MORPHOLOGICAL ANALYSIS OF THE LOBULA PLATE TANGENTIAL CELLS VS1-6 AND H2 OF *DROSOPHILA MELANOGASTER*

BACHELOR'S-THESIS

BIOLOGY

MATTHIAS MEIER

Contents

Abstract/Zusammenfassung……..…..3

Introduction…….4

Methods……9
Flies……9
Preparation……10
Microscopy and intracellular dye filling……12
Confocal Microscopy……..……....14
Reconstruction…….....14

Results……...16
Vertically Sensitive Neurons…….....16
H2 Neurons……....19

Discussion…….....23
VS1 Results……..23
H2 Results……23

Acknowledgements 25

References 26

Zusammenfassung

Das visuelle System eignet sich aufgrund seiner eindeutigen und präzisen Antworteigenschaften, sowie der objektiven und reproduzierbaren Stimulierungsmöglichkeiten, hervorragend dazu, die Eigenschaften neuronaler Netzwerke zu erforschen. Mit dem Modellorganismus steht *Drosophila melanogaster* und den damit verbundenen genetischen Möglichkeiten ein ideales Werkzeug zur Verfügung, dieses System zu untersuchen. Mit Hilfe computergenerierter Modelle können die Antworteigenschaften einzelner Nervenzellen sowie ganzer Netzwerke simuliert werden. Zur Erstellung solcher Modelle benötigt man neben der elektrophysiologischen Charakterisierung der Antworteigenschaften der einzelnen Zellen, zunächst genauere Informationen über die morphologische Beschaffenheit dieser Neuronen. In der folgenden Arbeit wurden Zellen aus der Lobula Platte, einem Bereich in den optischen Loben der Fruchtfliegen, welcher als Schaltzentrum der visuellen Wahrnehmung, sowie der daraus resultierenden motorischen Reaktionen bekannt ist (Borst & Haag, 2002), untersucht. Für die Analysen wurden sowohl H2 Zellen (durch horizontale Bewegungen stimulierbare Neuronen) als auch VS Zellen (durch vertikale Bewegungsmuster erregbare Neuronen) betrachtet. Die Zellen wurden mit einem fluoreszierenden Farbstoff gefüllt und somit unter dem Fluoreszenzmikroskop sichtbar gemacht. Anschließend wurden diese mit einem konfokalen Mikroskop gescannt und schließlich am Computer rekonstruiert. Die so erstellten digitalen Abbildungen der Zellen konnten nun statistischen Tests unterzogen werden, wobei die Morphologie der axonalen Verzweigungen mit dem Verzweigungsmuster des Dendritenbaumes verglichen wurde. Hierbei wurde ein Hinweis auf die morphologischen Voraussetzungen für die speziellen Antworteigenschaften von VS1 Zellen gefunden. Des Weiteren wurde eine Überlappung von H2 axonalen Strukturen mit HS (horizontal sensitive Zellen) Dendriten festgestellt und rekonstruiert. Diese Überlappung deutet auf eine kontralateralen Input von HS Zellen über H2 (Schnell et al. 2010) hin.

Introduction

Insects have been dominating earth for millions of years as measured by number and diversity. Directly after metamorphosis into the imago, flies show enormous abilities in visual reaction and flight control. These inborn skills, based on neural networks are propably one reason for their evolutionary success. Learning how these abilities are processed neurally is a fundamental step towards understanding the insects' brain and behavior. On the way to a better comprehension of the brain, different parts have to be analyzed. One field of special interest is motion detection, which is one field of the neural computation of visual input in the optic lobes of flies. Compared to other sensory systems, the inputs to the visual system are very objective and precise.

One behaviorally relevant readout of motion detection is the bahvioral response to moving visual stimuli, the optomotor response. Optomotor responses represent the animal's tendency to follow the movement of the visual surround to compensate for its mistaken perception of self-motion in the opposite direction. The neural computation of visual stimuli leading to such behavior have been shown to take place in the optic lobes of the insect brain (review in Borst & Haag, 2002), an area that covers the biggest part of the dipteran brain. In *Drosophila*, precise information has been gathered from single units of the optic lobe using electrophysiological and imaging techniques (Joesch et al., 2008; Schnell et al., 2010; Reiff et al. unpublished). However, for a complete functional understanding of these behaviorally relevant circuits it is necessary to gain additional information about structure and anatomy of the neural units involved, preferentially in computational models of cell structures. Basic anatomical analyses have been performed on neural circuits of the visual system of flies like *Calliphora*, *Musca* and *Drosophila* (Scott & Raabe, 2002; Rajashekhar & Shamprasad, 2004). Nevertheless, only few computational models, which would allow a precise characterization of the networks, are available. In any case, the studies mentioned above have clearly shown close morphological similarities between dipteran insects (Scott & Raabe, 2002). These morphological and structural analogies of the neural networks provide evidence for evolutionary conserved circuits, which points out the optimized function of the flies' enormous visual skills, in particular in motion detection.

A basic mechanism of how motion detection is realized was proposed by Hassenstein and Reichardt in 1956. Their model called “correlation-type motion detector” predicts the motion-sensitive response via the interaction of two neighboring photoreceptor cells. There are two basic operations: asymmetrical temporal filtering and a nonlinear interaction stage where the low-pass filtered signal from one image location is multiplied with the high-pass filtered signal from the neighboring image location (review in Borst & Haag, 2002). However, to date no cellular model is known.

The eyes or rather the retina of an insect is composed of many facets, called ommatidia. In *Drosophila,* the retina consists of approximately 800 ommatidia (Chapmann 1998). Each ommatidium is made up of 8 photoreceptor cells (R1-R8). The inner group of photoreceptor cells (R7/R8) is thought to be primarily involved in color vision (Hardie 1984), whereas the outer group (R1-R6) has been shown to play an important role in motion detection. R1-R6 ramify into the lamina, the second neuropil of the visual system (Fig. 1) (Meinertzhagen & O'Neil). The lamina consists of five different lamina monopolar cells, two centrifugal cells and one T1 cell. The next optic neuropil, the medulla (Fig. 1) is constituted by several intrinsic cells (Mi) which ramify only within the medulla, transmedulla interneurons (Tm) connecting distinct layers of the medulla to the lobula and TmY cells, which have an additional ramification within the lobula plate. The lobula and the lobula plate (Fig. 1) form the lobula complex. In the lobula plate, the prominent group of giant cells called lobula plate tangential cells is found (review in Borst & Haag, 2002). These neurons are characterized as directionally selective to visual motion and are considered to be involved in course control (Borst & Haag, 2002). Interestingly, their motion responses can be modeled by motion detectors of the correlation type. Further processing of this motion input through the complex network of lobula plate tangential cells is thought to be the center for course control (review in Borst & Haag, 2002)

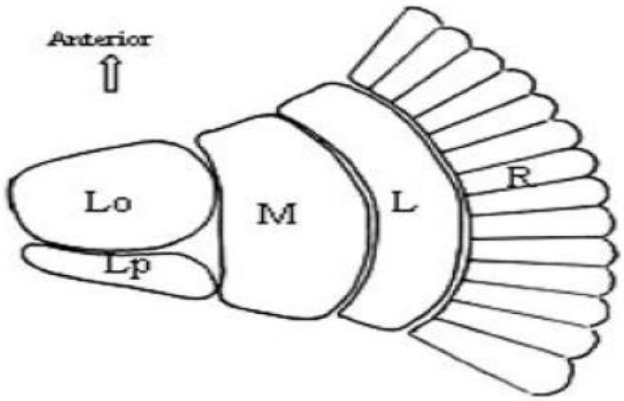

Fig. 1 Semi-schematic representation of the optic neuropils of *D. melanogaster* as seen in a horizontal section. The cellular cortex surrounding the neuropil is not shown. R, retina, consisting of ommatidia; L, lamina; M, medulla; Lo, lobula; Lp, lobula plate. (taken from Rajashekhar and Shamprasad, 2004)

Motion in the preferred direction (PD) of lobula plate tangential cells causes activation, i.e. depolarization of the membrane potential or an increase in firing rate, whereas movement in their anti-preferred direction (null-direction, ND) causes a hyperpolarization of the neurons. Lobula plate tangential neurons can be grouped in four different ways: (1) according to their preferred orientation, (2) their prevalent electrical response mode, (3) their projection area and (4) their spatial integration area (Borst & Haag, 2002). Below, tangential cells will be classified according to their preferred orientation. Lobula plate tangential cells in *Drosophila* can be subdivided in two classes: Horizontally sensitive cells and vertically sensitive cells. The most prominent cells within the horizontally sensitive cells are the HS cells. With 3 neurons, the HS cells *D. melanogaster* has same amount of cells as their bigger relatives (Heisenberg et al., 1978). According to their orientation in the lobula plate the HS cells are named HSN (northern), HSE (equatorial) and HSS (southern). Within the vertically sensitive neurons, the VS cells are most prominent, named – in *Drosophila* – with VS1 to VS6 from the most distally branching cell to the neuron that ramifies the most proximally (Heisenberg et al., 1978). In contrast to 9-11 neurons of the vertical system in bigger flies (Fischbach & Dittrich, 1989), the visual system of *Drosophila* contains only 6 neurons (Heisenberg et al., 1978; Scott & Raabe, 2002). From an evolutionary perspective, this numeric in VS tangential cells of the lobula plate is an interesting detail. It might allow multiple species to adapt to different niches by specializing their

flight control networks. The anatomical variability seems to be higher the lobula complex compared to the high structural conservation of the networks in the lamina and the medulla of the insects' brain. Apparently, evolutionary maximization of function is easier to accomplish in the lobula plate than in highly optimized structures, such as the lamina and the medulla. However, the exact computations performed within the lobula plate tangential cell network are not completely understood. In order to better understand this network, multi-compartment models of these circuits based on precise anatomical studies are required.

Due to sophisticated genetic tools, *Drosophila melanogaster* is an ideal organism for structural and electrophysiological analysis. Newly developed transgenic tool-sets can be used to modify and silence specific neuronal populations, or, as done in this study, visualize them by expressing fluorescent proteins. To further analyze the network properties of the visual system of *Drosophila* a Gal4/UAS system (Fig. 2) was used to genetically mark specific cell types in the optic lobes of fruit flies. Gal4 is a yeast transcription activator protein under the control of a driver gene, which can be specifically expressed in particular cell types, e.g. in VS or in HS cells.

Fig.2 Schematic representation of the Gal4/UAS System expressed in transgenic *Drosophila melanogaster*. (Luo et al. 2008)

When Gal4 is expressed it binds to a transgenically inserted upstream activation sequence (UAS), which in turn regulates the expression of any gene downstream of it, e.g. a fluorescent marker protein. In this project, cells were visualized using genetically encoded green fluorescent protein (GFP) (Luo et al., 2008). Thus, Gal4 determines in which population of cells the UAS and with it the marker protein is expressed. As genetically modified flies mostly express the marker protein not only in one single cell,

but in groups of neurons, the specificity of the marker expression in these neurons is not sufficient for making an exact conclusion about their morphology and interactions with other neurons. Hence single cells can hardly be imaged and reconstructed using only genetical methods. Furthermore, overlapping of dendritic and axonal branches of two neurons can be very infrequently resolved by genetically visualized cells. Therefore, single neurons will be filled with a synthetic dye using sharp electrodes. This method has the advantage of being directed, while other genetic methods for single cell visualization are random and less predictable. For instance, using the MARCM method (Scott & Raabe, 2002), hundreds of brains had to be dissected to get one single cell clone. Thus, filling distinct cells manually requires less time and is at the same time more precise than creating fluorescent single cell clones in a genetic way. Single dye filled cells were scanned individually using a confocal microscope, a special fluorescent technique of microscopy with a high resolution on the z-axis. This data was used for multi-compartmental reconstruction of tangential cells. Finally, this work will lead to realistic models of the tangential cell network and therefore, to a better understanding of the network used for visual navigation and course control in flies.

Methods

Flies:

Flies were raised on a standard cornmeal-agar medium with a 12 hr light/12 hr dark cycle, 25°C, and 60% humidity. We used female experimental flies, one day after eclosion. The line NP 0282 (established by the NP consortium; for screening see Otsuna and Ito 2006) expresses Gal4 in two of the three HS cells (HSN and HSE, Fig. 3/5) and in unidentified neurons of the central brain. To visualize these cells, mCD8-GFP was expressed via the Gal4/UAS - system. Furthermore, we used the line Gal4-DB331/+; UAS-mCD8-GFP/+; +/+, where GFP is expressed both in VS and HS cells (Fig 4/6), to fill vertical sensitive neurons.

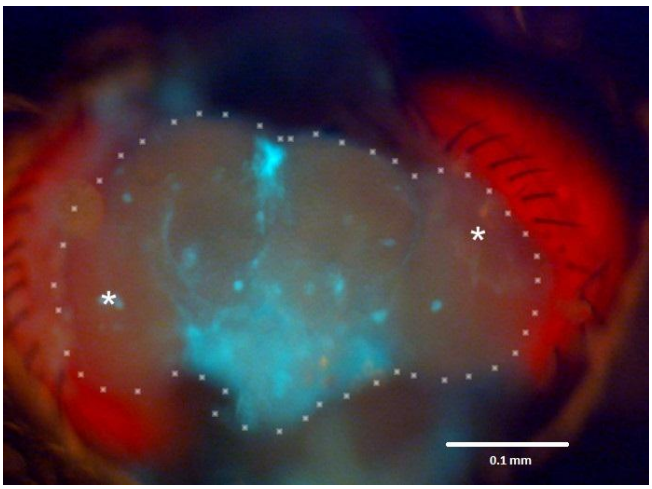

Fig 3: *Dorsal view of the brain of Drosophila melanogaster line NP 0282, boardered by white dots; *: optic lobe*

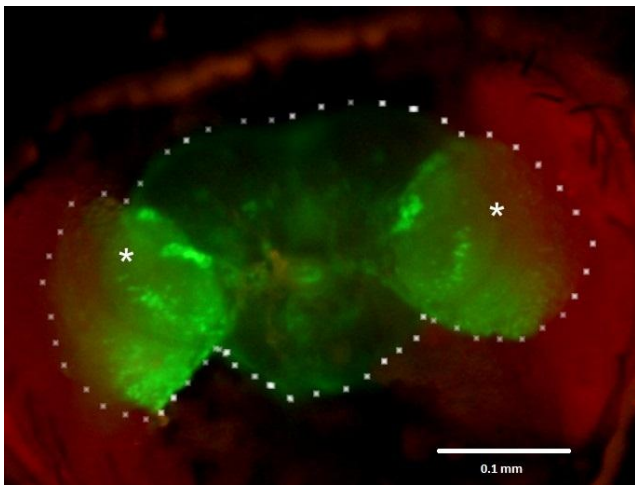

Fig 4: *Dorsal view of the brain of D. melanogaster line DB331-GFP, surrounded by white dots; *: optic lobe*

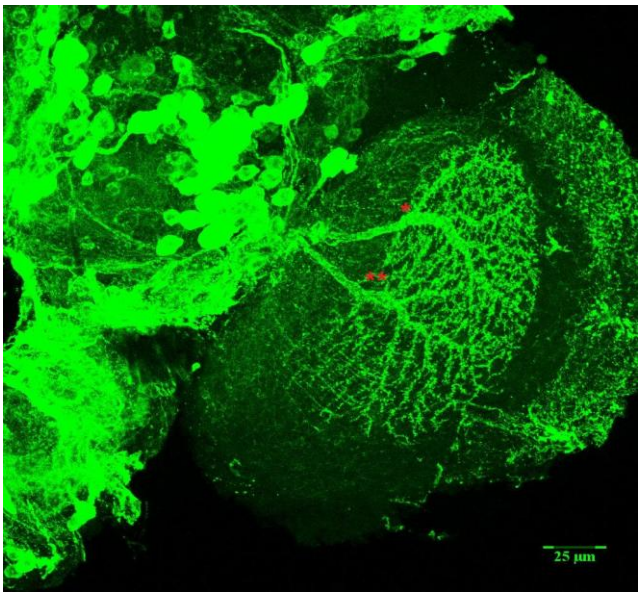

Fig. 5: *Right hemisphere of Drosophila brain (line NP 0282):*: HSN; **: HSE*

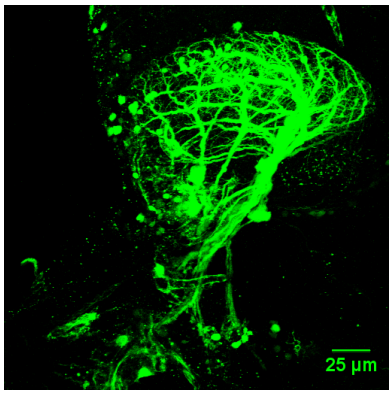

Fig. 6A: *Overview of GFP expression cell types in DB331 flies*

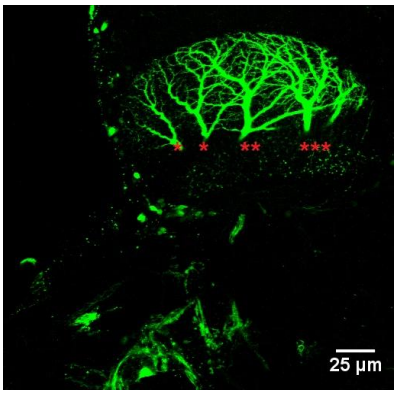

Fig. 6B: *Expression of HS cells in DB331 flies; *: HSS, **: HSE, ***: HSN*

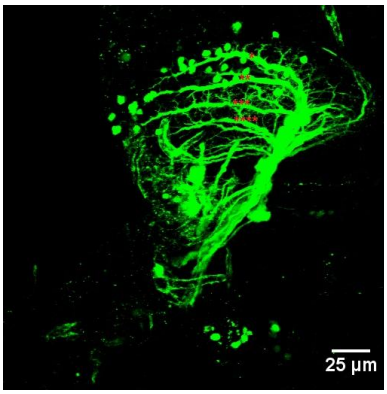

Fig. 6C: *Expression of VS cells in DB331 flies; *: VS1, **: VS2, ***: VS3*

Preparation:

One day old female flies were decapitated (Fig 7A+B). The fly heads were fixed in a layer of two-component glue (UHU Plus; UHU, Baden, Germany), with the compound eyes facing downward into the glue (Fig. 7C+D). After hardening of the glue (approx. 4 min) the specimen were covered with ringer-solution. The saline composition was (in mM): NaCl 103, KCl 3, TES 5, trehalose 10, glucose 10, sucrose 7, NaHCO3 26, NaH2PO4 1, MgCl2 (adjusted to pH 7.2-7.4). To reduce neuronal activity and apoptotic damage, no Calcium was included in the ringer-solution. The cuticle at the back side of the fly's head was opened with sharp needles (Fig. 7E+F) (Neolus, Gx3/4 in. 0.4 x 20 mm). Finally, fat bodies and the main tracheal branches were removed to get direct access to the brain (Fig. 7G-H).

Fig. 7A: *Decapitated fly (lateral view)*

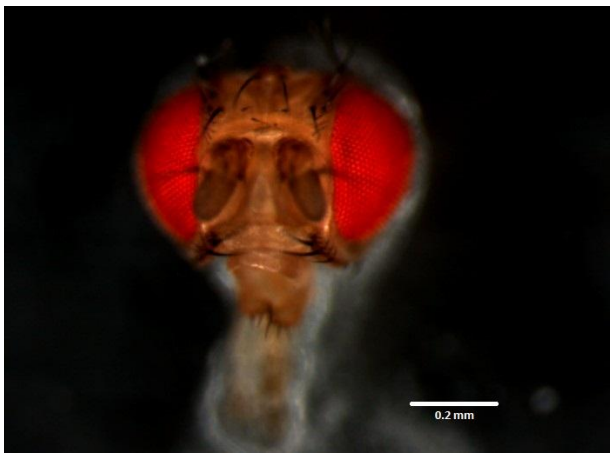

Fig. 7B: *Decapitated fly (frontal view)*

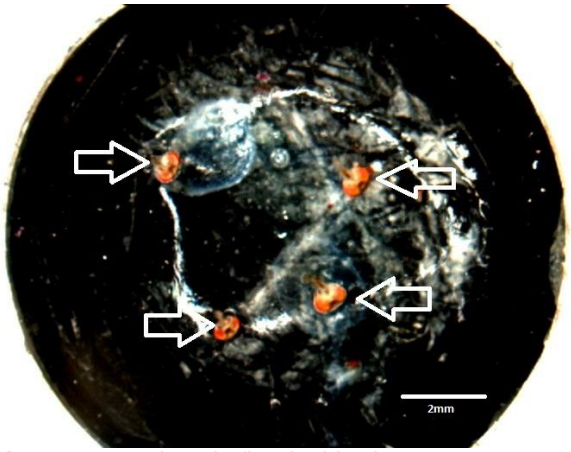

Fig. 7C: *Four heads fixed with glue to a custom made specimen stage; heads marked with arrows*

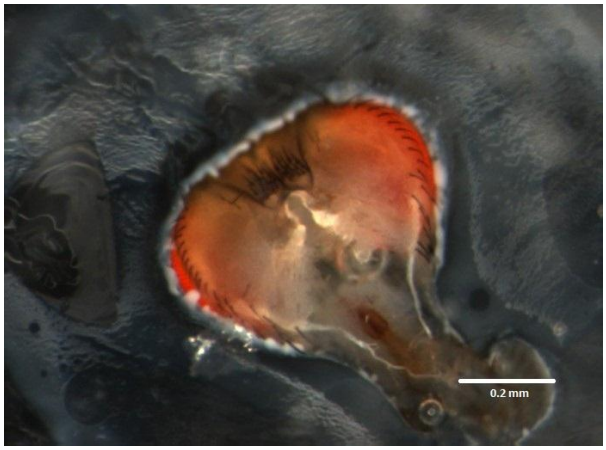

Fig. 7D: *Single head in glue*

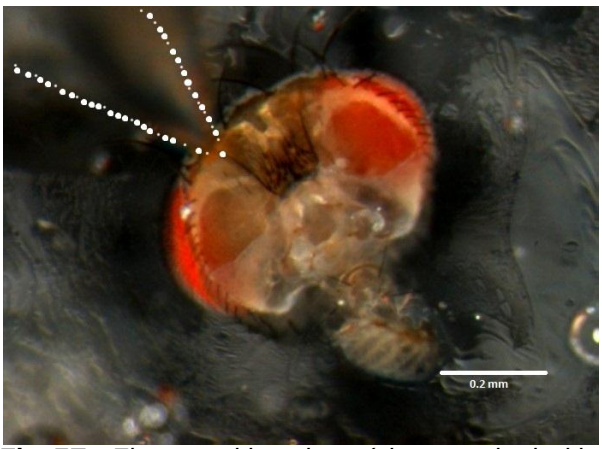

Fig. 7E: *First cut with a sharp (sharp marked with white dots)*

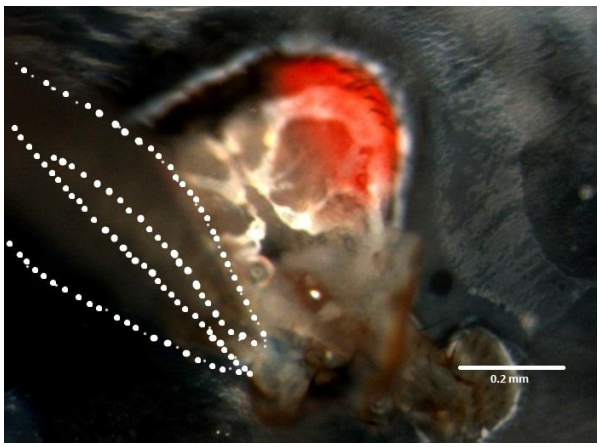

Fig.7F: *Removal of the cuticle with tweezers (marked with white dots)*

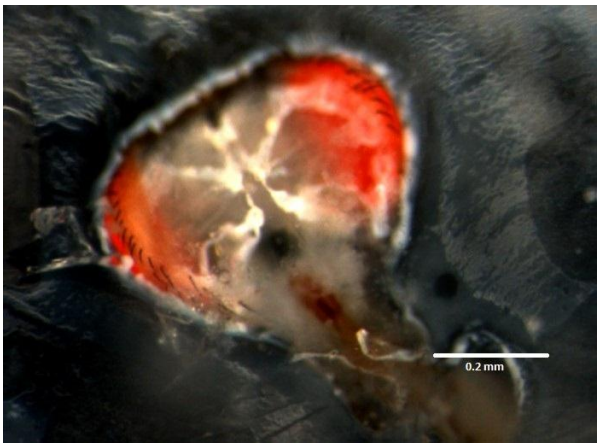

Fig. 7G: *Brain with main tracheal branches*

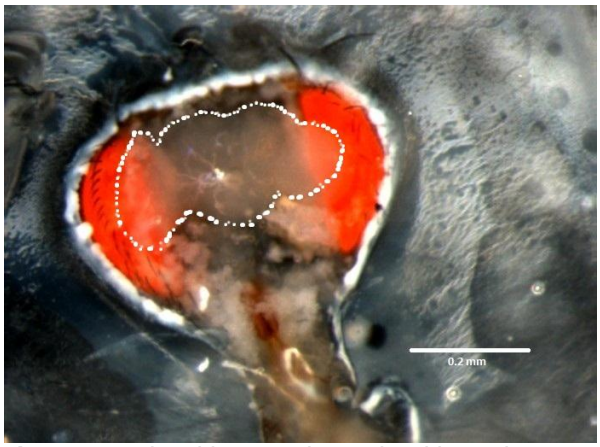

Fig. 7H: *Brain without main tracheal branches; removed with tweezers*

Microscopy and intracellular dye filling:

Dye fillings were performed using quartz electrodes (QF 100-70-10; Sutter Instrument) pulled with a laser puller (P-2000; Sutter Instrument). Electrodes were filled with 10mM Alexa Fluor 594 in 2M KCl solution (A10442; Invitrogen, Karlsruhe, Germany) and backfilled with 2 M KAc/0.5 M KCl solution.

Under a fluorescence microscope (Axiotec vario 100HD, Zeiss Jena, GER; fluorescence filterset EGFP/dsRed 1xF51-019, AHF Tuebingen, GER) with 40x magnification (Objective: Achroplan 40x/0,80 W; ∞/0; Zeiss Jena; GER), the electrode and the cell of interest were manually adjusted to the same focal level. Electrodes were positioned via a manipulator (MX-4, Narishige Micromanipulator Tokyo, JPN). Impaled cells were loaded by negative current pulses for a few seconds. The currents were generated by an amplifier (sec 10L, npi Tamm, GER).

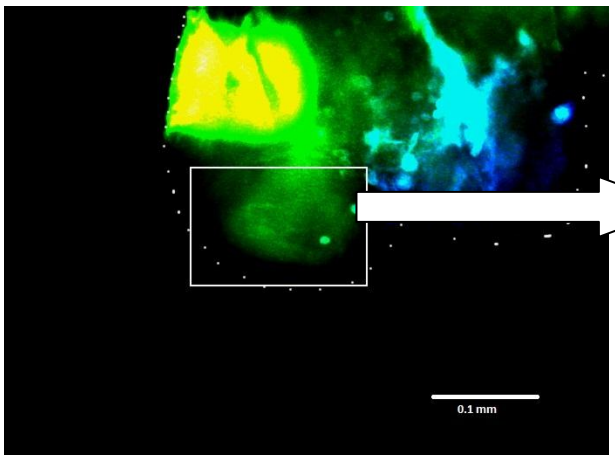

Fig. 8A1: *optic lobe of D. melanogaster line G73 under GFP2 excitation light*

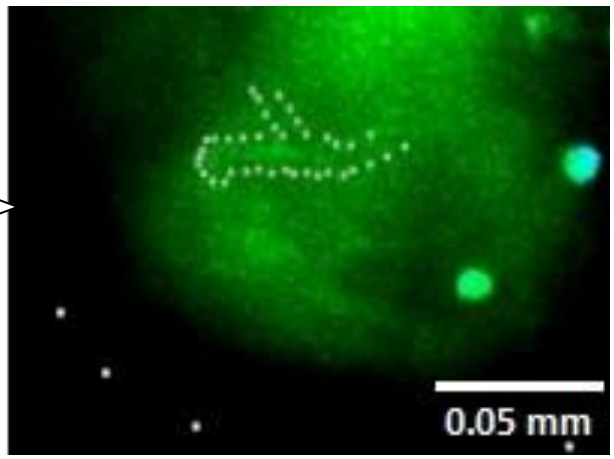

Fig. 8A2: *detailed view on Fig. 5A; pointed out: HSN-cell*

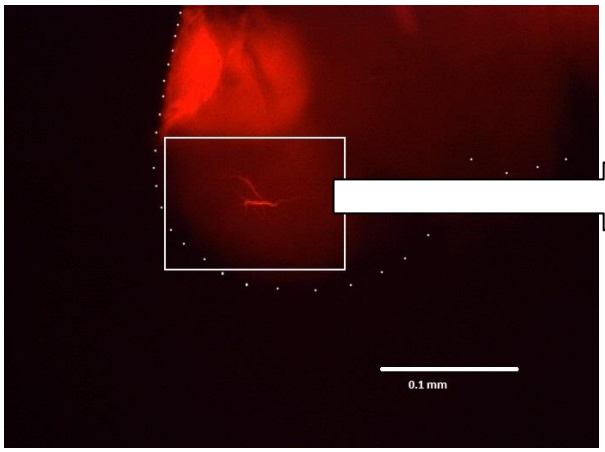

Fig. 9B1: *optic lobe of D. melanogaster line G73 under green excitation light; HSN cell filled with Alexa 594 (in white box)*

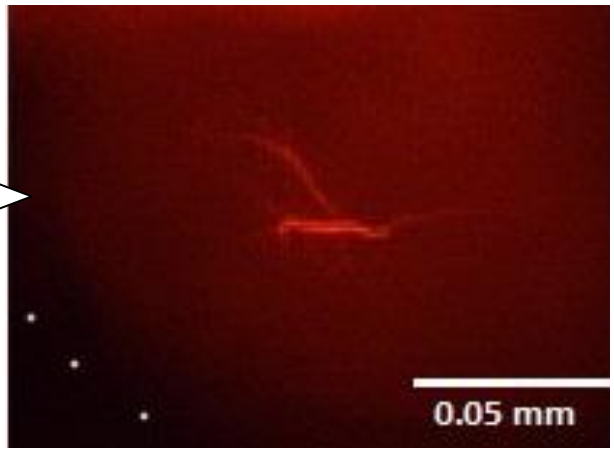

Fig. 9B2: *detailed view on Fig. 5B; dye filled HSN-cell*

Subsequently, the brains were fixed in 4% paraformaldehyde (PFA) for 30 minutes (Fig 10A). PFA binds to free amino groups of proteins and polymerizes them. Cross-linking all kinds of proteins, prevents the leak of the dye from the filled cells and preserves the tissue. After fixation the brains were washed 3 times in PBS (1x) and dissected from the head capsule (Fig. 10B/C). The brains were then transferred to an object slide and embedded in mounting medium (Fig. 10D-F) (Vecta Shield H-1000, Vector Laboratories, Inc. Burlingame, CA). To avoid crushing the brains, 2 layers of adhesive strip (Tesa) were used as place holders between the brains and the cover slip.

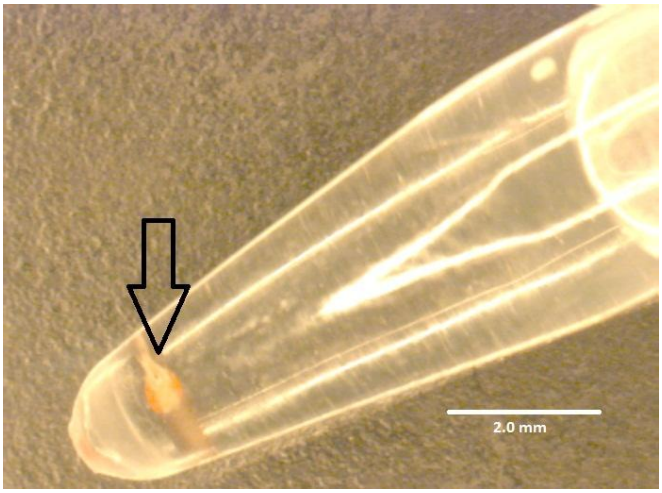

Fig. 10A: *Fixation of a head in 4% PFA*

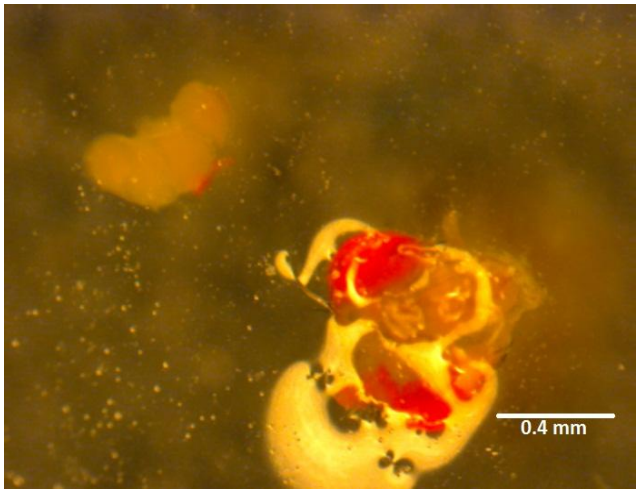

Fig. 10B: *Dissection of the fixed brain from the head capsule*

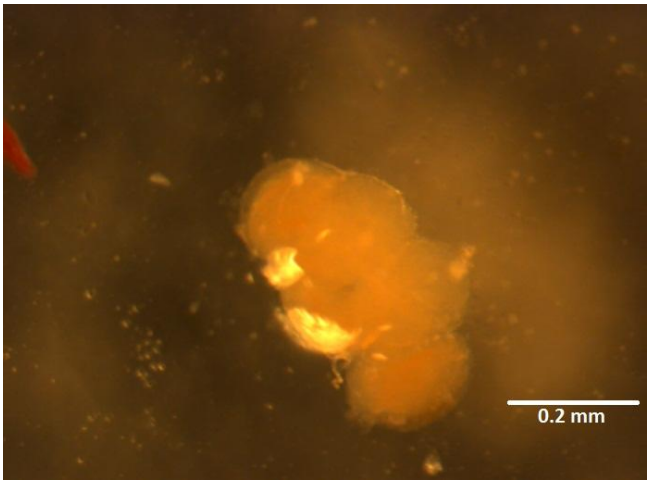

Fig. 10C: *Fixed and dissected brain in PBS*

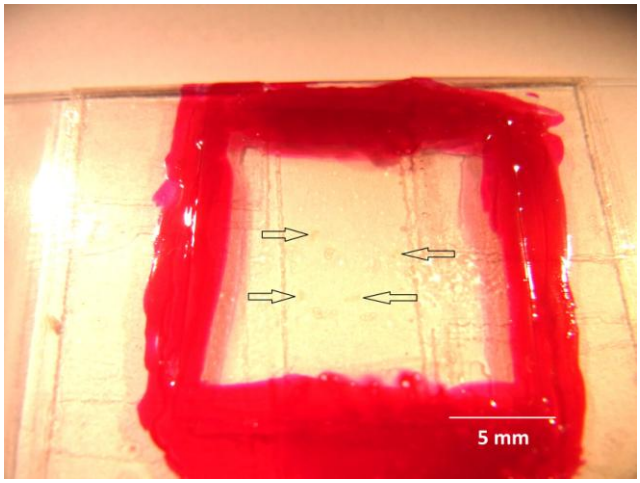

Fig. 10D: *heads on object slide, pointed with arrows, surrounded by nail polish*

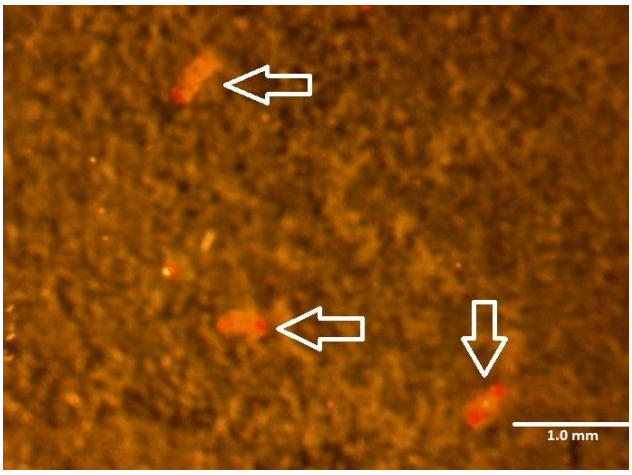

Fig.10E: *Three dissected brains in mounting medium*

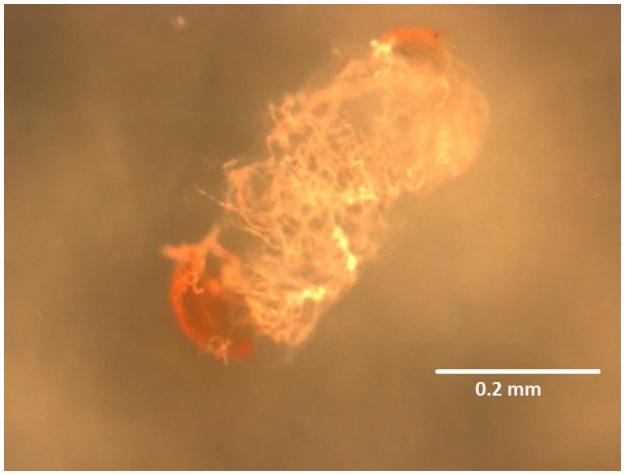

Fig. 10F: *Dissected brain in mounting medium*

Confocal Microscopy:

Serial optical sections were taken at 0.365 µm intervals with a resolution of 1024 x 1024 pixels using a confocal microscope (Leica SP2 respectively Olympus FV-1000) and oil-immersion X40 and X63 (numerical aperture [NA]=1.25) Plan-Apochromat objectives. The specialty of confocal microscopes compared to traditional wide-field fluorescence microscopes is the pinhole. The pinhole stays in an optically conjugate plane in front of the detector to eliminate out-of-focus signals. Thus, optical resolution along the z-axis is attained. Size, contrast, and brightness of the resulting image stacks were adjusted with ImageJ (National Institutes of Health, Bethesda, MD).

Reconstruction:

Cells were manually traced using Milana (Cuntz et al. 2008), a custom written software based on Matlab (Mathworks, Natick, MA) to translate the pixel based images (.tif) from the confocal scan, into a vector based model. Maps of maximum intensity and corresponding depth were computed along the Z-axis. This reduction from 3D-data to 2D images was sensible as there were no or very few 3D crossings of branches and all cells were planar. Based on these images, cylinder models of the branching structure were obtained in a semi-automated way: interactive software allowed switched viewing

of either the Z-projection or an individual slice of an image stack. The cells were reconstructed as a set of connected cylinders with variable length and diameter, representing the axonal or dendritic structure of the neurons. To adjust the diameter for the cylinders, the widths of two-dimensional rectangles were fitted by gauss functions. Switching through the 2D stacks of the three dimensional image, rectangles were manually defined via their end points, representing the center of the thickest part of the neural structure and their diameter. The depth-map according to their 2D location defined the Z-value of the cylinders. The images of the reconstructed cells were adjusted and visualized using Amira (Amira 5.2.2, Visage Imaging GmbH, Berlin, GER).

Results:

During the 10 weeks of laboratory work the handling and dissection of flies, the preparation of *Drosophila* brains, the work with confocal microscopy and the technique of targeted filling of tangential cells with fluorescent dye were learned. In this time 15 vertically sensitive cells (VS cells) were filled and fixed. Of these 15 cells, only approximately 50% were scanned with the confocal microscope. Among these 7 cells, the quality of only one VS1 cell was good enough to be reconstructed. Furthermore, the analysis of a filled H2 neuron was undertaken.

Vertically Sensitive Neurons:

In the Drosophila line DB331, VS cells were filled with Alexa-568 dye, digitalized with confocal microscopy and reconstructed using a Matlab based software. In figure 11A, a maximum intensity z-projection of a VS1 cell is shown. Furthermore, VS2 – VS6 maximum-intensity projections are shown in Figure 11B. Unfortunately, these fills did not have the required quality and were therefore not reconstructed. The cell illustrated in Fig. 11A was used for three dimensional reconstruction. Figure 11C shows a two dimensional snapshot of this reconstruction in a dorsal view on the fly's brain. The dendritic tree and the axon were scanned separately and rejoined on the computer after reconstruction. The planar structure of the vertically sensitive cell can be seen in Fig. 11D, whereas the 3D reconstruction is depicted from a lateral view.

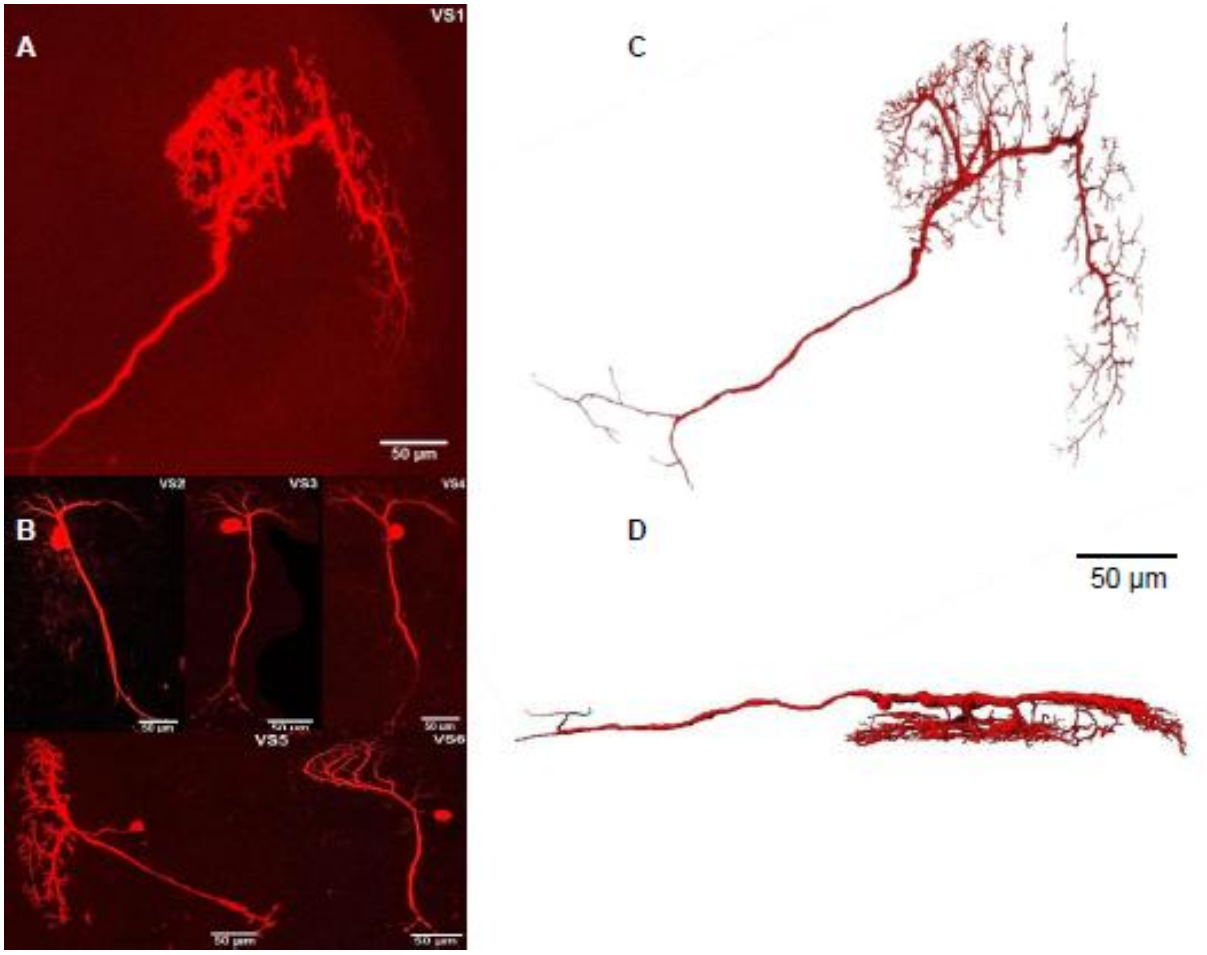

Fig. 11: VS cells filled with fluorescent dye; **A:** maximum intensity z-projection of VS1 cell (dorsal view); **B:** maximum intensity z-projections of VS2-VS6 cells (dorsal view); **C:** two dimensional image of the computer reconstruction of a VS1 cell (dorsal view); **D:** two dimensional image of the computer reconstruction of a VS1 cell (lateral view)

To statistically analyze the three dimensional model of the VS1 cell, six different parameters were compared within the cell. First a Sholl-analysis (Sholl DA, 1953) was performed. This method is based a series of concentric circles around the end point of the axon of the neuron. Counting the number of intersections with these circles allows a quantification and comparison of its branching structure. The width of one circle was calibrated to 10 µm. Figure 12A shows the reconstruction of the VS1 cell within the sholl plot. Furthermore the two distinguishable parts of the cell – the dendrite and the axon – were analyzed separately (Fig. 12B). The results plotted for the complete cell (blue line) and for the dendrite (red line) have similar characteristics within the first 120 µm whereas the graph for the axonal structure of the VS1 cell matches the plot for the whole cell in the last 100 µm.

The second analysis performed was the comparison of the spanning area between the whole cell, the dendritic structures and the axon (Fig. 12C). To this end, the expansion of the axonal terminus and the dendritic branching of the reconstructed cell were determined. The spanning field of the axon, compared to the spanning field of the dendrite, is about five times (4.9x) smaller. The ratio between the cable lengths of dendrite and axon terminal (Fig. 12D) shows with a factor of 14.7 an even more pronounced difference.

The number of branches (Fig. 12E) of a neural structure is indicative for its branching complexity and, together with the information about the spanning area, about the receptive field of the neuron. With only seven bifurcations, the axon of the VS1 cell has a very low branch number, compared to 526 branches of the dendritic tree. This data matches the results of the spanning area and the cable length, and can be clearly seen in the reconstructed cell (Fig. 11C). The next analysis considers branching points by the calculation of branching orders. Every branch gets an order representing the number of branches between itself and the root. This analysis shows the complexity of neural trees and points out, once again the difference between the dendritic branching and the axonal ramifications (Fig. 12F). The sixth structural property analyzed here, is the mean diameter of the neuron (Fig. 12G). In this analysis, the difference between axon and dendrite is not as obvious as for the previous ones. In the future, similar analysis will have to be performed for each VS cell type several times to analyze the variance of their morphologies. If the properties of each cell types are conserved, a “standard” model cell could be proposed for neuronal network models of the lobula plate.

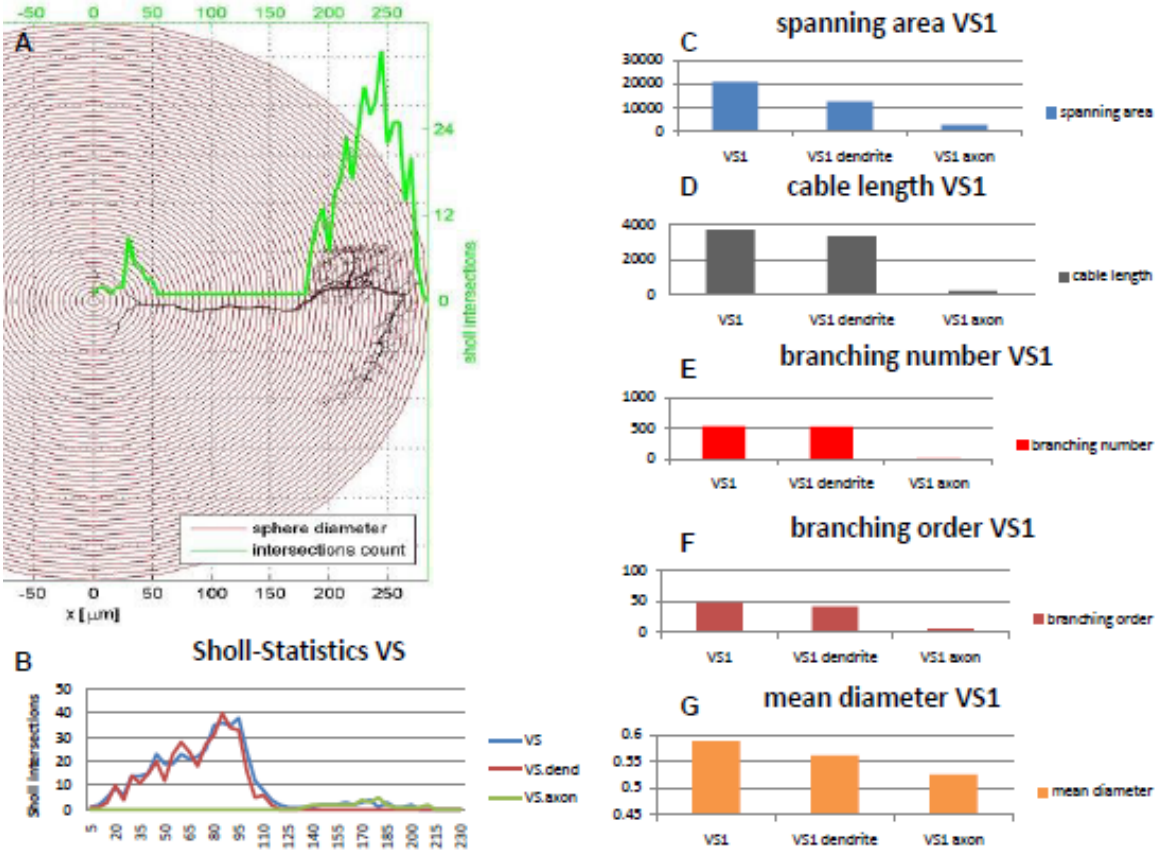

Fig. 12: Statistic analysis of a VS1 cell; A: Overlay of a VS1-cell reconstruction and a Sholl plot; **B:** Sholl plot comparison of a whole VS1 cell, a VS1-dendrite and a VS1-axon; **C-G:** Comparison of plots of the spanning area (**C**), cable length (**D**), branching number (**E**), maximum branching order (**F**) and the mean diameter (**G**) of a whole VS1 cell, a VS1-dendrite and a VS1-axon

H2 Neurons:

Besides VS cells, one other cell type was analyzed and reconstructed during the laboratory work. As shown in Fig. 13A, H2 cells get their dendritic input in one hemisphere – this optic lobe is depicted by * – and send their axon to the contralateral side of the brain – depicted by **. Figure 13B shows a close up on the axonal terminal of the H2 cell depicted in 13A. To resolve the branching layers of the dye filled H2 and a genetically labeled HS cell, the image stack was flipped by 90 degrees in the x-axis (13C). This rotation shows the deeper branching dendrites of the H2 cell compared to the layer of HS cells. Two dimensional images of the reconstructed H2 neuron are depicted in a dorsal view (Fig. 13D) and a lateral view (Fig. 13E). The dorsal view on the reconstruction allows a nice comparison with the maximum intensity projection above (Fig. 13A) and presents,

together with the lateral view on the cell (Fig. 13E), the three dimensional structure of H2.

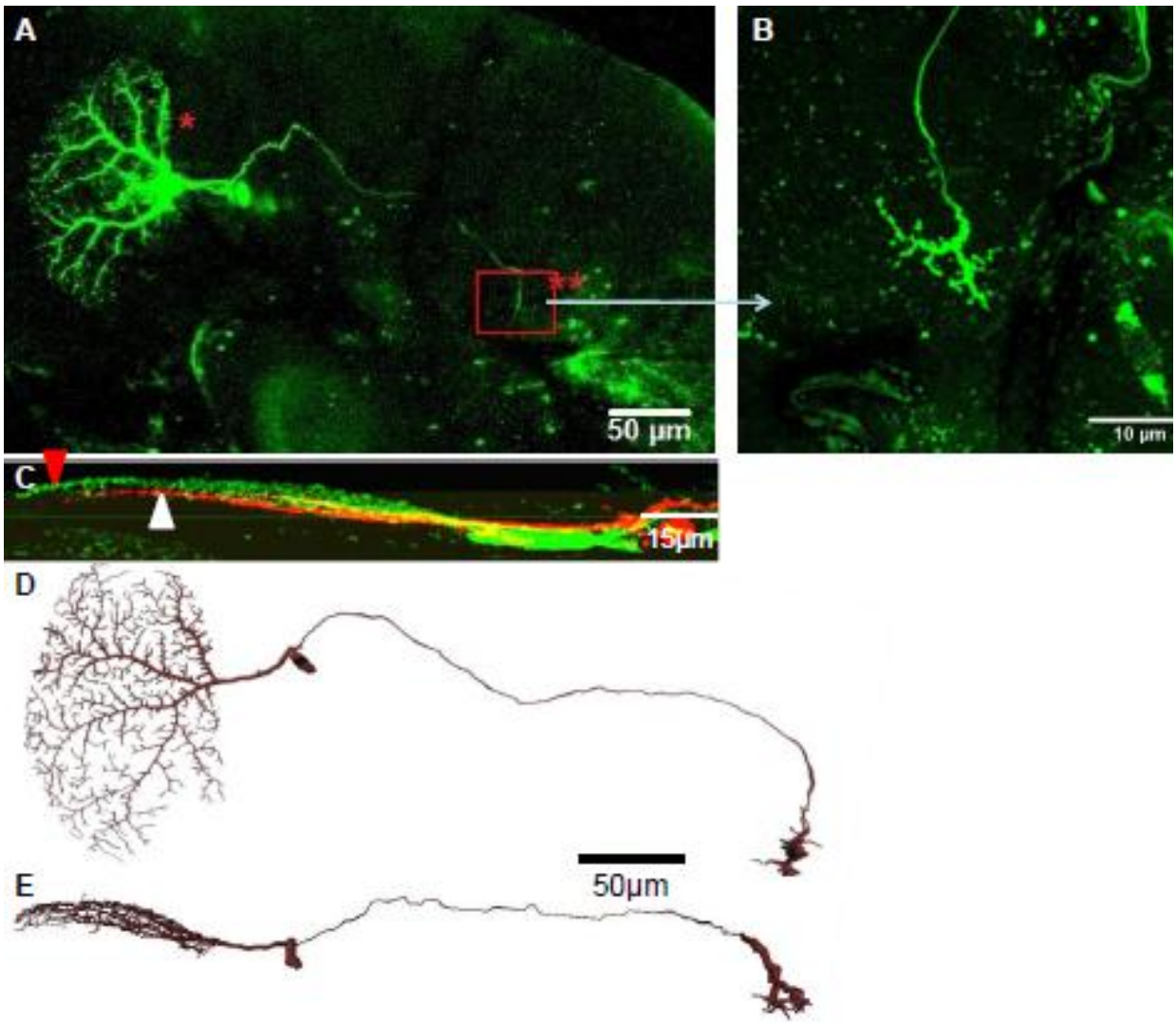

Fig.13 H2 cell from *D. melanogaster*; **A:** Maximum intensity z-projection of H2 cell (dorsal view); **B:** Maximum intensity z-projection of axon terminal of H2 cell (dorsal view), close up section from 'A'; **C:** Maximum intensity z-projection (lateral view) of merged H2 (red with white arrow) and HS (green with red arrow) dendrites cell layers **D:** two dimensional image of computer reconstruction of H2 cell (dorsal view); **E:** two dimensional image of computer reconstruction of H2 cell (lateral view).

The previously described contralateral projection of the H2 axon terminal is shown and described in Figure 14. Since this H2 cell was filled in a fly that expresses GFP in HSE and HSN, the overlap between HS and H2 axonal termini could be analyzed. Fig. 14A shows a maximum intensity z-projection of two merged stacks, where GFP expressing HSE and HSN cells (green) and an Alexa-filled H2 cell were scanned separately. This is possible using the same settings but different filter-sets to detect red and green fluorescence. A close up image was taken to better resolve the overlapping parts of the two cells (Fig. 14B). Therefore the maximum intensity of only four stacks was used to create a z-projection. The yellow color, resulting of the merge of the green HS and the red H2 cell (white triangles) indicates parts, were the cells are supposed to interact. For a better

visualization of the loci of overlap, the branches of the HS and H2 cells shown in Fig. 14A and B were reconstructed as described (Fig. 14 C-E). The separately reconstructed neural structures were joined together via Matlab in order to then be configured and adjusted in Amira. Figure 14E was rotated by approximately 180 degrees to get a better view of the overlapping parts of the two cells.

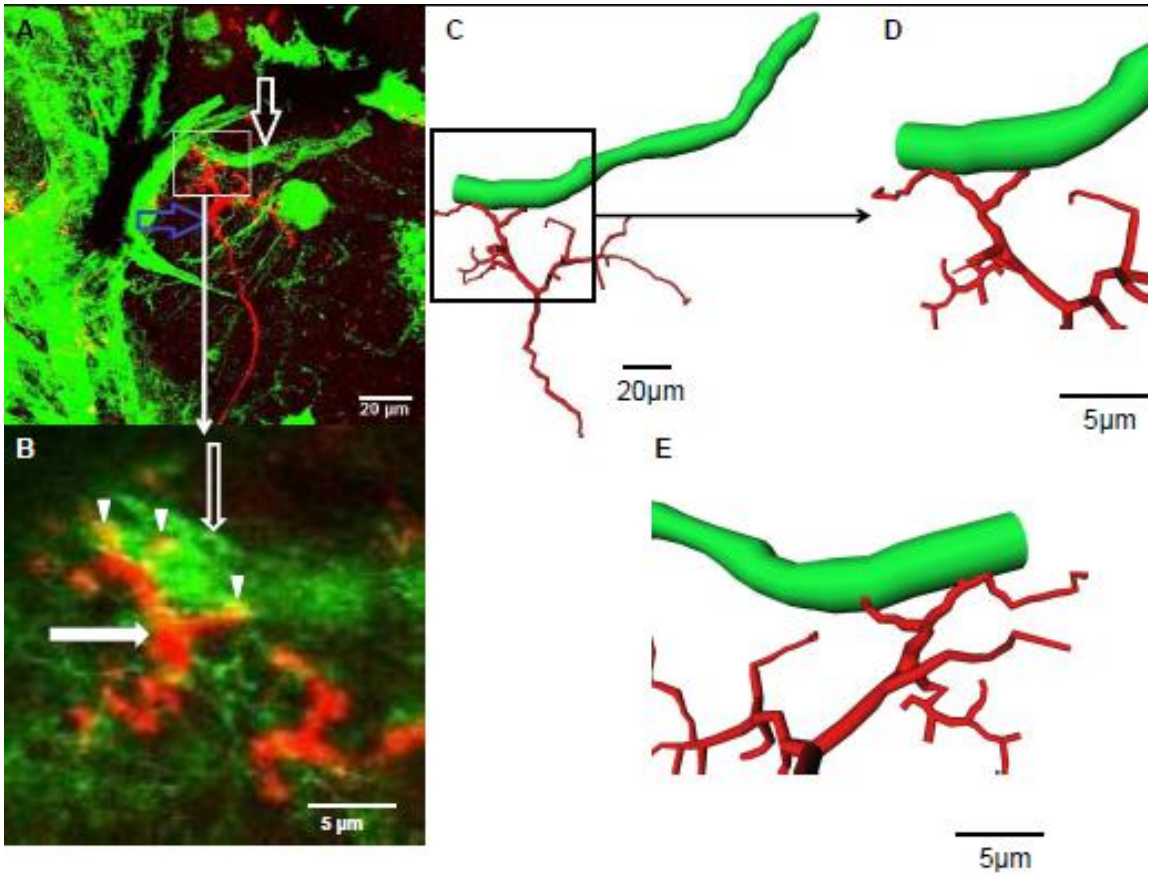

Fig. 14 Overlapping of axon terminal of H2 and HS dendrite; **A:** Merged maximum intensity z-projection of axon terminal of H2 (red, with blue arrow) and HS dendrite (green with white arrow); **B:** Merged maximum intensity z-projection close up of potential overlap (solid filled arrow: H2; unfilled arrow: HS; white triangles: loci of overlap); **C-E:** Two dimensional images of 3D reconstructions of H2-HS interaction

For the H2 cell, the same statistic analyses were performed as described previously for the VS1 neuron. This analysis showed comparable results between both cell types. The Sholl statistics, plotted in Fig. 15A and 15B visualize the number of intersections of the cell and the Sholl circles. The total length of the cell, from the axon terminal to the final branches of the dendritic tree, is approximately 300 µm. The cable length of the H2 dendrite is about 14 times bigger than the length of the axonal cable. In this H2 cell, the mean diameter of the axon is 2.3 times bigger than the dendritic diameter. This might be caused by the much longer cable of the dendrite: many small dendrites have to be calculated for the mean

diameter, whereas the axon consists of only 14 thicker branches (Fig. 15F). In addition, the branching order of the H2 dendrite is more than 6 times bigger than the axonal branching order (Fig. 15E). The last property of H2 cells, considered in this statistics, is the spanning area of H2, where the dendrite is more than 16 times bigger than the axon (Fig. 15G).

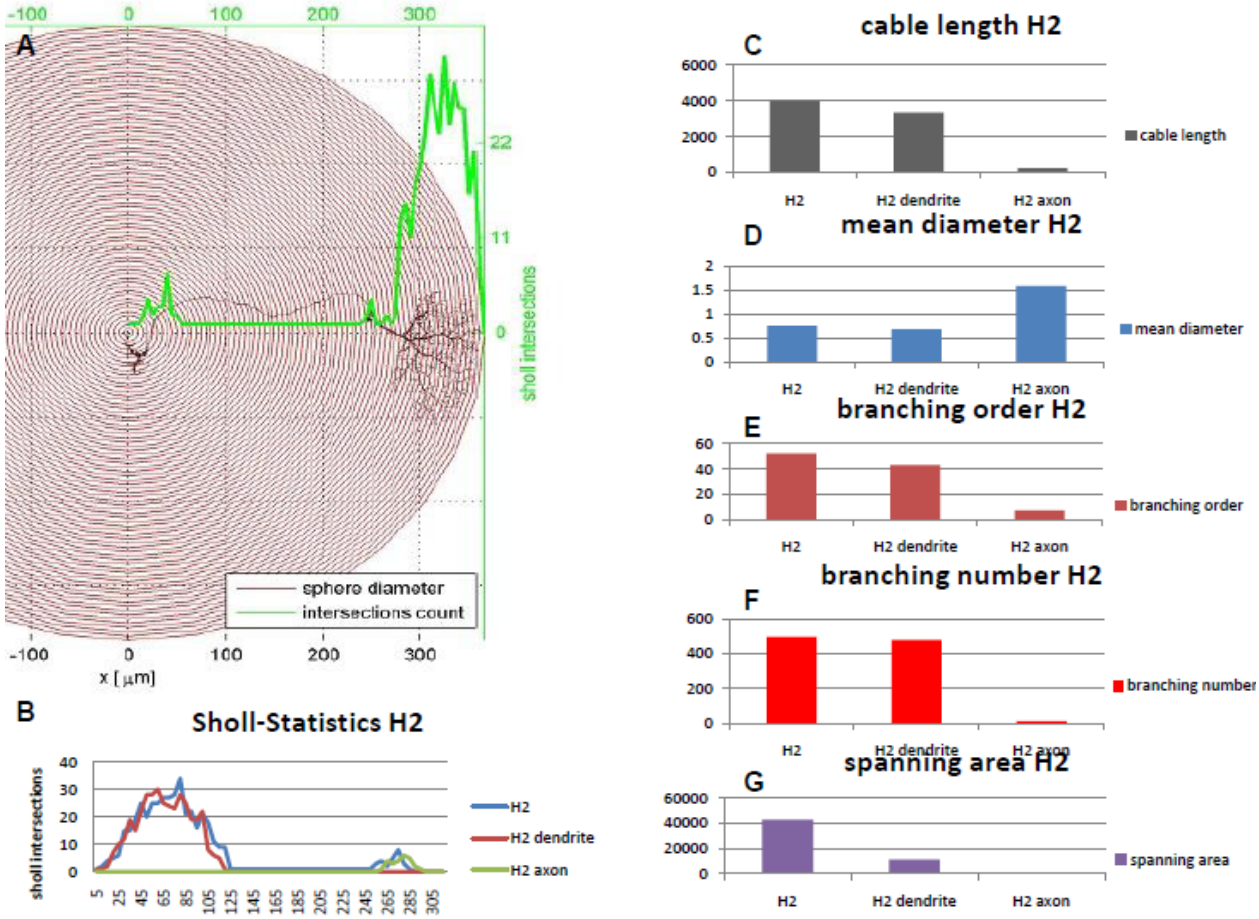

Fig. 15 Statistic analysis of a H2 cell; **A:** overlay of a H2-cell reconstruction and Sholl plot; **B:** Sholl plot comparison of a whole H2 cell, a H2-dendrite and a H2-axon; **C-G:** Comparison of plots of the cable length (**C**), mean diameter (**D**), maximum branching order (**E**), branching number (**F**) and the spanning area (**G**) of a whole H2 cell, a H2-dendrite and a H2-axo

Discussion

Morphological analysis of neuronal cell types is essential for their functional understanding, as response properties of neurons strongly depend on their anatomy. Electrical resistance for instance depends on the diameter of the dendrites and the axon. In this work, fluorescent targeted lobula plate tangential cells in *Drosophila* were dye filled, digitalized via confocal microscopy and reconstructed to extract their relevant anatomical information. Translating the voxel based pictures from the laser scanning microscope to a vector based three dimensional model of the cells retains the important morphological information. Moreover, it reduces the size of this information drastically. An image stack of a lobula plate tangential cell in Tiff format acquired with a confocal microscope has an average size of 200 MB. The size of the same cell transformed into a vector based model and saved as SWC file, is only about 70 KB in size. This reduction in storage size of factor ~4000 simplifies the computational handling of the data and allows a precise and simple anatomical analysis of the cell characteristics. In addition, these vector based models are indispensable for the construction of multi-compartmental models. In future, these models will be used in realistic network models of the lobula plate. Thus, this work is an important step for a better understanding of *Drosophila's* lobula plate tangential cell network. In following, the results will be discussed separately for VS and H2 cells.

VS1 results:

As electrophysiological studies have shown, VS cell respond primarily to vertical stimulation. In the case of VS1, a small but reproducible response can also be seen to horizontal whole field stimuli (Joesch, personal communication). Interestingly, the reconstructed VS1 cell shows branching patterns out of the VS cell layer (Fig.11). These z-axis branches might be responsible for the unusual, horizontal response properties of VS1 compared to the other vertically sensitive neurons (VS 2 –VS4) of the lobula plate.

H2 results:

Electrophysiological studies have shown that HS cells, in addition to ipsilateral stimuli, also respond to contralateral stimuli (Fig. 16) (Schnell et al. 2010) although these cells do not possess contralateral branches. Moreover, the contralateral response properties have an inverse preferred direction: whereas HS-cells respond preferentially to front-to-back stimuli on the ipsilateral side, they respond preferentially to back-to-front on the contralateral side.

This tuning allows them to be preferentially tuned to whole field rotational stimuli. This fact indicates that a horizontal sensitive neuron of the contralateral hemisphere is connected to HS-cells of the ipsilateral optic lobe. Based on the anatomical analysis of a single dye filled H2 cell in a fly were HSE and HSN were genetically visualized via GFP, H2 appears to be an excellent candidate. First, H2 dendrites stratify in a deeper layer of the lobula plate then HS cells (Fig.13C). Based on radioactive labeling with 2-deoxy-glucose, this layer has been previously shown to be preferentially activated by back-to-front stimuli (Buchner et al. 1984). Secondly, the axonal termini of H2 projects to the contralateral side and most interestingly, co-localize with the axonal termini of HS cells (Fig. 13/14). Taking these facts into account, H2 seems to be the missing link for the understanding of HS-cell response properties. It has been previously shown via neurobiotin injections that a contralateral tangential cell is coupled via electrical synapses with HS-cells (Schnell et al. 2010). To further investigate if this coupled cell is actually H2, neurobiotin injections into H2 dendrites will be performed in future experiments into H2 dendrites. If there is any electrical connection, HS-cells are expected to be visualized while staining against neurobiotin.

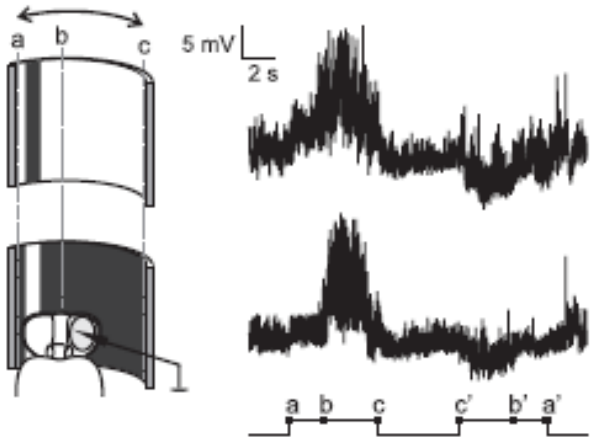

Fig. 16: Example trace of an HS ell responding to a light bar on a dark background and a dark bar on a light background moving in PD and ND. *a*, *b* and *c* and *a*', *b*' and *c*' mark the time points at which the bar occupied the respective positions on the arena. Note that HS cells respond to motion on the contralateral side as well. (Taken from Schnell et al. 2010)

Acknowledgements

I greatly thank Maximilian Joesch for supervising this thesis and teaching me the techniques that I needed for this work and how to write such a thesis; Friedrich Foerstner for his help with the morphological analysis and correction of the thesis; Hubert Eichner for helpful suggestions and correction and Axel Borst for making it possible to accomplish the thesis in the Department of Systems and Computational Neurobiology at the Max Planck Institute.

References

- Borst A, Haag J (2002) Neural networks in the cockpit of the fly, *J Comp Physiol A, 188: 419-437*
- Buchner E, Buchner S (1984) Deoxyglucose mapping of nervous activity induced in *Drosophila* brain by visual movement, *J Comp Physiol A, 155: 471-483*
- Chapman R.F (1998) The insects: structure and function, *Cambridge University press*
- Cuntz H (2008) The morphological identity of insect dendrites, *PLoS Computational Biology, Volume 4, Issue 12: 1-7*
- Heisenberg R, Wonnenberger R and Wolf R (1978) Optomotor blind^{H31} – a *Drosophila* mutant of the lobula plate giant neurons. *J. Comp. Physiol. 124, 287-296*
- Fischbach K.F and Dittrich A.P.M (1989) T he optic lobe of *Drosophila melanogaster.* I. A golgi analysis of wild-type structures, *Cell Tissue Res. 258, 441-475*
- Hassenstein B, Reichardt W, (1956) Systemtheoretische Analyse der Zeit-, Reihenfolgen- und Vorzeichenauswertung bei der Bewegungsperzeption des Rüsselkäfers *Chlorophanus*. Z., *Naturforsch. 11b, 513-524*
- Joesch M (2008) Response properties of motion-sensitive visual interneurons in the lobula plate of *Drosophila melanogaster, Current Biology 18, 1-7*
- Luo L, Callaway E (2008) Genetic dissection of neural circuits, *Neuron 57: 634-655*
- Meinertzhagen I.A and O'Neil S.D (1991) Synaptic organization of columnar elements in the lamina of the wild type in *Drosophila melanogaster, J. Comp. Neurol. 305: 232-263*
- Otsuna H and Ito K (2006) Systematic analysis of the visual projection neurons of *Drosophila melanogaster*. I. Lobula-specific pathways, *J. Comp. Neurobiology 497, 928-958*
- Rajashekhar K, Shamprasad V (2004) Golgi analysis of tangential neurons in the lobula plate of *Drosophila melanogaster*, *J. Biosci. Vol.29, 1: 93-104*
- Schnell B (2010) Processing of horizontal optic flow in three visual interneurons of the drosophila brain, *J Neurophysiol 103: 1646-1657*
- Scott E, Raabe T (2002) Structure of the vertical and horizontal system neurons of the lobula plate in *Drosophila, The journal of comparative neurology 454: 470-481*
- Sholl DA (1953) Dendritic organization in the neurons of the visual and motor cortices of the cat. *J Anat 87: 387-406*