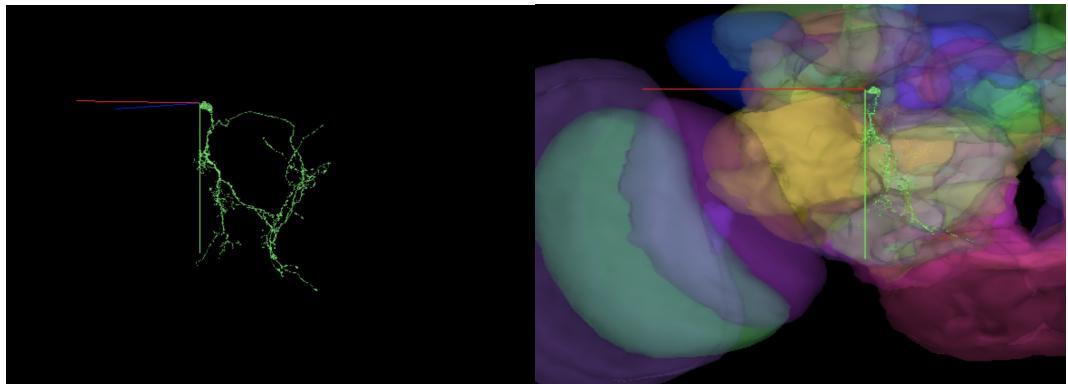
Also, I looked up to the Codex database to find similar neurons to the ones I was searching for, as well as used Fischbach paper to be able to identify neurons [Fischbach and Dittrich, 1989]. For neurons that I couldn’t find in either of them, that were completely new and foreign for me, I named them according to their dendritic arbor and axonal arbor locations. I could add or remove neuropil regions in the Flywire, to be sure which locations dendrites and axons from specific neurons were passing through, then naming them accordingly, for ex: PVLP\_to\_AVLP, which means their dendrites were in the PVLP segment and axons were in AVLP region. In Figure 4.3a and 4.3b you can see some examples of neurons I labeled



(a) PVLP\_LH\_to\_SLP

(b) Frontal neuropil view

**Figure 4.3:** Fly brain neuron identified as PVLP\_LH\_to\_SLP by myself



(a) PLP\_SPS\_IPS\_WED\_to\_SPS\_IPS\_EPA\_LAL\_CRE

(b) Dorsal neuropil view

**Figure 4.4:** Fly brain neuron identified as PLP\_SPS\_IPS\_WED\_to\_SPS\_IPS\_EPA\_LAL\_CRE by myself

according to this description. This means the dendritic arbor of this specific neuron was passing through the Posterior Ventral Protocerebrum (PVLP) and Lateral Horn (LH), and its axonal arbor was passing through the Superior Lateral Protocerebrum (SLP) [Schubert et al., 2018].

Another example is to be seen in Figure 4.4a and Figure 4.4b. I identified this neuron as PLP\_SPS\_IPS\_WED\_to\_SPS\_IPS\_EPA\_LAL\_CRE , because its dendrites were located in Posterior Lateral Protocerebrum (PLP), Superior Posterior Slope (SPS), Inferior Posterior Slope (IPS) and in Wedge (WED) and its axons were located in Superior Posterior Slope (SPS), Inferior Posterior Slope (IPS), Epaulette (EPA), Lateral Accessory Lobe (LAL) and in Crepine (CRE). Names of this brain region abbreviations were taken mostly from Schubert paper [Schubert et al., 2018] and some others from Codex (codex.flywire.ai). Even though this name was too long and somewhat unpractical, I decided the exact regions the neuron passes by are more important than the name, which can always be modified later on.

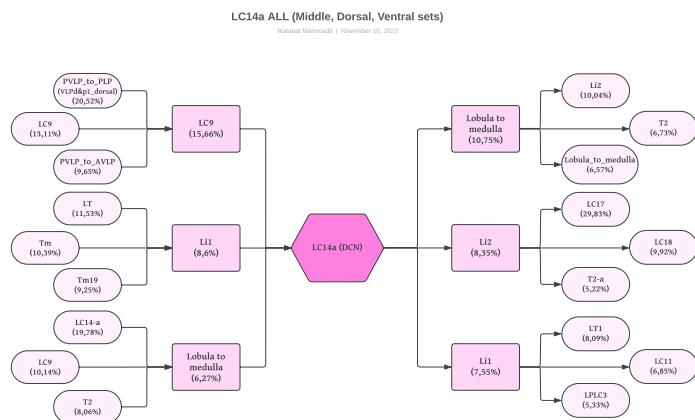
Using these methods, I identified over 250 cells, contributing to the Flywire dataset.

Defining the position of the cell bodies in each neuron is principal because there is often a correlation between the locations of the cell bodies and their developmental origin. If VPs in different species share the same innervation target, the same arborization pattern in the optic lobe, and the trajectory of projection in the central brain, they could be determined as identical. Nevertheless, in distant species, the cell body position might be shifted, sometimes crossing the border between the central brain, lateral cell body region, and the optic lobe. There are, however, exceptional cases, where the correlation between the number range and the cell body location is not maintained [Otsuna and Ito, 2006].

## 4.2 Three most important LC14a input neurons

To analyze LC14a cells, I received 6 example cells, 2 Middle sets, 2 Dorsal sets, and 2 Ventral set LC14a cells in R Code format with their IDs. Updating these neurons involved adding them to my R-formatted tracing script, obtaining their latest IDs, and using the links in the R Code to access their Excel sheets. These sheets contained pre- and post-IDs, partner types and names, and their weights (synapse counts) for each partner. During each update, I ran all the required libraries mentioned in the 'Materials and Methods' section and updated our lab database, 'Flywire' Excel sheet, before proceeding to update other cells.

In total, I updated all 6 sets (Middle, Dorsal, ventral) of LC14a cells, extracting information on their three most important input and output partner neurons. These neurons, forming the highest number of synapses with LC14a cells, are crucial for our research. The results most relevant to our thesis are these input and output cells, along with their synapse percentages (refer to Diagram 4.5).



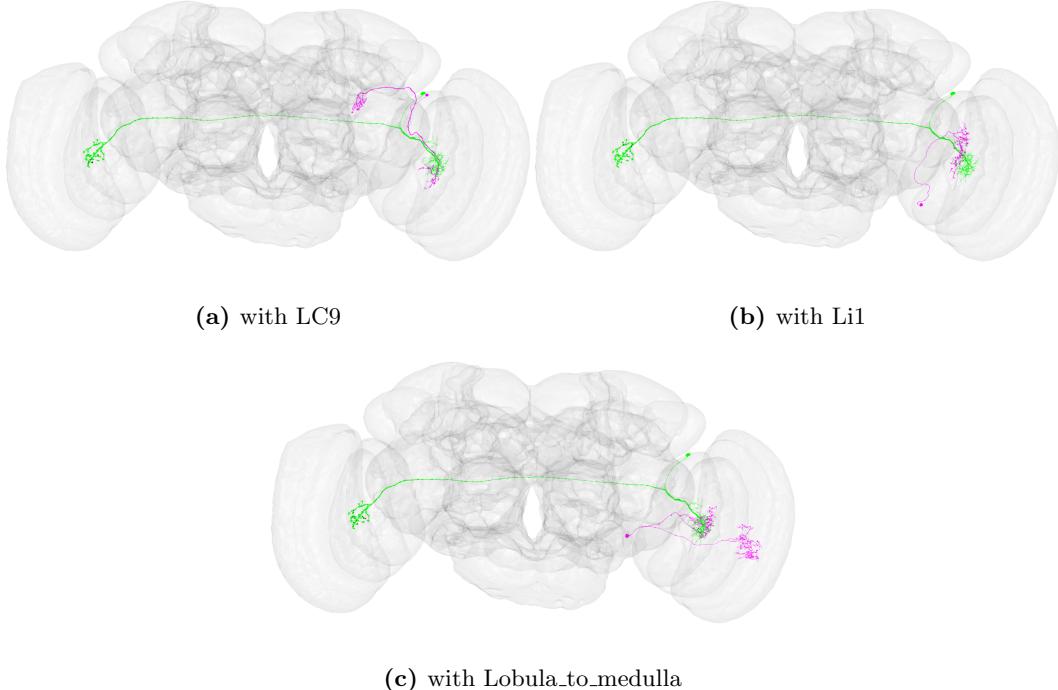
**Figure 4.5:** 3 most important input and output partner neurons (and their partners) of all 6 set (Middle, Dorsal, Ventral) of LC14a cells

First, we will discuss the three most important **input** (presynaptic) neurons of LC14a cells, which are LC9, Li1, and Lobula\_to\_medulla neurons (see Figure 4.6). To avoid confusion, we will color coordinate **input** and **output** neurons, input synapse partners being plotted with green and magenta colors and output synapse partners are going to be plotted with blue and red colors.

### 4.2.1 LC9 - First most important input neuron of LC14a and its most important input neuron

As we can see from the Diagram above (see Diagram 4.5), the first most important partner input neuron of LC14a cells is LC9, which creates 15.66% (1054 synapses) of all input synapses of LC14a neuron, which is the highest percentage compared to other input presynaptic neurons of LC14a.

As the name suggests, the Lobula Columnar 9 neuron is located in the Lobula brain region and is classified as a columnar neuron. Lobula columnar neurons connect Lobula with the central brain. LC9 neuron has this characteristic half-loop look to it.



**Figure 4.6:** 3D image of LC14a neuron (green) building presynaptic connections with its most important input partners (magenta)

Columnar neurons in general establish multiple and stacked retinotopic maps in the optic lobe. They connect the distinct cellular regions: Retina, Lamina, Distal Medulla, Proximal Medulla, Lobula, Lobula Plate, and Optic Foci in the central brain [Fischbach and Dittrich, 1989].

From all visual neuropiles, the Lobula is most intimately connected to the central brain. Various sets of isomorphic Lobula Columnar neurons project via various routes into different regions of the central brain.

I could plot LC14a presynaptic connection with LC9 in 3D format with the help of `plot3D()`, `view3d()`, and RGL functions mentioned in the "Material and Methods" part, see sub-figure 4.6a.

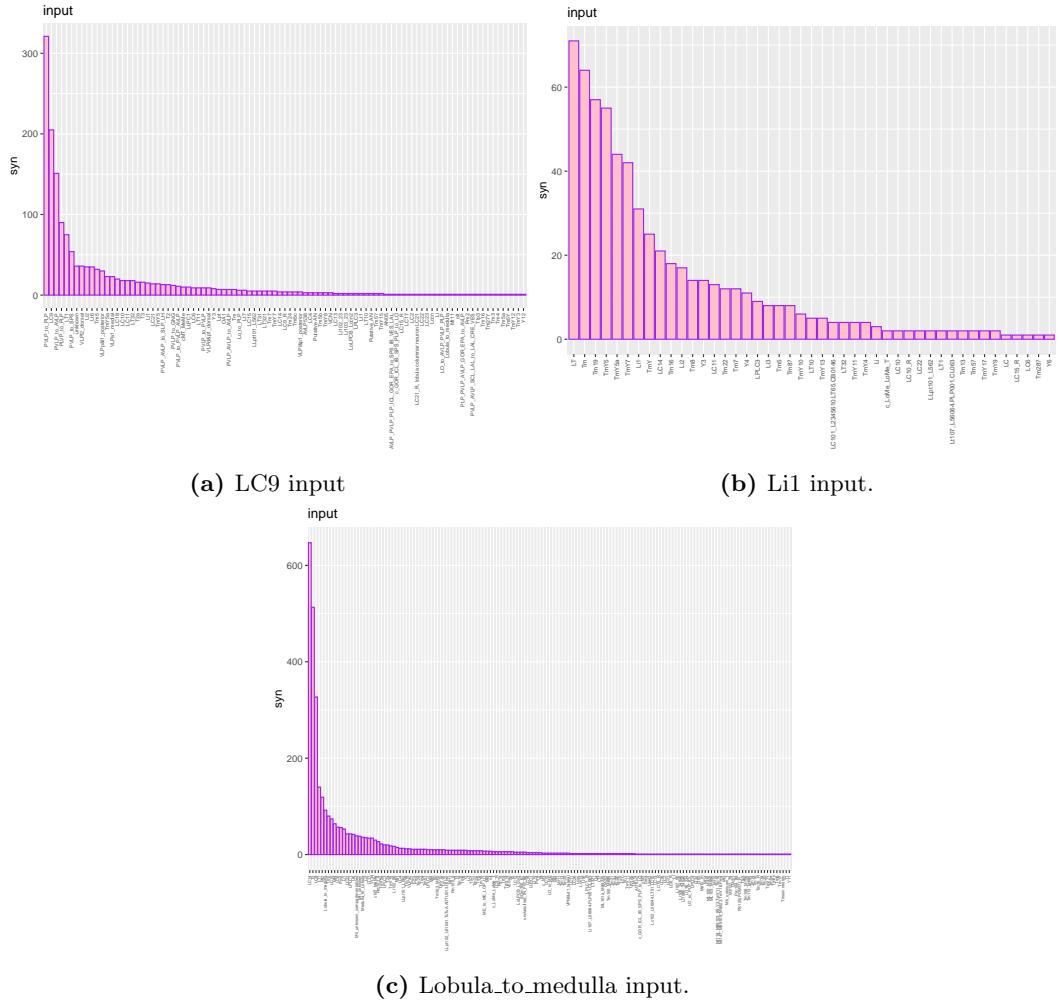
Now we are going to analyze the most important input neurons of the Lobula Columnar 9 neuron itself. I ran the summary statistics Rmd code on this neuron and got its statistics. LC14a input LC9 neuron has a higher percentage of input synapses compared to the output synapses (91,57% as opposed to 42,84%). Also as we can see from the Barplot 4.7a, the three most important LC9 input neurons are PVLP\_to\_PLP (with 20,52% of all LC9 input synapses, highest number), LC9 (with 13,11% of all input synapses) and PVLP\_to\_AVLP (making up to 9,65% of all input synapses).

Here is the plot of LC9 (LC14a input) connecting with its first most important input partner, PVLP\_to\_PLP (also called VLPd&p1\_dorsal), and creating pre-synapse (Figure 4.8). I could also plot presynaptic connections of the LC9 neuron with its second and third most important input neuron, LC9 and PVLP\_to\_AVLP.

Running the summary Rmd file that I was working on during my thesis, I got the summary statistics of the first most important LC14a input cell LC9. I used the table (see 4.1) from that code output in this thesis and the numbers correspond to percentages in Diagram 4.5. We can observe the top 3 cells contributing the most input synapses to LC9, along with other important input cells and their respective statistics.

I'm also going to discuss the most important input partners of the most important input neuron of LC9, which is PVLP\_to\_PLP (or VLPd&p1\_dorsal).

PVLP\_to\_PLP has its dendrites in the posterior ventral protocerebrum (PVLP) and



**Figure 4.7:** Barplots of most important input partners and input synapse count of LC14a most important input neurons (LC9, Li1, Lobula\_to\_medulla)

its axons in the posterior lateral protocerebrum (PLP). Below are the Barplot (see 4.9), statistics table (see table 4.2) and 3D plots of presynaptic connections that PVLP\_to\_PLP builds with its most important input neurons (see 4.10), which are LC9 with 44,79% (2437 synapses) of all input synapses, AVLP\_PVLP\_SLP\_SCL\_SIP\_ICL\_to\_SMP with 12,81% (697 synapses) of all input synapses, and LO\_to\_AVLP\_PVLP\_PLP with 4,41% (240 synapses) of all input synapses.

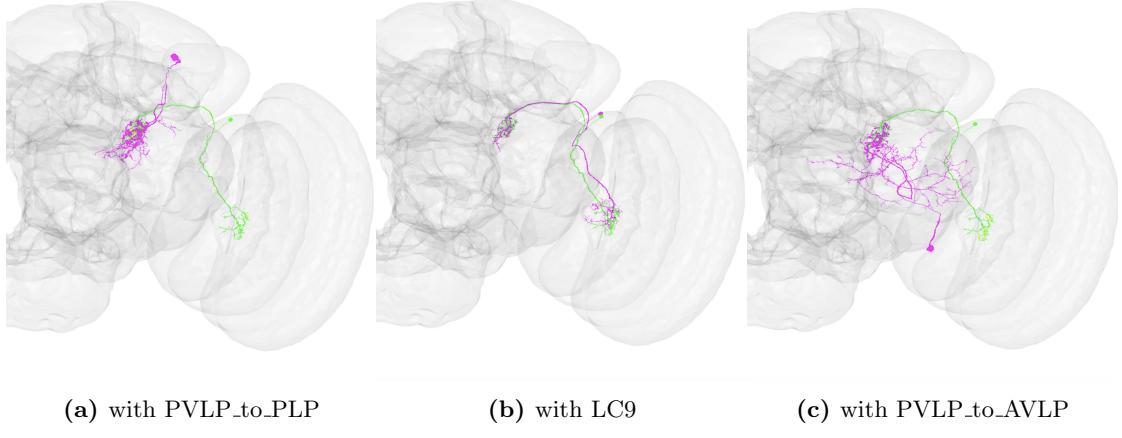
#### 4.2.2 Li1 - Second most important input neuron of LC14a

The second most important LC14a input neuron is Lobula intrinsic 1, creating 8,6% (579 synapses, second highest pre-synapses number) of all input synapses.

In Figure 4.6b you can see the 3D plot of LC14a presynaptic connection with Li1. As we can also notice from the image, Li1 is located inside the Lobula fly brain segment and therefore it's called Lobula intrinsic.

Different from the Medulla brain section, the Lobula and Lobula plate of Drosophila do not seem to contain many intrinsic neurons that stain. This agrees with what is known from other Diptera. For the lobula, only two true candidates Li1 and Li2 have been detected in Drosophila and are the most significant [Fischbach and Dittrich, 1989].

I also analyzed the most important input and output neurons of Li1 (LC14a input). With the help of the summary statistics Rmd code, I could get its statistics. However, Li1



**Figure 4.8:** 3D image of LC9 (LC14a input) (green) presynaptic connections with most important input partners (magenta)

**Table 4.1:** Statistics of broad input partner types of LC9. Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to LC9 in %.

type	partner_type_broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
input	PVLP_to_PLP	32	3	16.9	321	19	4.5%	20.52%
input	LC9	10	1	2.3	205	88	20.85%	13.11%
input	PVLP_to_AVLP	33	13	25.2	151	6	1.42%	9.65%
input	PLVP_to_PLP	20	7	12.9	90	7	1.66%	5.75%
input	LT1	36	1	7.5	75	10	2.37%	4.8%
input	PVLP_to_SPS	23	4	10.8	54	5	1.18%	3.45%
input	VLPl2_dorsal	9	1	3.3	36	11	2.61%	2.3%
input	Li2	7	1	2.5	35	14	3.32%	2.24%
input	Lt6	34	1	17.5	35	2	0.47%	2.24%

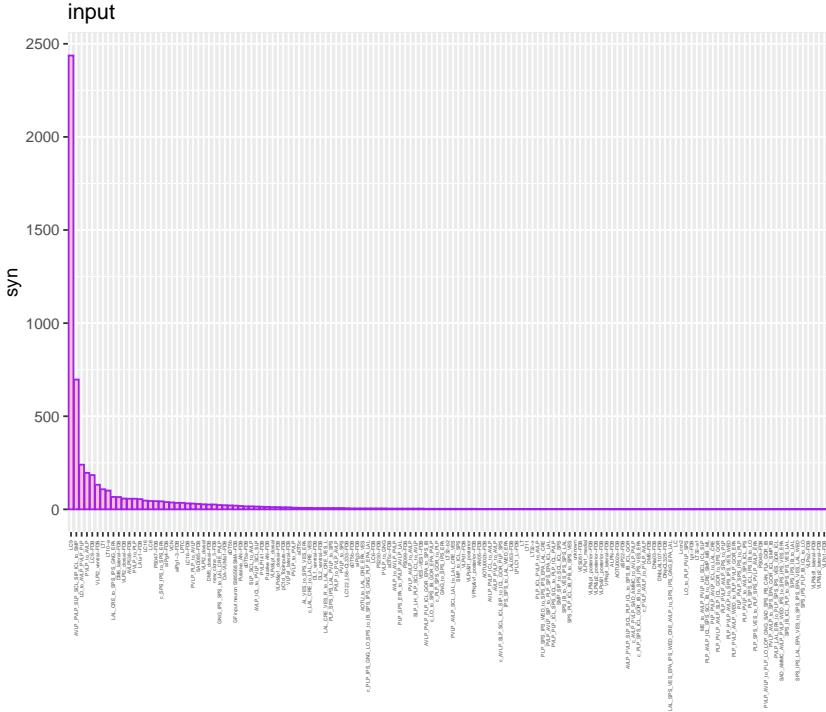
neurons had a lower percentage of input synapses compared to the output synapses (38,72% as opposed to 39,81%). Also as we can see from the Barplot 4.7b three most important input neurons to the Li1 neuron itself, which are: LT, Tm, and Tm19.

In Figure 4.11 we can see presynaptic connections of Li1 (LC14a input) with its most prominent input partners. Figure 4.11a is a pre-synapse of Li1 and Lobula Tangential neuron, Figure 4.11b describes the pre-synapse of Li1 with Transmedullar neuron. For the last image, Figure 4.11c, I chose another Li1 neuron for my R code to plot 3D images, because the previous Li1 didn't provide good images of pre-synapse with Tm19 (Transmedullar 19) cell, therefore the picture is the left part of the brain hemisphere as opposed to previous two images, where it was right hemisphere.

I could also get the statistics of the second most important LC14a input cell Li1 in table form. As you can see from the table 4.3, numbers correspond to percentages in Diagram 4.5 and to distribution in Barplot ???. Lobula Tangential is the first most important Li1 input neuron with 11,53% creating the highest number of presynapses, the second most important input cell is Transmedullar with 10,39% of all input synapses and the third most important input neuron for Li1 was Transmedullar 19 with 9,25% input synapses. In addition to the top 3 cells that contribute the most input synapses to Li1, the table 4.3 provides information about other significant input cells along with their respective statistics.

#### 4.2.3 Lobula\_to\_medulla - Third most important input neuron of LC14a

The third most crucial presynaptic input neuron of LC14a is Lobula\_to\_medulla, contributing 6,27% (422 synapses) of all input synapses. As implied by its name, this neuron traverses



**Figure 4.9:** PVLP\_to\_PLP input

**Table 4.2:** Statistics of broad input partner types of PVLP\_to\_PLP. Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to PVLP\_to\_PLP in %.

type	partner.type.broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
input	LC9	26	1	10.5	2437	233	35.36%	44.79%
input	AVLP_PVLP_SLP_SCL_SIP_ICL_to_SMP	269	159	232.3	697	3	0.46%	12.81%
input	LO_to_AVLP_PVLP_PLP	148	12	80	240	3	0.46%	4.41%
input	PVLP_to_AVLP	39	1	10.3	196	19	2.88%	3.6%
input	LC9-FDB	25	1	8.4	184	22	3.34%	3.38%
input	VLPi2_ventral-FDB	80	1	12	132	11	1.67%	2.43%
input	LT1	100	1	18	108	6	0.91%	1.98%
input	LT10-a	94	7	50.5	101	2	0.3%	1.86%
input	LAL,CRE_to_SPS_IPS_GNG_EPA	25	10	16.8	67	4	0.61%	1.23%
input	DM6_lateral-FDB	30	2	13.2	66	5	0.76%	1.21%

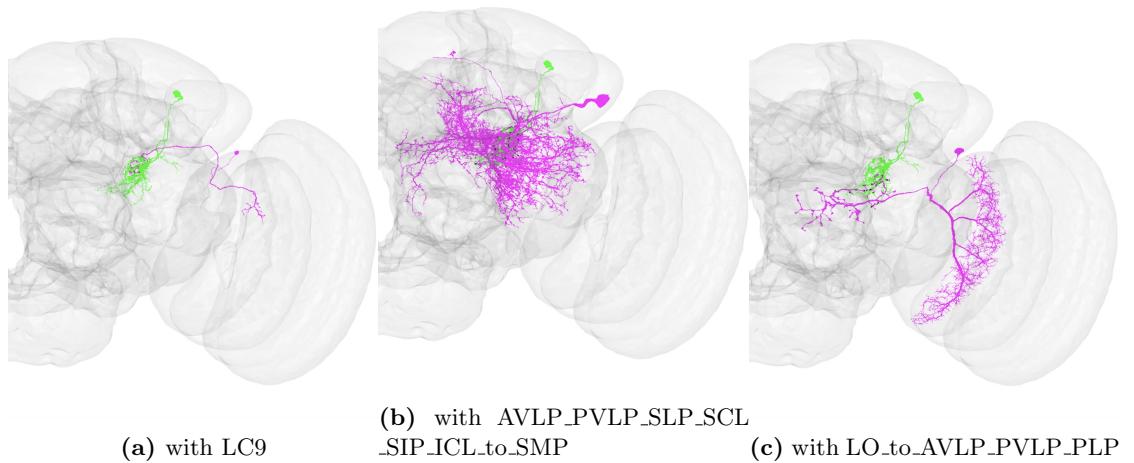
the Lobula and Medulla brain segments, with dendrites located in the Lobula and axons in the Medulla. It establishes a connection between the Lobula and Medulla.

Figure 4.6c illustrates LC14a presynapse with Lobula\_to\_medulla.

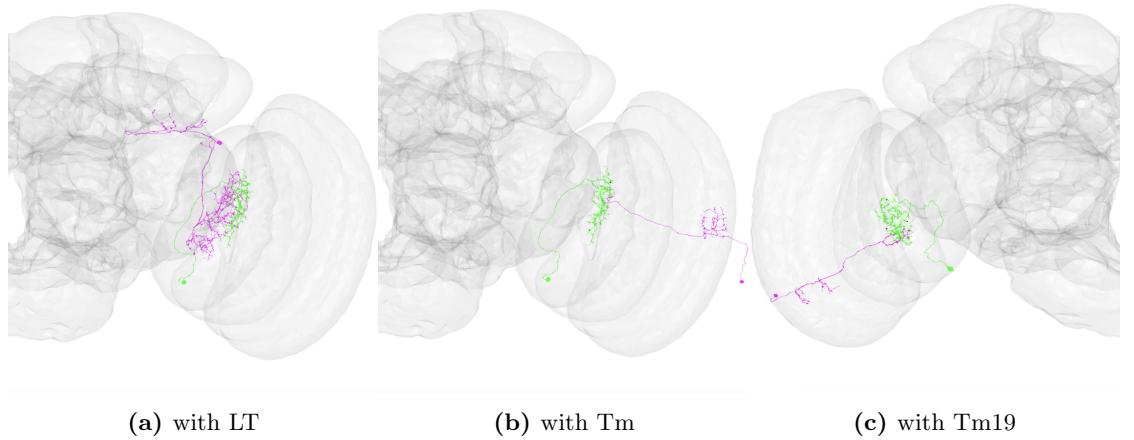
By executing the summary statistics Rmd code, I obtained information about the most important input neurons of Lobula\_to\_medulla (LC14a input) and their statistics. Lobula\_to\_medulla has more input synapses compared to output synapses (82,19% input and 57,65% output). Barplot 4.7c further displays the three most significant input neurons of Lobula\_to\_medulla: LC14-a, LC9 and T2.

I created 3D images of pre-synapses that Lobula\_to\_medulla forms with its input partners, see Figure 4.12. Specifically, Figure 4.12a shows the pre-synapse of Lobula\_to\_medulla with LC14-a, Figure 4.12b shows input synapse of Lobula\_to\_medulla with LC9 and the last Figure 4.12c displays pre-synapse of Lobula\_to\_medulla with the T2 neuron. The order of these images corresponds to the importance of the input partners, from the first most important to the third.

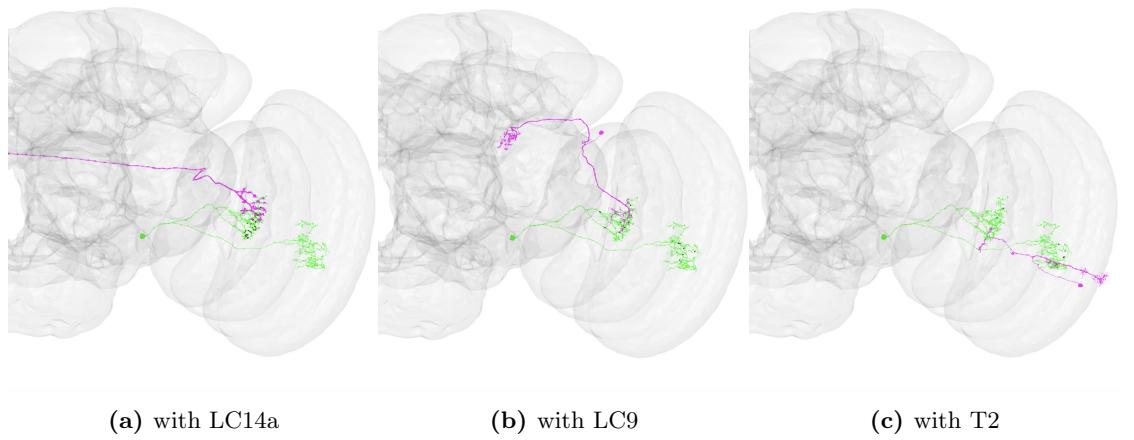
I also obtained the table (see table 4.4) presenting the statistics of input partners of Lobula\_to\_medulla. As we can observe, the numbers in the table correspond to the percentages in Diagram 4.5 and are visually represented in Barplot 4.7c. LC14-a stands out as



**Figure 4.10:** 3D image of PVLP\_to\_PLP (LC9 input (LC14a input)) (green) presynaptic connections with most important input partners (magenta)



**Figure 4.11:** 3D image of Li1 (LC14a input) (green) presynaptic connections with most important input partners (magenta)



**Figure 4.12:** 3D image of Lobula\_to\_medulla (green) presynaptic connections with most important input partners (magenta)

**Table 4.3:** Statistics of broad input partner types of Li1 (second most important LC14a input cell). Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to Li1 in %.

type	partner.type.broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage.ided	percentage.ided.syn
input	LT	67	2	23.7	71	3	1.4%	11.53%
input	Tm	10	1	3	64	21	9.81%	10.39%
input	Tm19	16	1	8.1	57	7	3.27%	9.25%
input	TmY5	10	1	2.9	55	19	8.88%	8.93%
input	TmY5a	19	1	3.4	44	13	6.07%	7.14%
input	TmY7	8	1	3	42	14	6.54%	6.82%
input	Li1	6	1	1.9	31	16	7.48%	5.03%
input	TmY	13	1	5	25	5	2.34%	4.06%
input	LC14	4	1	2.6	21	8	3.74%	3.41%
input	Tm16	4	1	3	18	6	2.8%	2.92%

the primary and most important input neuron to Lobula\_to\_medulla, contributing 19,78% of all input synapses. Following closely, LC9 is the second most important input neuron to Lobula\_to\_medulla with 10,14% of all input synapses and T2 secures the third position, contributing 8,06% of all pre-synapses. This table also details other noteworthy input partners of the Lobula\_to\_medulla neuron along with their respective statistics.

**Table 4.4:** Statistics of broad input partner types of Lobula\_to\_medulla (third most important LC14a input cell). Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to Lobula\_to\_medulla in %.

type	partner.type.broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage.ided	percentage.ided.syn
input	LC14-a	101	1	30.4	638	21	2.95%	19.78%
input	LC9	42	1	16.4	327	20	2.81%	10.14%
input	T2	14	1	5.4	260	48	6.73%	8.06%
input	T2-FDB	19	1	4	242	60	8.42%	7.5%
input	LT1	49	1	9.3	140	15	2.1%	4.34%
input	Mt2	79	1	23.8	119	5	0.7%	3.69%
input	Lobula_to_medulla	22	1	5.1	92	18	2.52%	2.85%
input	Tm2-FDB	7	1	2.5	77	31	4.35%	2.39%
input	Mt4	18	1	9.1	64	7	0.98%	1.98%
input	LC6	14	1	5.7	57	10	1.4%	1.77%

## 4.3 Three most important LC14a output neurons

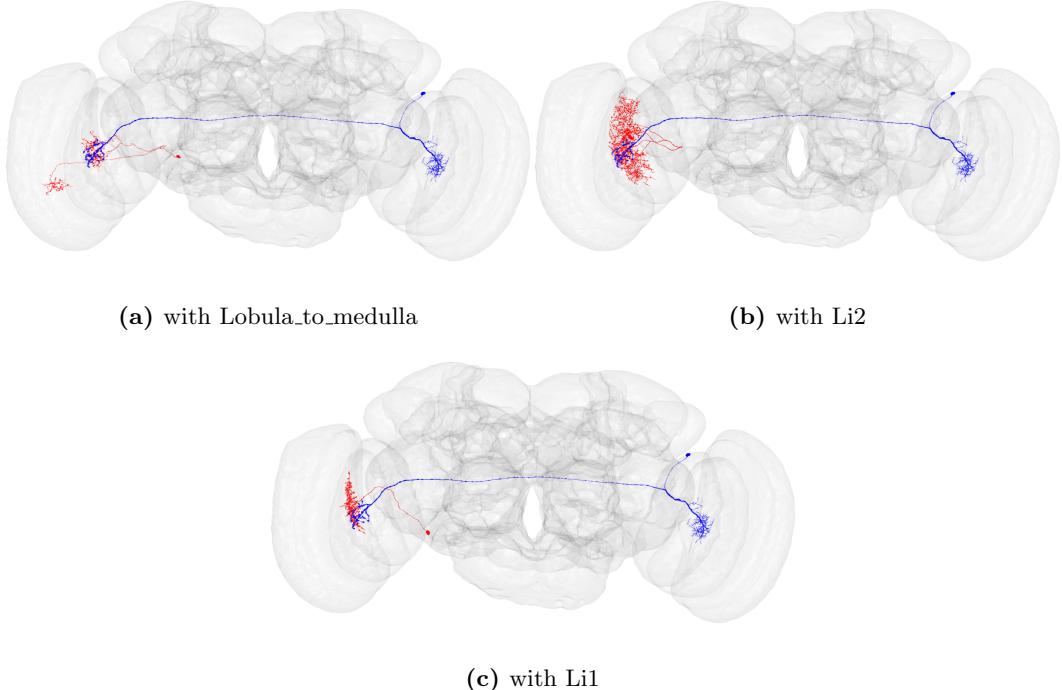
Now we are going to discuss the three most important **output** (postsynaptic) neurons of LC14a cells, which are Lobula\_to\_medulla, Li2, and Li1 (see Figure 4.13).

### 4.3.1 Lobula\_to\_medulla - First most important output neuron of LC14a

The first most important output neuron of LC14a is Lobula\_to\_medulla with 10,75% (highest percentage) of all LC14a post-synapses (see Diagram 4.5).

Figure 4.13a showcases the 3D plot of the postsynaptic connection of LC14a with Lobula\_to\_medulla.

I'm going to describe the three most important output neurons of Lobula\_to\_medulla (LC14a output) with their statistics, which are: Li2 with 10,04% (the highest percentage of post-synapses) of all output synapses, T2 with the second highest percentage of post-synapses with 6,73% and third most important output neuron is Lobula\_to\_medulla with 6,57% of all output synapses. Summary statistics Rmd code output showed that Lobula\_to\_medulla (LC14a output) generally has a higher percentage of input synapses compared to output synapses (70,71% input as opposed to 54,92% output).



**Figure 4.13:** 3D image of LC14a neuron (blue) building postsynaptic connections with its most important output partners (red)

These numbers correspond to the Diagram 4.5 results and also to the Barplot 4.14 distribution.

Figure 4.15 contains three subfigures, which are output synapses Lobula\_to\_medulla builds with its three most important postsynaptic partners. Sub-figure 4.15a shows the output synapse of Lobula\_to\_medulla with Li2, next sub-figure 4.15b displays the postsynaptic connection of Lobula\_to\_medulla with second most important output partner T2 and last sub-figure 4.15c demonstrates the output synapse of Lobula\_to\_medulla with its third most important output neuron Lobula\_to\_medulla. For the last sub-figure I used another LC14a output Lobula\_to\_medulla neuron on my R Code to plot this 3D image, that's why it shows the opposite (right) hemisphere compared to the first two sub-figures.

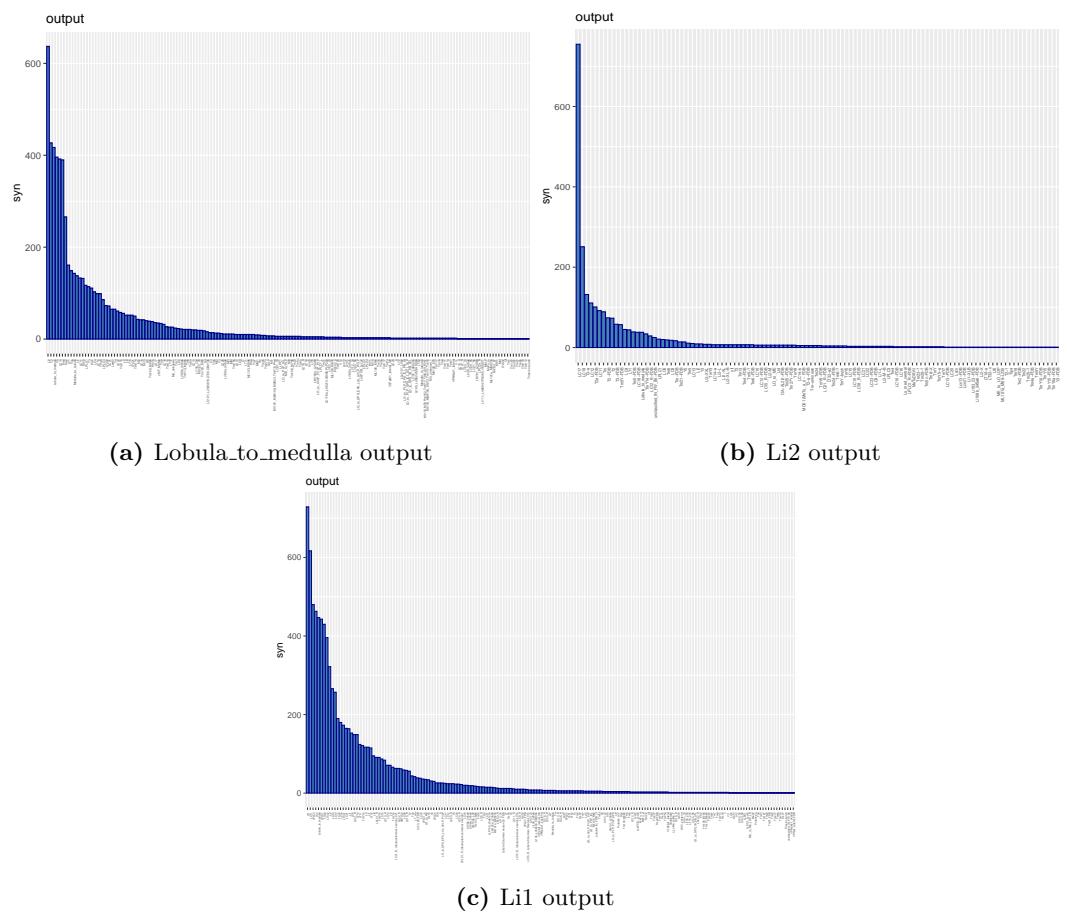
Running the summary Rmd file, I could get the statistics table (see 4.5 of the most important output neurons of Lobula\_to\_medulla (Li2, T2, and Lobula\_to\_medulla). In this table, we observe not just the three primary cells with the highest output synapse counts to Lobula\_to\_medulla but also additional significant output cells along with their respective statistics.

### 4.3.2 Li2- Second most important output neuron of LC14a

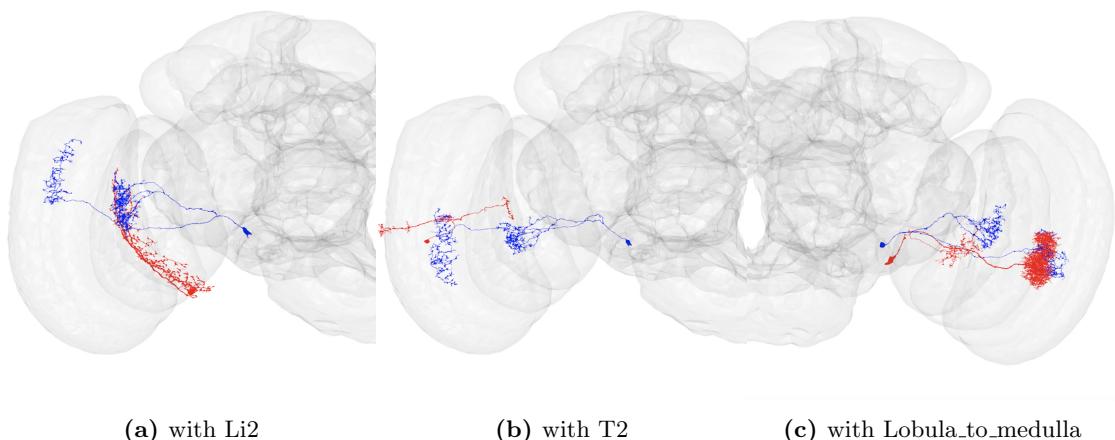
The second most important output neuron of LC14a is Lobula intrinsic 2, contributing to 8,35% of all LC14a post-synapses (as a reference, see Diagram 4.5). As implied by its name, this neuron is situated within Lobula, with both dendrites and axonal arbors.

In Sub-figure 4.13b, you can find the 3D plot illustrating the postsynaptic connection between LC14a and Li2.

Now, let's delve into the three most important output neurons of Li2 (LC14a output) along with their statistics. Lobula Columnar 17 is the first most important L2 output neuron, constituting 29,83% of all output synapses, the Lobula Columnar 18 is the second most relevant output neuron with 9,92% of all post-synapses, followed by T2-a as the third one with 5,22% of all output synapses. Examining the statistics of Li2 neurons itself, it is noted that Li2 has more input synapses compared to output synapses (53,34% input



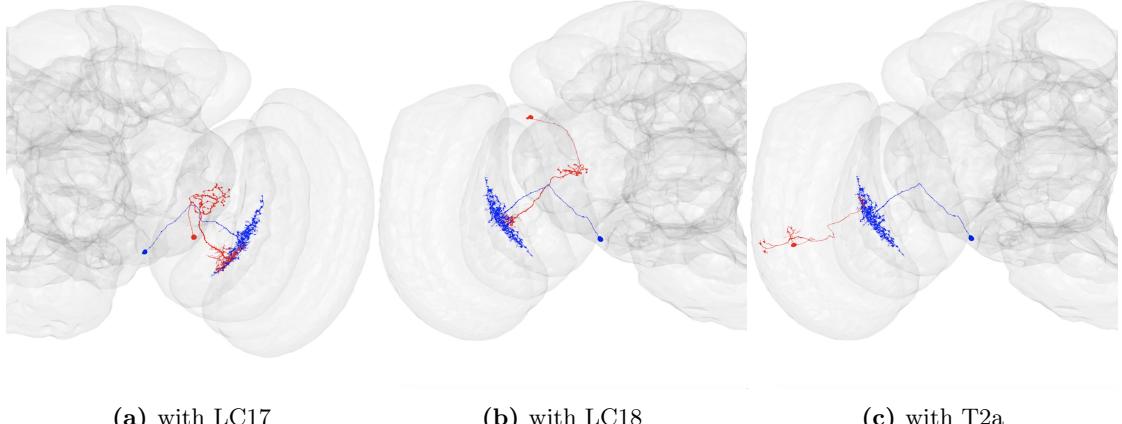
**Figure 4.14:** Barplots of the most important output partners and output synapse count of LC14a most important output neurons (Lobula\_to\_medulla, Li2, Li1)



**Figure 4.15:** 3D image of Lobula\_to\_medulla (green) postsynaptic connections with most important output partners (magenta)

**Table 4.5:** Statistics of broad output partner types of Lobula\_to\_medulla (first most important LC14a output cell). Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to Lobula\_to\_medulla in %.

type	partner_type_broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
output	Li2	117	1	11.8	637	54	4.97%	10.04%
output	T2	16	1	4.8	427	89	8.2%	6.73%
output	Lobula_to_medulla	66	1	11.3	417	37	3.41%	6.57%
output	Y5	35	2	13.7	396	29	2.67%	6.24%
output	LC14	63	1	10.3	392	38	3.5%	6.18%
output	Pm4	84	1	24.4	390	16	1.47%	6.15%
output	Pm2	37	1	6.8	266	39	3.59%	4.19%
output	LT1	31	1	7.3	161	22	2.03%	2.54%
output	T2a	9	1	3.7	149	40	3.68%	2.35%
output	Medulla_to_Lobula	79	5	47.7	143	3	0.28%	2.25%



**Figure 4.16:** 3D image of Lobula intrinsic 2 (blue) postsynaptic connections with most important output partners (red)

compared to 43,99% output). These statistics align with those presented in Diagram 4.5.

Additionally, I generated the Barplot 4.14b showcasing the most important output partners of Li2 neuron. Here, you can observe that the three neurons forming the most postsynapses with L2 are LC17, LC18, and T2-a. This Barplot illustrates the synapse count for each output partner neuron with Li2.

Figure 4.16 demonstrates the postsynaptic connections that Lobula intrinsic 2 neuron establishes with its most relevant output partners. The first sub-figure displays the output synapse of Li2 with LC17 (see 4.16a, the second sub-figure (4.16b shows output synapse with LC18 and the third sub-figure(4.16c) shows post-synapse with the third most important output neuron of Li2, T2a.

The statistics table 4.6 for the most important output neurons of Li2 also corroborates the same results. In this table, you'll find not only the top three cells contributing the highest number of output synapses to Li2 but also other noteworthy output neurons along with their respective statistics.

### 4.3.3 Li1- Third most important output neuron of LC14a

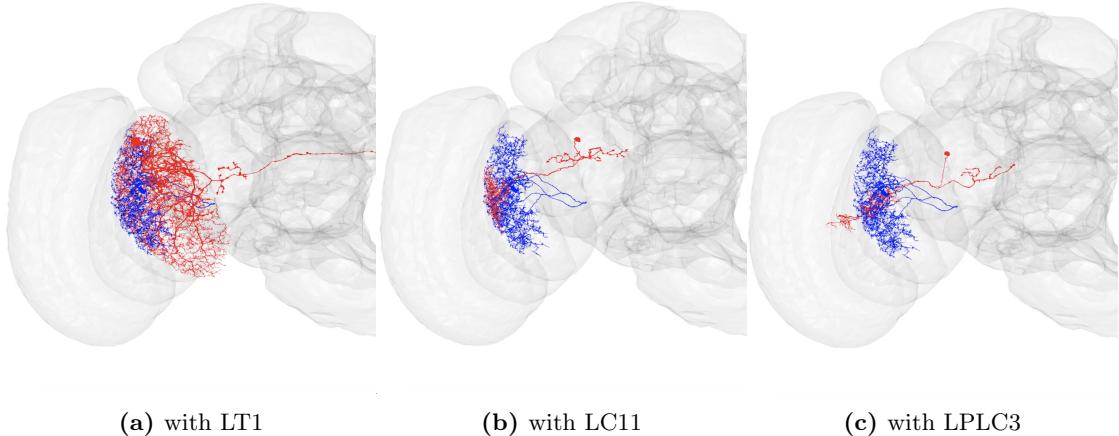
Lobula intrinsic 1 is the third most important output neuron of LC14a, contributing to 7,55% of all LC14a post-synapses (refer to Diagram 4.5).

Sub-figure 4.13c) presents a 3D plot illustrating the postsynaptic connection between LC14a and Li1.

The three most important output neurons of Li1 (LC14a output) include Lobula Tangential 1, accounting for 8,09% of all output synapses, Lobula Columnar 11 with 6,85% of all

**Table 4.6:** Statistics of broad output partner types of Li2 (second most important LC14a output cell). Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to Li2 in %.

type	partner_type_broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
output	LC17	36	1	11.3	755	67	10.5%	29.83%
output	LC18	28	2	8.1	251	31	4.86%	9.92%
output	T2-a	11	1	3.3	132	40	6.27%	5.22%
output	LC12	11	1	4.8	111	23	3.61%	4.39%
output	T2a-FDB	7	1	2.9	101	35	5.49%	3.99%
output	LC11	13	1	4	92	23	3.61%	3.63%
output	Tm	10	1	4	89	22	3.45%	3.52%
output	T2-FDB	7	1	3.2	74	23	3.61%	2.92%
output	T2	7	1	2.2	73	33	5.17%	2.88%
output	T3-FDB	4	1	2	58	29	4.55%	2.29%



**Figure 4.17:** 3D image of Lobula intrinsic 1 (blue) postsynaptic connections with most important output partners (red)

post-synapses, and Lobula Plate - Lobula Columnar 3 neuron generating 5,33% of all output synapses to Li1. Li1, in general, establishes more input synapses (pre-synaptic connections) than output synapses with its partner neurons (59,64% input vs 40,42% output). These percentages are also reflected in Diagram 4.5.

Barplot 4.14c visually displays the most important output partners of Li1, highlighting the top three neurons building the most post-synapses compared to others.

We can see output synapses Lobula intrinsic 1 builds in Figure 4.17 with its most important output partner neurons. Sub-figure 4.17a depicts the post-synapse between Li1 and LT1 (first most important output neuron), sub-figure 4.17b shows output synapse between Li1 and second most important output partner LC11, and last sub-figure 4.17c is an image of the post-synapse between Li1 and its third most important output neuron LPLC3.

I also obtained the statistics table 4.7 of the most important output partners of Li1 (LC14a output). In this table, we can observe additional significant output neurons that establish post-synapses with Lobula Intrinsic 1, along with their respective statistics.

#### 4.4 Three most important LC14b input neurons

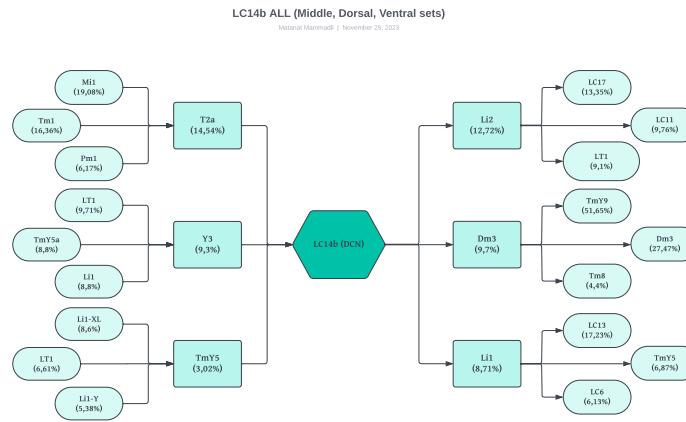
Now, let's delve into the key input and output neurons of LC14b. While LC14b neurons share similarities with LC14a, such as their appearance, their axonal and dendritic arbors exhibit slight differences - being somewhat longer with neurites branching further down before creating an arbor. Furthermore, the axons of LC14b traverse both the Lobula and the Medulla, occupying positions in both brain regions.

**Table 4.7:** Statistics of broad output partner types of Li1 (third most important LC14a output cell). Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to Li1 in %.

type	partner_type_broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
output	LT1	168	1	22.8	729	32	2.18%	8.09%
output	LC11	41	1	10.6	617	58	3.96%	6.85%
output	LPLC3	49	1	8.4	480	57	3.89%	5.33%
output	LC4	35	1	11	463	42	2.86%	5.14%
output	Lobula_to_medulla	85	1	8.9	447	50	3.41%	4.96%
output	LPLC1	47	1	13.8	443	32	2.18%	4.92%
output	Tm24	30	1	5.6	430	77	5.25%	4.77%
output	LT322	156	25	99	396	4	0.27%	4.39%
output	Li2	98	1	7.7	322	42	2.86%	3.57%
output	LC14	34	1	5.5	266	48	3.27%	2.95%

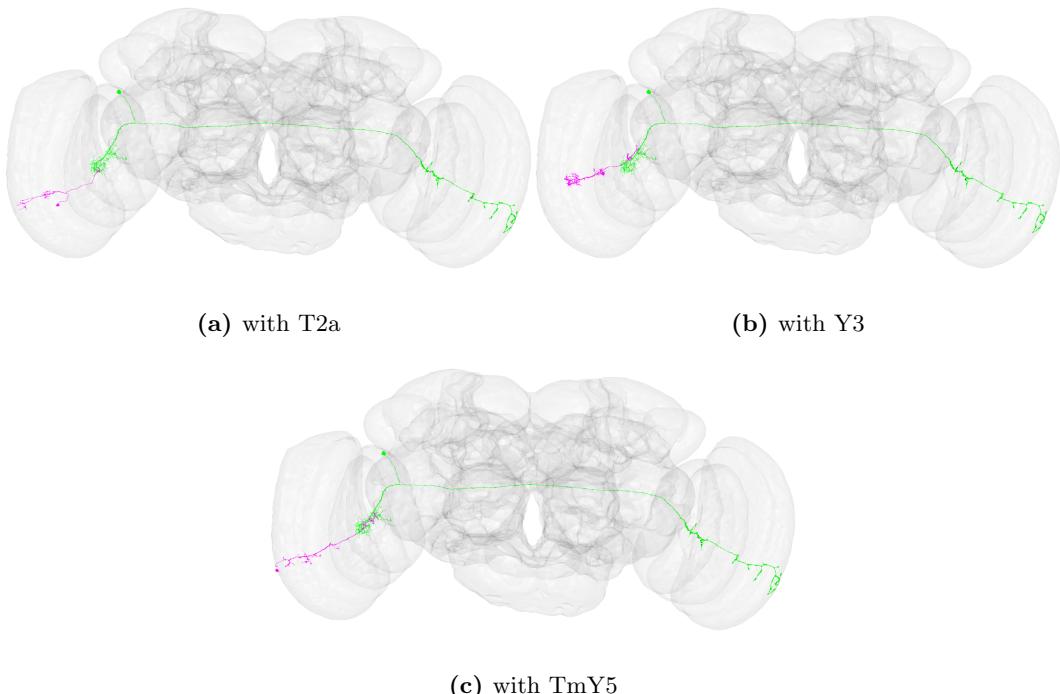
Similar to LC14a cells, I received 6 example cells, 2 Middle set, 2 Dorsal set, and 2 Ventral set LC14b cells. I updated these LC14b cells in my tracing R Code and summary Rmd code using their IDs. After obtaining their latest IDs, I accessed their Excel sheets containing crucial information such as pre- and post-IDs, partner types, partner names, and their weight (synapse count). Before updating each neuron, I performed several tasks, including running all required libraries, conducting a diagnostic run of the `fafbseg()` package, updating our lab database "Flywire" Excel sheet, and updating all cells before the specific cell I was going to update.

In total, I updated all 6 sets (Middle, Dorsal, ventral) of LC14b cells, revealing their three most important input and output partner neurons. When we say 'most important,' we refer to neurons exerting the highest impact on LC14b cells, constructing the most synapses compared to other input and output neurons. These results are pivotal for our research, and you can observe their synapse percentages in Diagram 4.18.



**Figure 4.18:** 3 most important input and output partner neurons (and their partners) of all 6 set (Middle, Dorsal, Ventral) of LC14b cells

Initially, we will discuss the three most important **input** (presynaptic) neurons of LC14b cells, which are T2a, Y3, and TmY5 neurons (see Figure 4.19). Just as we did previously, to prevent confusion, we will employ color coordination: **input** synapse partners will be



**Figure 4.19:** 3D image of LC14b neuron (green) building presynaptic connections with its most important input partners (magenta)

represented with green and magenta colors, while **output** synapse partners will be depicted with blue and red colors.

#### 4.4.1 T2a - First most important input neuron of LC14b and its most important input neuron

The primary input neuron to LC14b, T2a, contributes to 14,54% (549 synapses) of all input synapses received by LC14b.

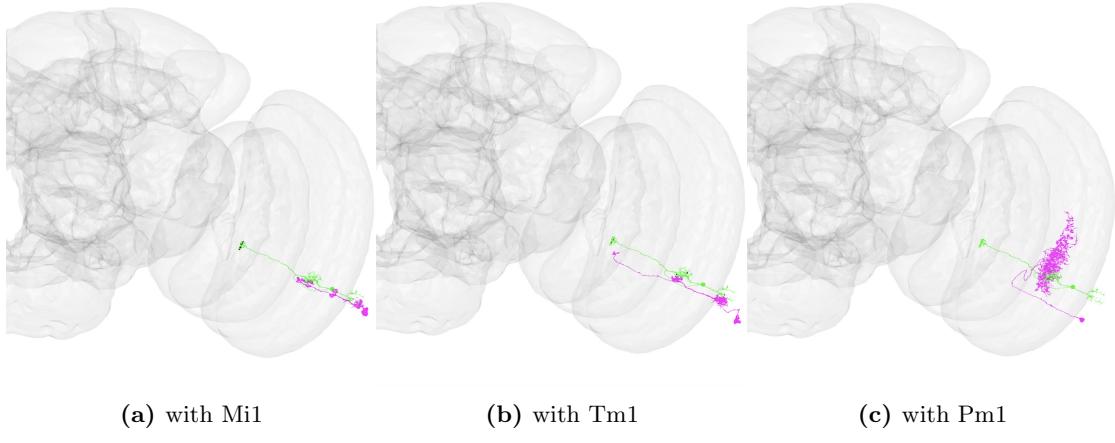
T2a is a special form of T2 cells. T2 neurons, alongside T3 neurons, belong to neurons that connect the Proximal Medulla with the Lobula. The cell bodies of T2 cells are clustered posteriorly in the space left by the Medulla and Lobula Plate neuropiles. Their fibers travel along the inner face of the Medulla to their respective columns where they bifurcate in a T-like fashion (hence the name T cells), sending one branch distally into the Medulla and the other into the isotopic column of the Lobula [Fischbach and Dittrich, 1989].

T2 neurons distinguish themselves from T3 neurons in two aspects. While their arborizations in the Proximal Medulla are very similar to those of T3 neurons, T2 cells possess additional dendrites in the Distal Medulla.

The axons of T2a cells terminate in the Lobula at the same level as T3 axons; their terminals have, however, a larger lateral extent. The terminals of T2 are located in the second layer of the Lobula.

In sub-figure 4.19a, the 3D plot illustrates the presynaptic connection of the LC14b neuron with T2a.

Furthermore, 3D plots depicting presynaptic connections of T2a cells with their most significant input neurons were obtained. These include Medulla intrinsic 1 being the first most important input neuron of T2a with 19,08% (133 synapses) of all input synapses, followed by Transmedullar 1 neuron as the second most important input neuron of T2a with 16,36% (114 synapses) of all input synapses and Proximal medullar 1 neuron as the third most important input neuron of T2a with 6,17% (43 synapses) of all input synapses.



**Figure 4.20:** 3D image of T2a (LC14b input) (green) presynaptic connections with most important input partners (magenta)

Respectively, Figure 4.20 shows all these input neurons of T2a with the right order of sub-figures (from the first most important to the third).

Barplot 4.21a provides a visual representation of the key input partners for T2a (LC14b input). In this illustration, the top three neurons (Mi1, Tm1, and Pm1) are highlighted, indicating their substantial contribution to the highest number of input synapses compared to other partners.

Following the execution of the summary Rmd code, I obtained the statistical data for the most important input neurons of T2a. The results are presented in Table 4.8.

**Table 4.8:** Statistics of broad input partner types of T2a (first most important LC14b input cell). Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to T2a in %.

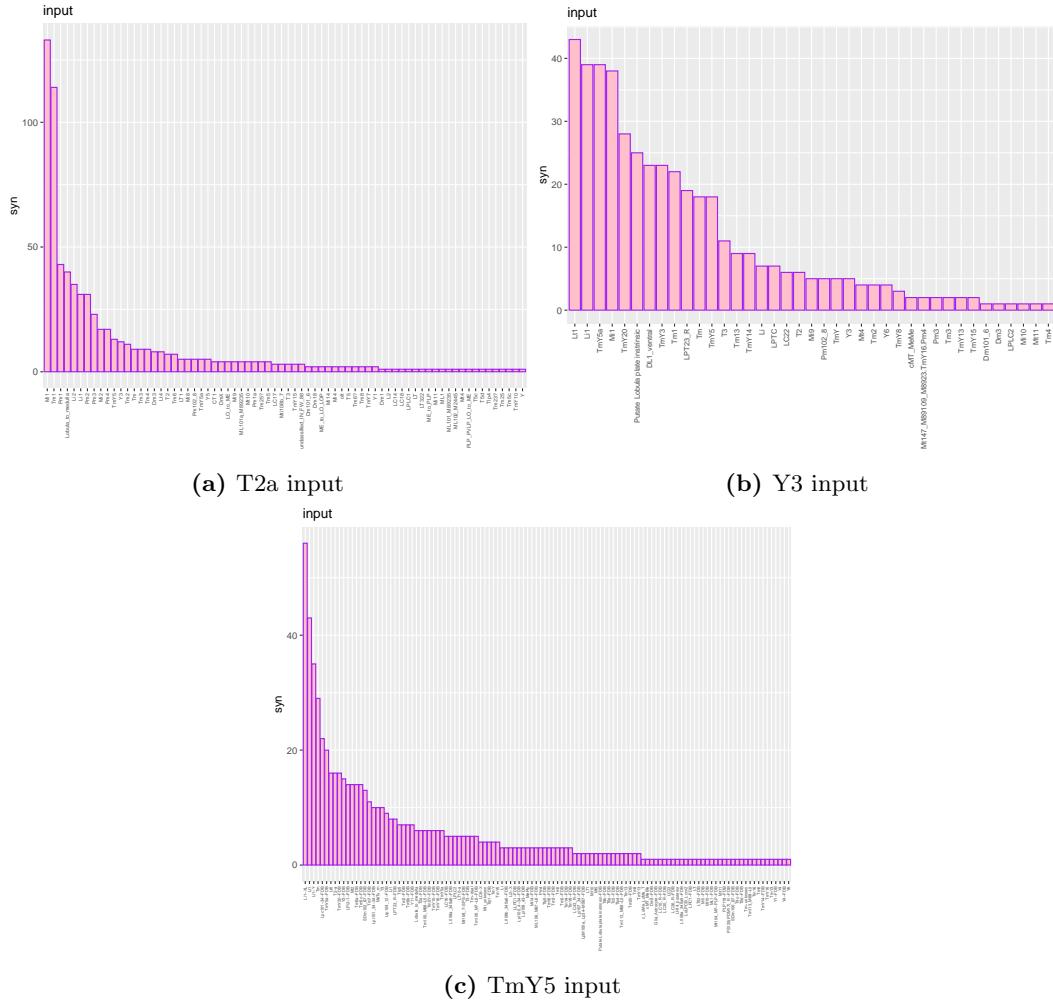
type	partner_type_broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
input	Mi1	24	1	8.9	133	15	7.35%	19.08%
input	Tm1	31	1	8.8	114	13	6.37%	16.36%
input	Pm1	11	1	3.6	43	12	5.88%	6.17%
input	Lobula_to_medulla	9	1	3.3	40	12	5.88%	5.74%
input	Li2	11	1	4.4	35	8	3.92%	5.02%
input	Pm2	6	1	2.4	31	13	6.37%	4.45%
input	Li1	14	1	4.4	31	7	3.43%	4.45%
input	Pm3	8	4	5.8	23	4	1.96%	3.3%
input	Pm4	6	1	3.4	17	5	2.45%	2.44%
input	Mi2	7	1	4.2	17	4	1.96%	2.44%

I will also delve into the discussion of the primary input partners for the most important input neuron of T2a (LC14b input), which is Mi1.

Mi1 neuron, as its name suggests, is located inside the Medulla in the optic lobe. The corresponding Barplot is illustrated in the Figure 4.22, statistical details are outlined in the table 4.9, and 3D plots of the presynaptic connections that Mi1 builds with its most important input neurons are presented in Figure 4.23, which are Pm2 with 22,88% (140 synapses) of all input synapses, Pm1 with 18,63% (114 synapses) of all input synapses, and T2 with 8,33% (51 synapses) of all input synapses.

#### 4.4.2 Y3 - Second most important input neuron of LC14b

Y3 is the second most important input neuron of LC14b, contributing to 9,3% (351 synapses) of all input synapses.



**Figure 4.21:** Barplots of most important input partners and input synapse count of LC14b most important input neurons (T2a, Y3, TmY5)

Y cells connect three optic lobe regions, the Lobula Plate with the Proximal Medulla (retinotopic regions) and Lobula [Fischbach and Dittrich, 1989]. The cell bodies of Y cells are located in the rind of the lobula plate. They typically have dense arborizations within several columns of the lobula plate neuropile. Y cells do not extend into the distal medulla neuropile. Their dendrites within the lobula plate are not much stratified.

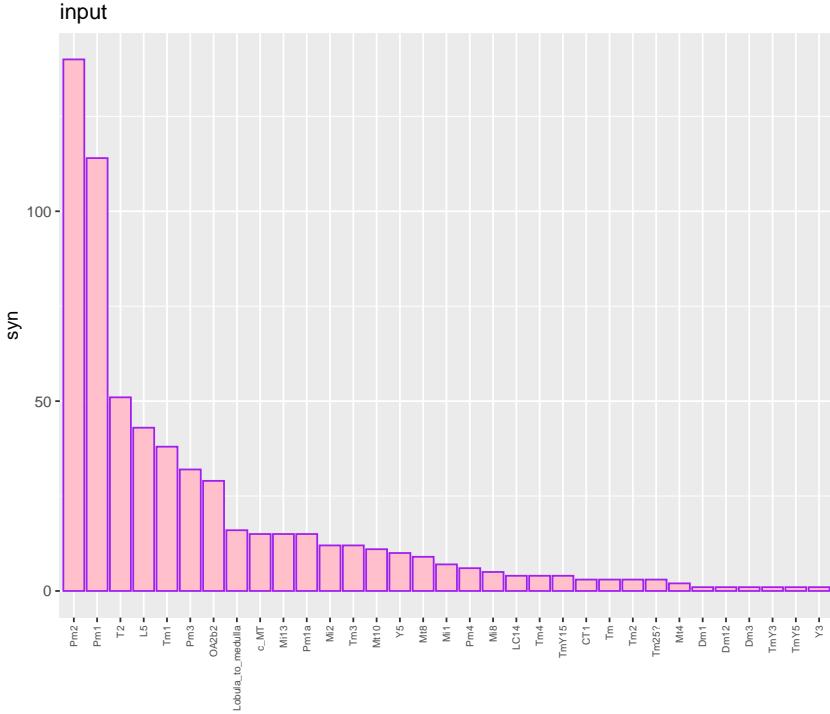
The main fiber of the Y cells bifurcates in the inner chiasm, sending one or several branches into the lobula and the other one upstream into the proximal medulla. In wild-type *Drosophila*, no Y cell has ever been seen to enter the distal medulla.

The sub-figure 4.19b visually represents the input synapse formed by LC14b with its second most significant input neuron, Y3.

Figure 4.24 showcases 3D plots of pre-synapses Y3 builds with its three most important input neurons. These include Lobula Tangential 1, accounting for 9,71% (43 synapses, highest amount) of all input synapses, Transmedullar Y5a neuron, creating 8,8% (39 synapses) of all input synapses and third most important input neuron, Lobula intrinsic 1 neuron, contributing to 8,8% (39 synapses) of all input synapses.

Barplot 4.21b reflects the outcomes corresponding to the top three input neurons of Y3. Notably, in our case, both TmY5a and Li1 contribute the same number of input synapses and percentages, resulting in a switch in their positions.

Furthermore, the statistics table for the most significant input neurons of Y3 can be found in the table 4.10.



**Figure 4.22:** Mi1 input

**Table 4.9:** Statistics of broad input partner types of Mi1. Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to Mi1 in %.

type	partner_type_broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
input	Pm2	23	1	7.4	140	19	14.96%	22.88%
input	Pm1	17	1	5.2	114	22	17.32%	18.63%
input	T2	9	1	4.6	51	11	8.66%	8.33%
input	L5	43	43	43	43	1	0.79%	7.03%
input	Tm1	37	1	19	38	2	1.57%	6.21%
input	Pm3	12	1	4	32	8	6.3%	5.23%
input	OA2b2	13	7	9.7	29	3	2.36%	4.74%
input	Lobula_to_medulla	13	1	5.3	16	3	2.36%	2.61%
input	Mi13	4	1	2.1	15	7	5.51%	2.45%
input	Pm1a	6	1	3	15	5	3.94%	2.45%

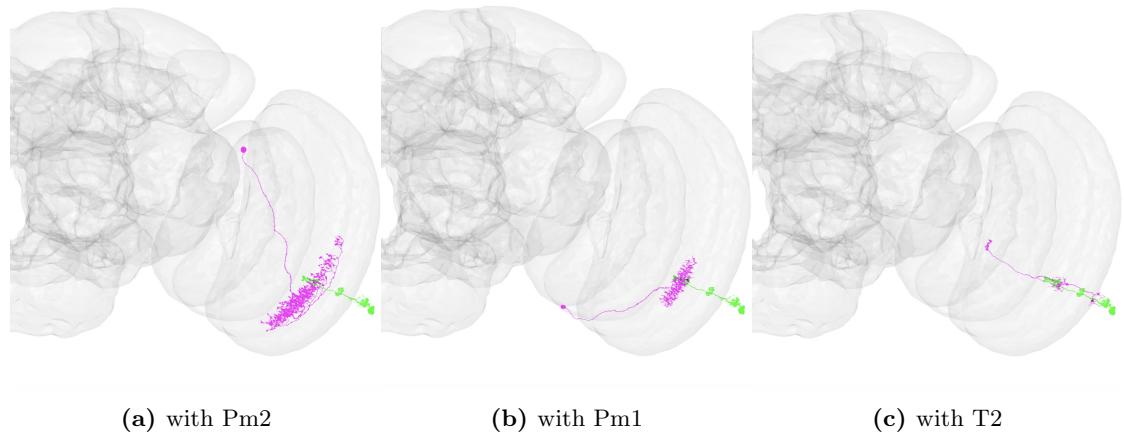
#### 4.4.3 TmY5 - Third most important input neuron of LC14b

The third most important input partner of LC14b cells is the Transmedullary Y5 neuron, contributing to 3.02% of all input synapses (114 synapses) to LC14b.

Transmedullary cells connect the distal medulla with the lobula. They are columnar cell types with their soma in the distal rind of the medulla that penetrates the medulla neuropile into the lobula. A common characteristic of Tm neurons is that they arborize only at certain levels of the medulla neuropile, with different types choosing different layers. They are also distinguished by the lateral extent of their arborizations and by the depth of their projections into the lobula [Fischbach and Dittrich, 1989].

TmY cells are a little different than typical Tm cells, by their shape and the brain segments they connect. Different than usual Tm cells, TmY cells connect three brain segments, distal medulla, lobula, and lobula plate (they branch out a little bit towards the lobula plate, creating a special angle in the top view).

The 3D image depicting the synaptic connection between LC14b and the TmY5 neuron is presented in the sub-figure 4.19b.

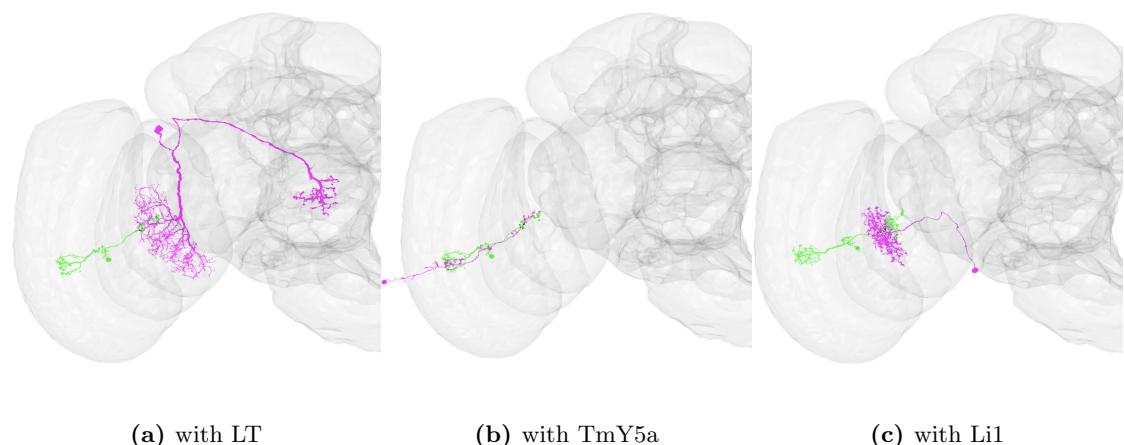


(a) with Pm2

(b) with Pm1

(c) with T2

**Figure 4.23:** 3D image of Mi1 (T2a input (LC14b input)) (green) presynaptic connections with most important input partners (magenta)



(a) with LT

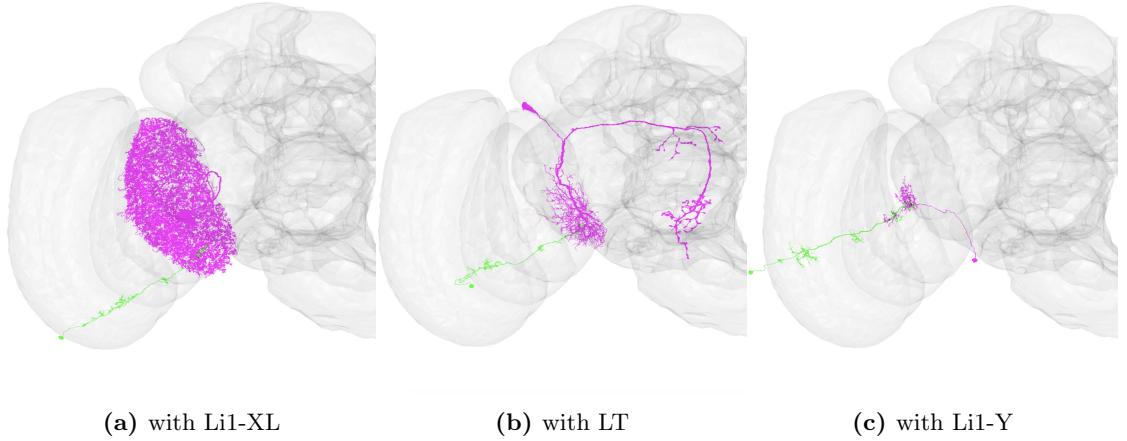
(b) with TmY5a

(c) with Li1

**Figure 4.24:** 3D image of Y3 (LC14b input) (green) presynaptic connections with most important input partners (magenta)

**Table 4.10:** Statistics of broad input partner types of Y3 (second most important LC14b input cell). Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to Y3 in %.

type	partner_type_broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
input	Lt1	17	13	14.3	43	3	2.68%	9.71%
input	TmY5a	18	1	6.5	39	6	5.36%	8.8%
input	Li1	13	1	7.8	39	5	4.46%	8.8%
input	Mi1	13	1	5.4	38	7	6.25%	8.58%
input	TmY20	11	3	7	28	4	3.57%	6.32%
input	Putate Lobula plate intrinsic	9	7	8.3	25	3	2.68%	5.64%
input	TmY3	9	4	5.8	23	4	3.57%	5.19%
input	DL1_ventral	8	7	7.7	23	3	2.68%	5.19%
input	Tm1	9	4	7.3	22	3	2.68%	4.97%
input	LPT23_R	13	3	6.3	19	3	2.68%	4.29%



**Figure 4.25:** 3D image of TmY5 (LC14b input) (green) presynaptic connections with most important input partners (magenta)

Furthermore, I generated 3D plots of TmY5 pre-synapses (Figure 4.25) involving its most important input neurons. The first most important TmY5 input partner is Li1-XL with 8,6% (56 synapses) of all input synapses (see 4.25a), the second most important TmY5 input neuron is LT1, contributing to 6,61% (43 synapses) of all input synapses (see 4.25b) and the third most important TmY5 input cell is L1-Y, generating 5,38% (35 synapses) of all input synapses (described in the sub-figure 4.25c).

The same results, featuring the same top three input partners of TmY5, are visualized in Barplot 4.21c.

Additionally, you can find the detailed statistics in the table 4.11 of the most important input neurons of TmY5 (LC14b input). This table not only displays the top three input partners but also includes information about other important input neurons along with their specific statistics.

## 4.5 Three most important LC14b output neurons

Next, we will explore the three most important output neurons of LC14b. Figure 4.26 illustrates the postsynaptic connections of LC14b with its most significant output neurons in a 3D format.

**Table 4.11:** Statistics of broad input partner types of TmY5 (third most important LC14b input cell). Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to TmY5 in %.

type	partner_type_broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
input	Li1-XL	34	22	28	56	2	0.94%	8.6%
input	Lt1	26	17	21.5	43	2	0.94%	6.61%
input	Li1-Y	35	35	35	35	1	0.47%	5.38%
input	Tm	6	1	2.4	29	12	5.66%	4.45%
input	Lpc101-34-FDB	11	5	7.3	22	3	1.42%	3.38%
input	TmY5a-FDB	8	1	4	20	5	2.36%	3.07%
input	T2-a	5	1	3.2	16	5	2.36%	2.46%
input	TmY20-FDB	6	1	3.2	16	5	2.36%	2.46%
input	Lt6	16	16	16	16	1	0.47%	2.46%
input	Y3-FDB	8	1	3	15	5	2.36%	2.3%

#### 4.5.1 Li2 - First most important output neuron of LC14b and its most important output neuron

Lobula intrinsic 2 is the first and most important output neuron of LC14b, constituting 12.72% (644 synapses) of all output synapses. As mentioned previously, Li2 is a neuron entirely situated within the Lobula brain segment of *Drosophila melanogaster*.

The postsynaptic connection between LC14b and Li2 is visualized in the 3D format in plot 4.26a.

Li2 has the three most important output partners, the first one being the Lobula Columnar 17 neuron with 13.35% (499 synapses) of all output synapses, the second one being the Lobula Columnar 11 neuron with 9.76% (365 synapses) of all output synapses, and the third one being the Lobula Tangential 1 neuron with 9.1% (340 synapses) of all output synapses. 3D plots depicting their synaptic connections with Li2 are presented in Figure 4.27, with LC17 as the first most important output neuron (see sub-figure 4.27a), LC11 as the second most important output neuron of Li2 (see sub-figure 4.27b), and LT1 as the third most important output neuron of Li2 (refer to sub-figure 4.27c). The last two sub-figures feature different Li2 neuron IDs, resulting in slight variations compared to the first sub-figure, but all represent LC14b output Li2 neurons.

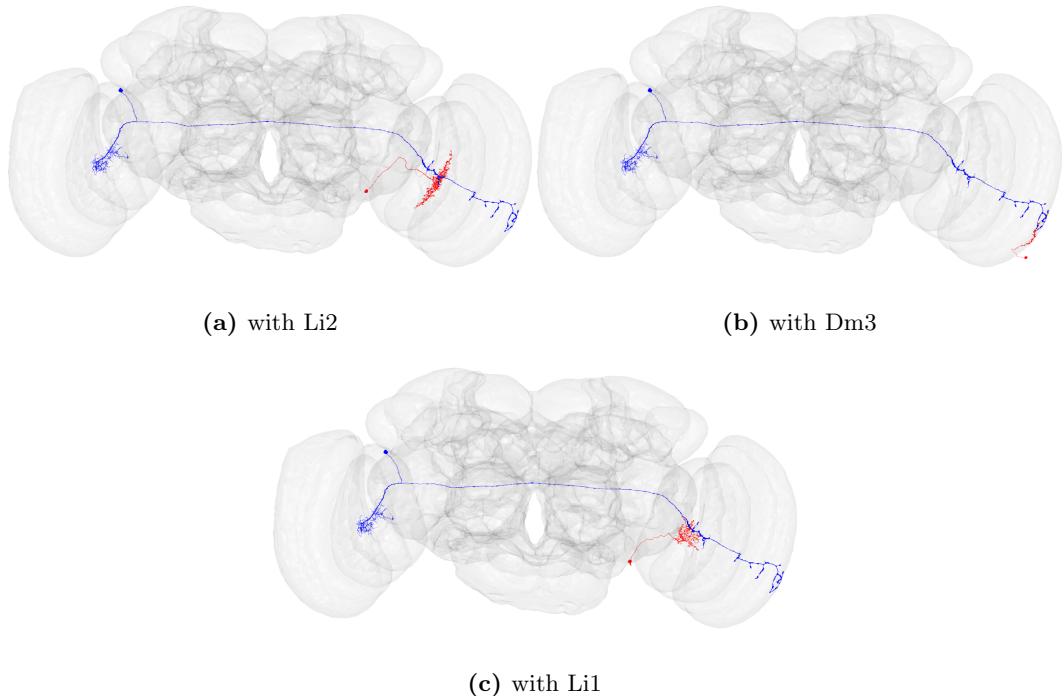
In the Barplot (refer to 4.28), we observe the same three primary output neurons, along with other prominent output partners, contributing to the highest number of output synapses for Li2.

I also obtained the statistics table (refer to 4.12) for the most important output partners of Li2 (LC14b output). In this table, we can observe the same percentages and synapse counts mentioned earlier, including those from the top three output neurons, as well as other notable output neurons of Li2.

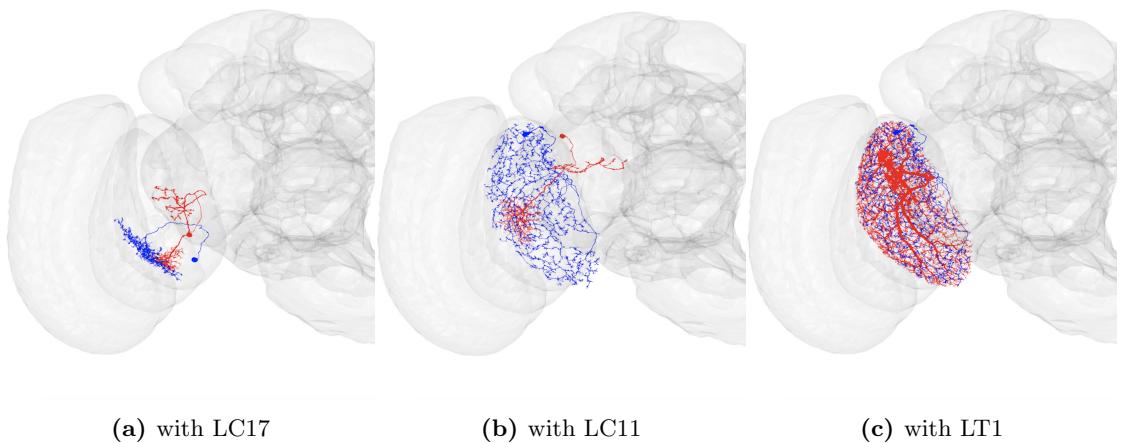
**Table 4.12:** Statistics of broad output partner types of Li2 (first most important LC14b output cell). Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to Li2 in %.

type	partner_type_broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
output	LC17	57	1	9.4	499	53	6.66%	13.35%
output	LC11	18	1	4.7	365	77	9.67%	9.76%
output	LT1	89	1	16.2	340	21	2.64%	9.1%
output	LC18	28	1	6.5	266	41	5.15%	7.12%
output	LPLC1	17	1	5.7	228	40	5.03%	6.1%
output	T2-a	12	1	4.2	173	41	5.15%	4.63%
output	LT	80	1	27.2	136	5	0.63%	3.64%
output	Tm24	12	1	4.8	110	23	2.89%	2.94%
output	LC12	13	1	6.1	104	17	2.14%	2.78%
output	LT11	90	1	34.3	103	3	0.38%	2.76%

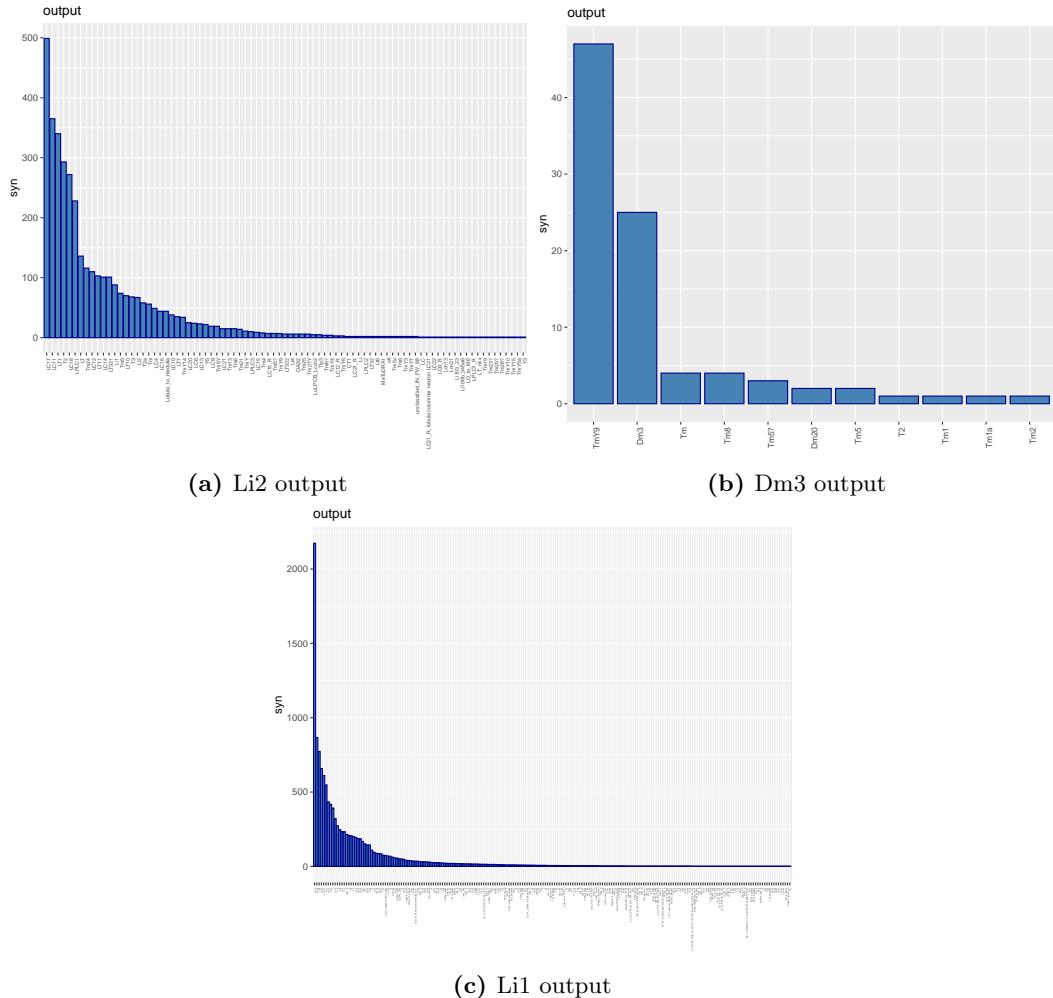
Now I'm going to discuss the most important output partners of the most important



**Figure 4.26:** 3D image of LC14b neuron (blue) building postsynaptic connections with its most important output partners (red)



**Figure 4.27:** 3D image of Li2 (LC14b output) (blue) postsynaptic connections with most important output partners (red)



**Figure 4.28:** Barplots of most important output partners and output synapse count of LC14b most important output neurons (Li2, Dm3, Li1)

output neuron of Li2 (LC14b output), which is LC17.

LC17, or Lobula Columnar 17 neuron, is categorized as a Lobula Columnar neuron. Lobula columnar (LC) cells are a class of *Drosophila* VPNs (Visual Projection Neurons) that project to distinct central brain structures called optic glomeruli [Wu et al., 2016].

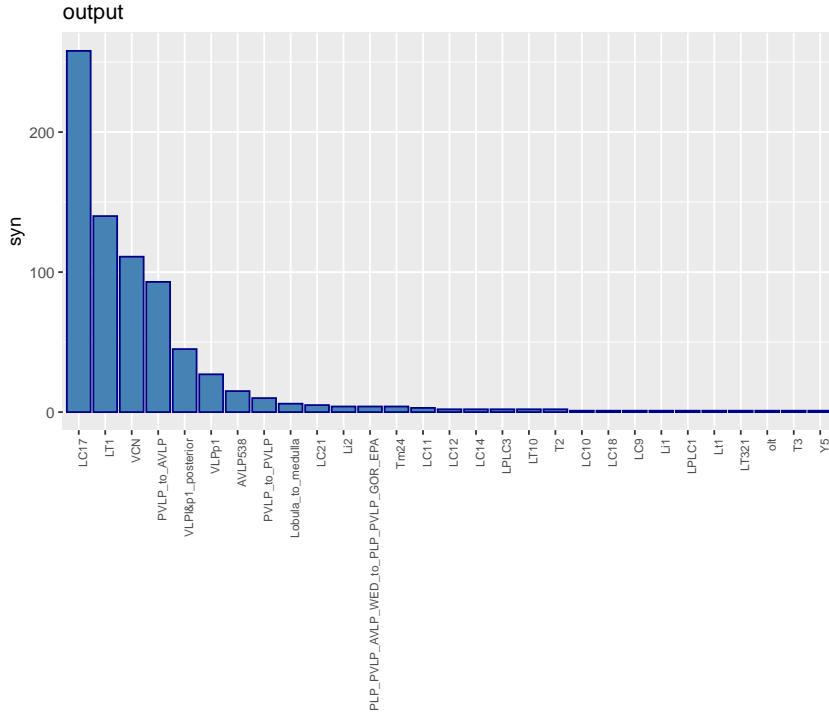
Below, you'll find the Barplot (refer to 4.29), statistics table (see table 4.13), and 3D plots illustrating the postsynaptic connections established by LC17 with its most significant output neurons (see 4.30). These key partners include LC17 itself, contributing 34.63% (258 synapses) of all output synapses, LT1 with 18.79% (140 synapses), and VCN with 14.9% (111 synapses). The image of the output synapse that LC17 builds with LC17 was taken from above, it's a top view of *Drosophila melanogaster* brain.

#### 4.5.2 Dm3 - Second most important output neuron of LC14b

Distal medullar 3 neuron is the second most important output neuron of LC14b, contributing to 9,7% (491 synapses) of all output synapses. As implied by its name, this neuron is situated in the distal region of the medulla in the fly brain.

This 3D plot (refer to Figure 4.26b) illustrates the synaptic connection between LC14b and its second most significant output partner, Dm3.

The three most important output neurons of Dm3 are TmY9 with 51,65% (47 synapses) of all output synapses, Dm3 with 27,47% (25 synapses) of all output synapses, and Trans-



**Figure 4.29:** LC17 output

medullar 8 neuron with 4,4% (4 synapses) of all output synapses. I generated 3D plots of the post-synaptic connections of these neurons to Dm3, see Figure 4.31. Sub-figure 4.31a shows the post-synaptic connection of Dm3 with TmY9 (has this characteristic look of longer, downwards going arbors), sub-figure 4.31b demonstrates the post-synaptic connection of Dm3 with Dm3, its second most important output partner and sub-figure 4.31c displays the post-synaptic connection of Dm3 with Tm8 neuron. For the final sub-figure, a different LC14b output Dm3 neuron was used, resulting in the image being from the right brain hemisphere, in contrast to the left hemisphere in the preceding sub-figures.

The Barplot (see 4.28b) displays the same results, featuring the top three Dm3 output neurons (TmY9, Dm3, Tm8), along with other prominent output partners of Dm3. On the Y-axis, you can observe the number of synapses these neurons create with Dm3.

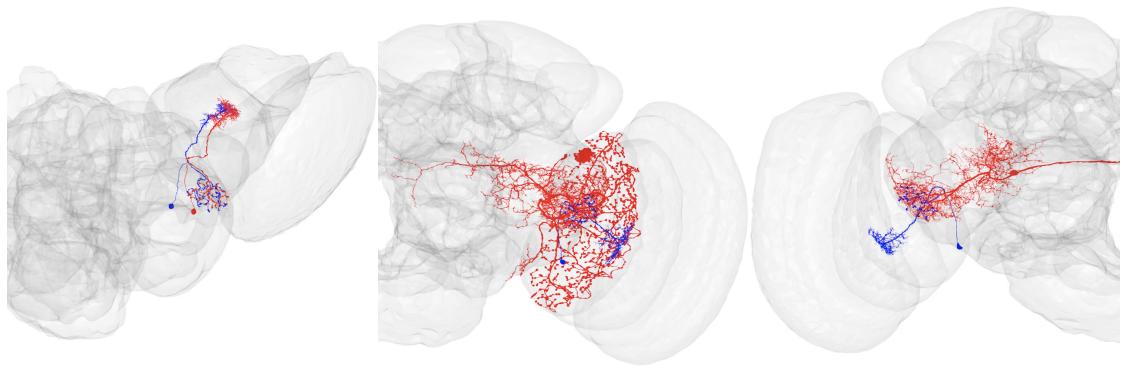
The statistics table 4.14 provides details about the most important output neurons of Dm3 (LC14b output) and their relevant statistics.

#### 4.5.3 Li1 - Third most important output neuron of LC14b

Lobula intrinsic 1 is the third most important output neuron of LC14b, building 8,71% (441 synapses) of all output synapses to LC14b.

The 3D plot in Image 4.26c demonstrates the post-synaptic connection formed by LC14b with its third most important output neuron, Li1.

I could also generate 3D plots of post-synaptic connections of Li1 with its three most important output partner neurons: Lobula Columnar 13 neuron being the first most important output neuron with 17,23% (2173 synapses) of all output synapses to Li1, Transmedullar Y5 neuron being the second most important output neuron with 6,87% (867 synapses) of all output synapses to Li1 and Lobula Columnar 6 neuron being the third most important output partner of Li1 creating 6,13% (773 synapses) of all output synapses. Figure 4.32 with its three sub-figures demonstrates these results as well, sub-figure 4.32a showing the output synapse between Li1 and LC13, sub-figure 4.32b displaying the output synapse between Li1 and TmY5 and finally, sub-figure 4.32c showing the output synapse between Li1 and its third most important output partner, LC.

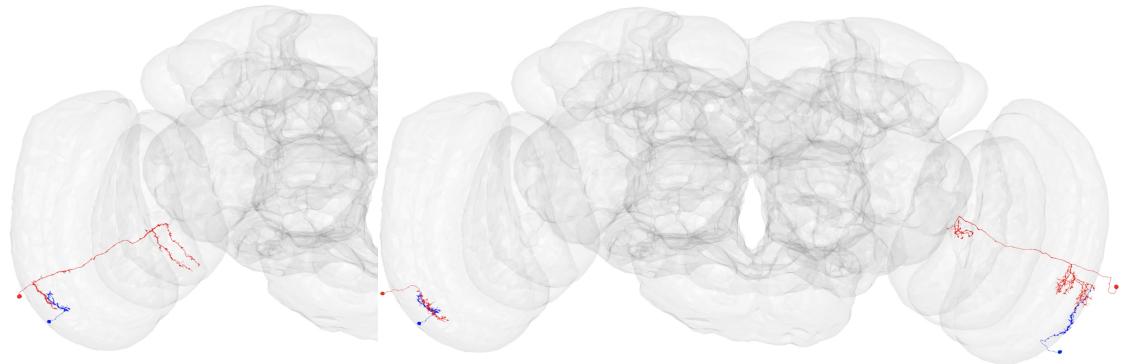


(a) with LC17 (top view)

(b) with LT1

(c) with VCN

**Figure 4.30:** 3D image of LC17 (Li2 output (LC14b output)) (blue) postsynaptic connections with most important output partners (red)

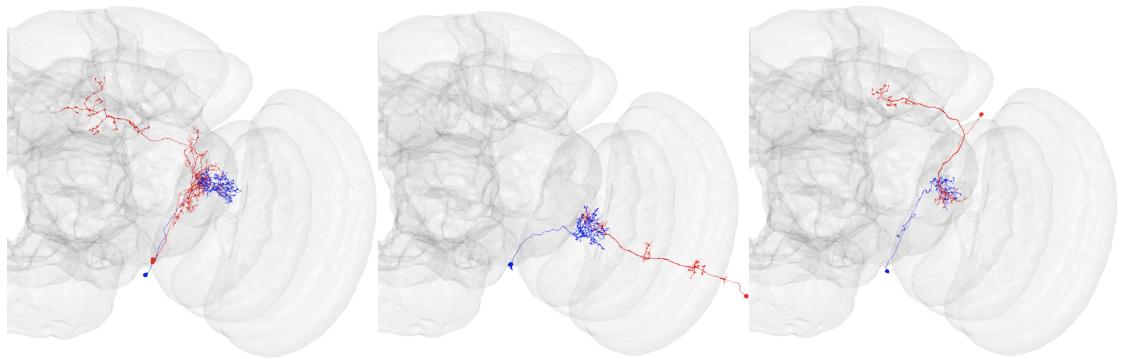


(a) with TmY9

(b) with Dm3

(c) with Tm8

**Figure 4.31:** 3D image of Dm3 (LC14b output) (blue) postsynaptic connections with most important output partners (red)



(a) with LC13

(b) with TmY5

(c) with LC

**Figure 4.32:** 3D image of Li1 (LC14b output) (blue) postsynaptic connections with most important output partners (red)

**Table 4.13:** Statistics of broad output partner types of LC17 (first most important Li2 (LC14b output) output cell). Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to LC17 in %.

type	partner_type_broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
output	LC17	8	1	1.8	258	144	67.29%	34.63%
output	LT1	38	1	14	140	10	4.67%	18.79%
output	VCN	16	1	5.6	111	20	9.35%	14.9%
output	PVLP_to_AVLP	85	1	31	93	3	1.4%	12.48%
output	VLPl&p1-posterior	23	22	22.5	45	2	0.93%	6.04%
output	VLPP1	14	13	13.5	27	2	0.93%	3.62%
output	AVLP538	15	15	15	15	1	0.47%	2.01%
output	PVLP_to_PVLP	10	10	10	10	1	0.47%	1.34%
output	Lobula_to_medulla	4	1	2	6	3	1.4%	0.81%
output	LC21	5	5	5	5	1	0.47%	0.67%

**Table 4.14:** Statistics of broad output partner types of Dm3 (second most important LC14b output cell). Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to Dm3 in %.

type	partner_type_broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
output	TmY9	15	1	3.9	47	12	29.27%	51.65%
output	Dm3	3	1	1.6	25	16	39.02%	27.47%
output	Tm	2	1	1.3	4	3	7.32%	4.4%
output	Tm8	4	4	4	4	1	2.44%	4.4%
output	Tm5?	2	1	1.5	3	2	4.88%	3.3%
output	Dm20	1	1	1	2	2	4.88%	2.2%
output	Tm5	2	2	2	2	1	2.44%	2.2%
output	T2	1	1	1	1	1	2.44%	1.1%
output	Tm1	1	1	1	1	1	2.44%	1.1%
output	Tm1a	1	1	1	1	1	2.44%	1.1%

In the Barplot 4.28c, these outcomes are also depicted, showcasing the top three output partners: LC13, TmY5, and LC6, which construct the highest number of output synapses to Li1 compared to other output neurons.

Additionally, I obtained the statistics table 4.15 for Li1 output neurons. This table not only presents the top three output neurons of Li1 and their synapse percentages but also includes information on the maximum, minimum, and average synapse counts that they generate. Furthermore, it provides statistics for other significant Li1 output partners.

**Table 4.15:** Statistics of broad output partner types of Li1 (third most important LC14b output cell). Listed are the maximum and minimum number of synapses from a cell type, the average number of synapses, the total number of synapses, the number of cells (n), the number of identified cells in %, and the number of identified synapses connected to Li1 in %.

type	partner_type_broad	Maxsyn	Minsyn	averagesyn	syn	n	percentage_ided	percentage_ided_syn
output	LC13	51	1	19.8	2173	110	6.28%	17.23%
output	TmY5	73	1	27.1	867	32	1.83%	6.87%
output	LC6	22	1	7	773	111	6.34%	6.13%
output	LC21	31	2	15	658	44	2.51%	5.22%
output	TmY17	88	20	55.5	611	11	0.63%	4.84%
output	TmY	71	1	22.8	548	24	1.37%	4.34%
output	TmY7	69	1	24.1	434	18	1.03%	3.44%
output	LT32	401	1	59.7	418	7	0.4%	3.31%
output	Li	375	1	65.3	392	6	0.34%	3.11%
output	LC10	17	1	2.9	321	110	6.28%	2.54%

# Discussion

In this research, we investigated and identified the most important input and output partner neurons of LC14a and b cells (Dorsal Cluster Neurons) in *Drosophila melanogaster*.

The visual system of the adult *Drosophila* makes up to 150,000 neurons and glial cells of its whole brain [Nériec and Desplan, 2016]. The optic lobe being the main focus of the visual system, where the most processing and computational work is done, contains more than 60% of the fly brain's neurons. The Lobula of the optic lobe is one of the highest-order visual neuropils and main motion centers and is responsible for the optomotor responses of the fly, visual fixation, and tracking behavior.

Recent studies have revealed that Lobula Columnar (LC) and Lobula Plate-Lobula Columnar (LPLC) neurons, a group of columnar output neurons of the Lobula, are tuned to visual features resembling conspecifics and predators. Their activity can provoke a variety of behavioral responses. These neurons attain their specific visual tuning by combining the feature selectivity of their presynaptic partners. Nevertheless, due to the lack of connectomic reconstruction covering the Lobula and its upstream neuropils, the presynaptic partners of these LC and LPLC neurons remain largely unknown. The tracing and proofreading of the FAFB (full adult fly brain) dataset is still in progress. This dataset contains almost the entire Lobula but it lacks nearly the entire Medulla, a region containing neurons providing inputs into the Lobula, which are as a result mostly unlabeled [Tanaka () and Clark, 2022].

This brings us back to our previous open question and problem, that it remains mostly unknown how the input and output neurons of the Lobula are connected.

Although LC14a and b cells are not considered the typical Lobula Columnar cells (that connect the Lobula to the central brain), because they connect the Lobulas (and Medullas (LC14b)) of the opposite optic lobes in the fly brain, they are still very significant for the visual system, as they are required for object orientation behavior in flies. Examining the most important input and output partners of these neurons, as well as their input's input and output's output partners, contributes significantly to our comprehension of their roles and functions in the visual circuitry of the *Drosophila melanogaster*.

The DCNs (LC14a and b cells) exhibit non-heritable variation in wiring in the fly brain [Linneweber et al., 2020]. Variability in neuron wiring affects individual behavioral responses. Understanding the specific input and output stimuli from partner neurons that influence the functionality of the DCNs could provide insights into the origin of visual individuality.

Using the FAFB connectome dataset, we could find out the most important input and output neurons (based on the number of synapses they build with DCNs) of LC14a and b cells. For LC14a, the most important input partner was the Lobula Columnar 9 neuron. These LCs are easily distinguishable by their distinct half-loop shape and the fact that they connect the central brain to the Lobula. LC9 neurons, like other Lobula Columnar neurons, belong to Visual Projection Neurons in *Drosophila*. One of its known functions is that the arousal state in male flies is integrated with visual information from LC9 visual neurons in DNp09 descending neurons to permit a locomotor walking program, ipsilateral turning, used in pursuit [Oram and Card, 2022]. LC9 neurons are also generally involved in motion detection in flies.

The most important input partner of LC9, based on the number of synapses it forms, is PVLP\_to\_PLP (or VLPD&p1\_dorsal). This neuron, identified by our lab members, still holds uncertainties regarding its function and role.

Moreover, PVLP\_to\_PLP neuron has three most important input neurons, the first one being LC9 (which we already discussed), the second one being AVLP\_PVLP\_SLP\_SCL\_SIP\_ICL\_to\_SMP, was identified by myself based on the brain segments its arbors traversed and the third one being LO\_to\_AVLP\_PVLP\_PLP, again another neuron identified by me. Their functionalities also remain unclear to us.

The second most important input partner of the LC9 neuron is the LC9 neuron, indicating a self-connective neuron. We have already discussed their functions in the optic lobe and visual circuitry.

The third most important input partner neuron of the LC9 neuron is PVLP\_to\_AVLP, identified by our lab members. Given the nomenclature, it suggests the dendrites' location in PVLP (posterior ventral protocerebrum) and the axon's location in AVLP (anterior ventrolateral protocerebrum). However, its exact functionality remains uncertain.

The second most important input neuron of LC14a was the Li1 (Lobula intrinsic 1) neuron. Li1 neurons play an important role in the color vision pathways of the fly brain. These neurons are located inside the Lobula.

Li1 has also three most important input partners, the first one being the Lobula Tangential neurons. LT neurons are generally involved in the motion pathways of the fly brain. For example, LT10 neurons are responsible for processing second-order motion, and LT32 neurons are accountable for detecting behaviorally relevant visual features.

The second most important input partners of Li1 are Tm (Transmedullar) neurons. They are comparably longer neurons that go through the Medulla. Tm neurons are also generally involved in color vision pathways, but some of them are also involved in motion detection circuits, for example, Tm4.

The third most important input neuron of Li1 is the Tm19 neuron. The Tm19 neurons are easily recognizable because of their shape; compared to other Tm neurons, they have two longer downwards-going arbors.

Lobula\_to\_medulla is the third most important input neuron of LC14a. It is consistently located between the Lobula and the Medulla. This is also one of those neurons labeled by our lab, so we are not sure about its exact functionality.

The first most important output neuron of the LC14a cell is also Lobula\_to\_medulla. The Lobula\_to\_medulla also has the three most important output neurons, the first one being Li2. Lobula intrinsic 2, just like Li1, also participates in color vision pathways.

The second most important Lobula\_to\_medulla output neuron is T2. They connect the proximal Medulla exclusively with the Lobula. As mentioned earlier in the Results section, their fibers travel along the inner face of the Medulla to their respective columns where they bifurcate in a T-like fashion (hence the name T-cells), sending one branch distally into the Medulla and the other into the isotopic column of the Lobula [Fischbach and Dittrich, 1989]. T2 cells are involved in the motion perception pathways.

The third most important Lobula\_to\_medulla output neuron is Lobula\_to\_medulla, indicating that it is a self-connective neuron.

The second most important LC14a output neuron is Li2. We talked about Li2 and its functions earlier.

The Li2 neuron also has the three most important output neurons, the first one being the LC17 neuron. The Lobula Columnar 17 neuron is involved in motion control and aggression regulation pathways. LC17 exhibits minimal responses to object motion stimuli [Klapoetke et al., 2022]. The second most important output neuron of Li2 is LC18. The Lobula Columnar 18 neuron is involved in motion detection pathways. This neuron responds strongly to moving objects but only weakly to looming object motion. LC18 detects the motion of very small objects by comparing contrast changes in time [Klapoetke et al., 2022].

The third most important output neuron of Li2 is T2a cells, a sub-type of T2 cells, implying similar functionalities. They are also involved in motion perception pathways. T2 cells in general respond strongly to a small moving object.

The third most important output neuron of LC14a is Li1. We have already discussed this neuron and its functions.

The three most important output neurons of Li1 are LT1, LC11, and LPLC3 neurons. The Lobula Tangential 1 neuron is involved in the detection of behaviorally relevant visual features. The Lobula Columnar 11 neuron participates in visual perception of the fly, it responds to the small moving objects. The Lobula Plate Lobula Columnar 3 neuron is most likely involved in motion control and danger detection pathways.

The first and most important input partner of LC14b cell is T2a, and we have discussed T2a earlier.

The three most important input partners of T2a neurons are Mi1, Tm1, and Pm1. The Medulla intrinsic 1 neuron is involved in motion and brightness detection pathways. Trans-medullar 1 neuron participates in motion detection pathways. Tm1, together with Tm2, responds selectively to brightness decrements, with the response of Tm1 delayed compared to Tm2. They both play analogous roles in the detection of moving dark edges [Behnia et al., 2014]. The Proximal medullar 1 neuron is involved in the color vision pathways of *Drosophila melanogaster*.

The three most important input partners of Mi1 neuron (T2a input) are Proximal medullar 2, Proximal medullar 1, and T2 neurons. The Medulla, composed of 40,000 cells, is the largest compartment in the optic lobe and is responsible for processing both motion and color information [Erclik et al., 2017]. Therefore, we assume that Pm2 is involved in the motion detection pathway and Pm1, as mentioned above, in the color vision pathway [Morante and Desplan, 2008]. T2 neurons, as mentioned before, are accountable for motion perception pathways.

The second most important input neuron of the LC14b neuron is the Y3 neuron. Y3 neurons participate in directional selectivity pathways.

The first and most important input neuron of the Y3 neuron is the LT1 neuron. We have already talked about the LT1 neuron and its role in the visual system. The second most important input partner of the Y3 is the TmY5a neuron. The TmY5a neuron is involved in color vision circuit pathways. The third most important input neuron of the Y3 neuron is the Li1 neuron, the functions of which have been thoroughly explored in prior discussions

Now we are moving on to the third most important input neuron of LC14b, which is the TmY5 neuron. Its function in the visual system of the fly brain is mentioned above.

The three most important input partners of TmY5 are Li1-XL, LT1, and Li1-Y. Li1-XL and Li1-Y are all Lobula intrinsic neurons of different sizes. We have already discussed the Lobula Tangential 1 neuron earlier.

Let's move on to the three most important output neurons of LC14b. The first one is the Li2 neuron. The three most important output neurons of Li2 are LC17, LC11, and LT1, all these neurons have been thoroughly discussed in earlier sections.

The three most important output partners of the LC17 neuron (Li2 output) are LC17 itself, LT1, and VCN neurons. The LC17 and The LT1 were already discussed previously, while VCN, standing for visual centrifugal neuron, remains a neuron with functions yet to be fully investigated.

The second most important output neuron of LC14b is the Distal medullar 3 neuron. Dm3 is involved in the color vision pathways.

The three most important output partners of Dm3 are TmY9, Dm3 itself, and Tm8 neurons. The TmY9 neuron plays an important role in motion computation pathways. Dm3's functions were previously mentioned, and the Tm8 neuron is most likely involved in color vision pathways according to existing literature [Morante and Desplan, 2008].

The third most important output partner neuron of the LC14b is the Li1 neuron. Its three most important output partners include the Lobula Columnar 13 neuron, the TmY5 neuron, and the Lobula Columnar 6 neuron. The LC13 neuron is likely involved in motion control pathways, it is tuned to distinct features of object motion [Klapoetke et al., 2022]. The TmY5 was already mentioned and the LC6 neuron, like most other LC neurons, contributes to motion control pathways.

As we can observe, most input and output neurons that are connected either directly or indirectly to the LC14a and b cells, are crucial for the visual system and the majority of them play an important role in various pathways, including different types of motion, object detection, behavioral or color vision pathways.

Once again, this confirms the pivotal role of DCNs in the visual circuitry. They establish indirect connections among nearly all neurons engaged in the visual system of the *Drosophila melanogaster*, serving to unite and integrate them.

There is still much more to analyze and discover on this topic. We can always extend this research to create a more detailed connectivity map of *Drosophila* brain neurons, specifically those related to visual circuitry.

I intend to explore this topic more thoroughly and conduct independent research in addition to my Bachelor's Thesis. I am actively involved in enhancing the connectivity map of *Drosophila melanogaster* brain neurons, continually updating the Codex and Flywire databases. My goal is to contribute regularly to these resources and actively participate in advancing the knowledge within this scientific community.

# Acknowledgements

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