University of Utah
UNDERGRADUATE RESEARCH JOURNAL

PRESYNAPTIC BOUTON AND POSTSYNAPTIC KENYON CELL CLAW MORPHOLOGY WITH SYNAPTIC PLASTICITY IN *DROSOPHILA*

by

Cody Orton

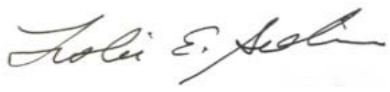
A Senior Honors Thesis Submitted to the Faculty of
The University of Utah
In Partial Fulfillment of the Requirements for the

Honors Degree in Bachelor of Arts

In

Biology

Sophie Caron, PhD
Thesis Faculty Supervisor



Dr. Leslie Sieburth
Co-Director, School of Biological Sciences

Dr. Naina Phadnis
Honors Faculty Advisor

Sylvia D. Torti, PhD
Dean, Honors College

April 2021

Abstract

My thesis aims at determining whether or not neural activity affects connectivity in the *Drosophila melanogaster* olfactory circuit. Specifically, I am testing whether sensory activity is required for Projection neurons and Kenyon cell morphology. Therefore, my primary and central question is: does sensory activity reduction increase or decrease the number of synapses between neurons, and does this affect cellular morphology in *Drosophila*?

There are more than 60 types of olfactory receptors within the sensory circuit[18]. Each sensory neuron that expresses the same type of olfactory receptor sends their axons to a specific type of projection neuron. These projection neurons send their axons to the secondary olfactory center, the calyx of the mushroom body. In the calyx, these projection neurons form presynaptic boutons, which are wrapped by postsynaptic claws of multiple Kenyon cells. Kenyon cells project to the mushroom body lobe, where they synapse with efferent neurons that produce a learned response to specific odors. In order to ablate olfactory receptors, we use knockout flies of *Orco* -/-, which is a gene required to form 80% of the olfactory receptor complexes[4,15].

My work focused on the presynaptic and postsynaptic connections between projection neurons and Kenyon cells. I examined if there are changes in the presynaptic sites of projection neurons and changes in postsynaptic sites in Kenyon cells in *Orco* -/- mutants, who are insensitive to many types of odors. To visualize presynaptic sites, I studied projection neurons in control flies and *Orco* -/- mutants using photoactivatable GFP, and the number of presynaptic sites were quantified. To analyze postsynaptic sites, I looked at data for both the control and *Orco* -/- knockout flies, comparing the number of Kenyon cells in each to determine if there is a change in the odor-insensitive flies. The results of this study show that through comparing control and *Orco* -/- fly Kenyon cell types, there is no significant difference between genotypes,

implying that it is not the *Orco* that accounts for synaptic plasticity. We can now focus on other genes to knockout in order to continue to study how it affects olfactory synaptic plasticity referring to Kenyon cell claw numbers.

Table of Contents

Introduction

The fruit fly, *Drosophila Melanogaster,* has an olfactory circuit similar to ours, just with fewer neurons. These fruit flies can also portray learning through olfactory systems by associating an odor with a reward. This is seen within the mushroom body, which is a center of the brain in *Drosophila* that houses over 2000 neurons[9], called Kenyon cells which receive most of their input from the olfactory circuitry. (Figure 1) The Kenyon cells form connections with pre-ganglionic sensory neurons of the olfactory circuit with postganglionic claws. It is this system of pre-ganglionic and postganglionic synaptic morphology that I am portraying in this thesis by comparing pre-ganglionic bouton numbers and each postganglionic Kenyon cell subtype for two separate genotypes. These Kenyon cell subtypes are AlphaBeta, Alpha'Beta', and Gamma neurons[3].

My research focused on characterizing the intrinsic and unique morphological presynaptic projection patterns with subsequent postsynaptic connections to Kenyon cell subtypes with regard to the olfactory circuit (Figure 1) in *Drosophila*. This morphology has links to synaptic plasticity itself in *Drosophila* memory integration, but it is also crucial for our own memory formation and retention as human beings. The way in which we form memories is through creating and strengthening neuronal connections in our brains. This allows us to store our beloved memories through many different mechanisms like long-term potentiation[6]. This is critical because the human brain is modified through experience and can be changed according to our subjective lives.

In order to properly understand the effects of ablated sensory factors in the olfactory circuitry of *Drosophila* fruit flies, the circuitry itself must be properly explained. The formation of olfactory associated memories is characterized by synaptic plasticity. Synaptic plasticity is

formed by strong intraneuronal interactions over time. As these interactions build up and more neurons participate in chemical signals, the synapses become stronger and more associative with memories in the brain. Fruit flies first sense odors at their antennae and maxillary palps on the front of their heads, and then these senses begin to move through olfactory sensory neurons (OSNs) to begin integration into the olfactory circuit to store memory of the detected odor[11]. These OSNs have already been studied and have shown that for each detected odor, a specific response profile is generated in the brain determined by a single unique class of olfactory receptor. There are more than 1400 *Drosophila* melanogaster OSNs, with many expressing one of 61 different olfactory receptors[6]. Once stimulated, these OSNs then activate glomeruli in the antennal lobe, which then connect to projection neurons. Canonical types of projection neurons receive inputs from a single glomerulus. As a set of OSNs expressing the same receptor always innervates the same glomerulus, each projection neuron can be attributed to a single receptor-glomerulus pair, with some exceptions[12]. Projection neurons then extend their axons to innervate the calyx of the mushroom body in *Drosophila*. The calyx of the *Drosophila* is formed by microglomeruli which each have a projection neuron bouton with Kenyon cells that contact and reveal the distinctive polymorphic claw shapes of these calyceal microglomerular connective elements in *Drosophila*[8].

The Kenyon cell claws can be, and most likely are, diverse in number as these claws contribute to connectivity. These calyceal microglomeruli have shown to be comprised by a single very large presynaptic projection neuron bouton that is surrounded by a large number of small postsynaptic claws of Kenyon cell dendrites and GABA-positive interneurons[14]. Again, these presynaptic boutons and postsynaptic claws are my major study elements as I characterize the connection from a projection neuron to a Kenyon cell population in a microglomerular

element within the mushroom body calyx of *Drosophila* brain. Figures 2-5 represent photolabled postsynaptic claws for each Kenyon cell type between the control and *Orco -/-* genotypes.

Within the olfactory circuit, there are three different receptor types: Odorant Receptors (ORs), Ionotropic Receptors (IRs), and Gustatory Receptors (GRs)[12]. My work focused on Olfactory Receptors. I quantified the number of presynaptic sites in projection neurons, and counted the number of postsynaptic claws in Kenyon cell subtypes for each genotype and documented the numbers of postsynaptic claws depending on the subtype of Kenyon cell. Using two specific genotypes of *Drosophila*, namely the control, which has transgenes of photoactivatable GFP, and *Orco -/-* mutant genotypes. 80% of olfactory receptors require *Orco* to function, and *Orco* knockout flies have been shown to have reduced behavioral responses to odors[4].

Sensory activity in presynaptic projection boutons and postsynaptic Kenyon cells has previously shown that activity-dependent plasticity in the calyx is mixed. Also, studies show that antenna removal at the virgin stage did not alter the volumes of projection neuron boutons later, suggesting that odor-evoked activity may be dispensable in calyx development[21]. However, artificial hyperpolarization of projection neurons with leaky potassium channel expression resulted in the increase of projection neuron bouton volumes[14]. In addition, optogenetic activation of projection neurons with channel rhodopsin upregulated the projection neuron bouton volumes. These results are seemingly contradicting, making a cohesive interpretation difficult. To solve this controversy of whether connectivity development is influenced by sensory activity. I visualized the boutons of projection neurons in *Orco -/-* mutants and control flies.

The olfactory circuit is being studied because of its critical importance and implications to memory, potentially both in *Drosophila* and humans. As we begin to understand the olfactory

circuit morphology, we see that different odors are encoded differently throughout the brain. This way of encoding olfactory memories will help us understand how memories themselves are stored and created in the brain. The morphology of the olfactory circuit is the first key step in further determining how synaptic plasticity is achieved in the brain.

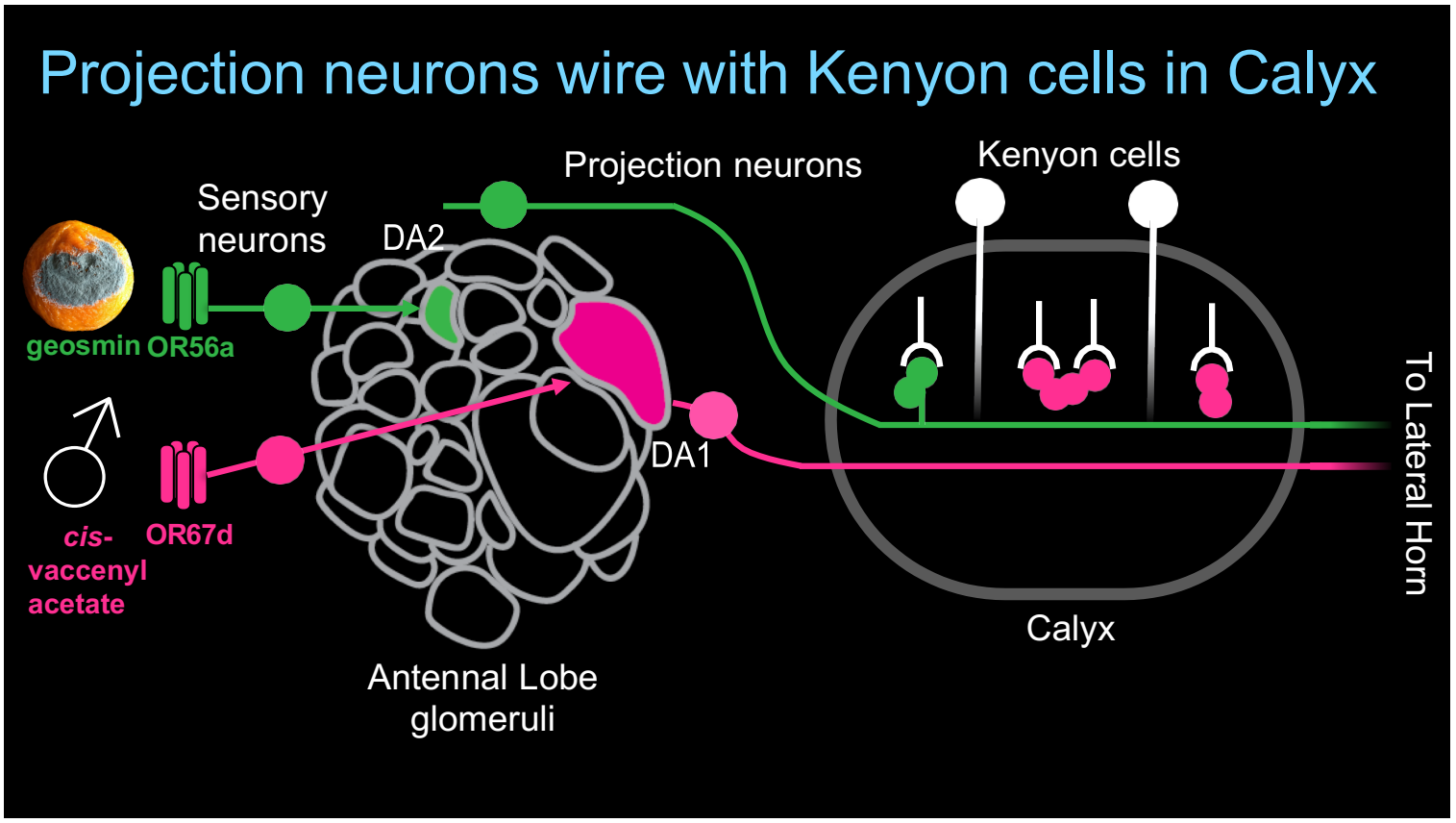


Figure 1. A diagram of the olfactory circuit in *Drosophila* fruit flies showing an overview of synapses between sensory neurons and projections neurons and Kenyon cells.

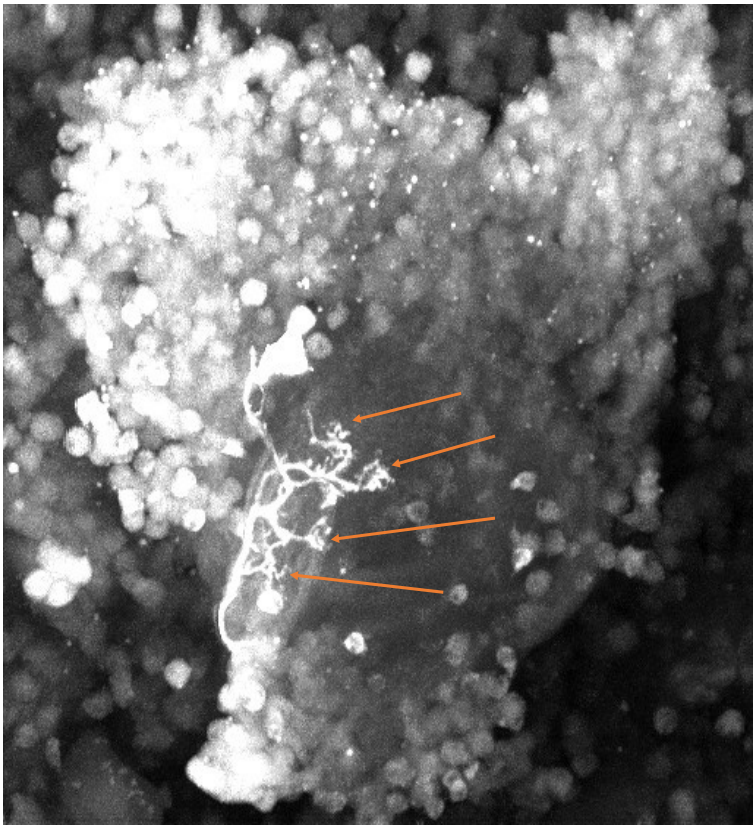


Figure 2. AlphaBeta Control

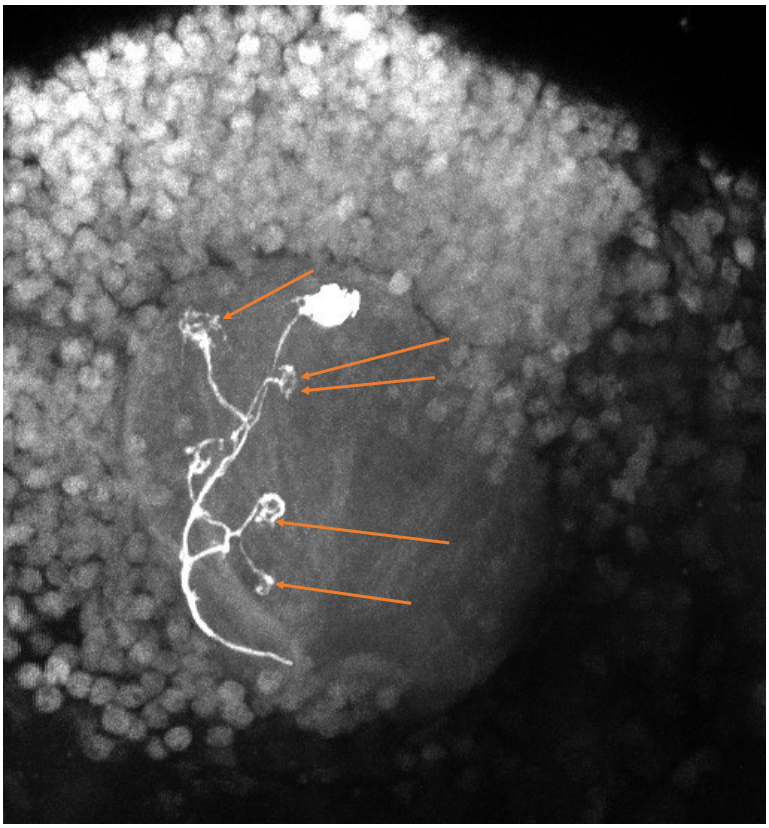


Figure 3. AlphaBeta *Orco -/-*

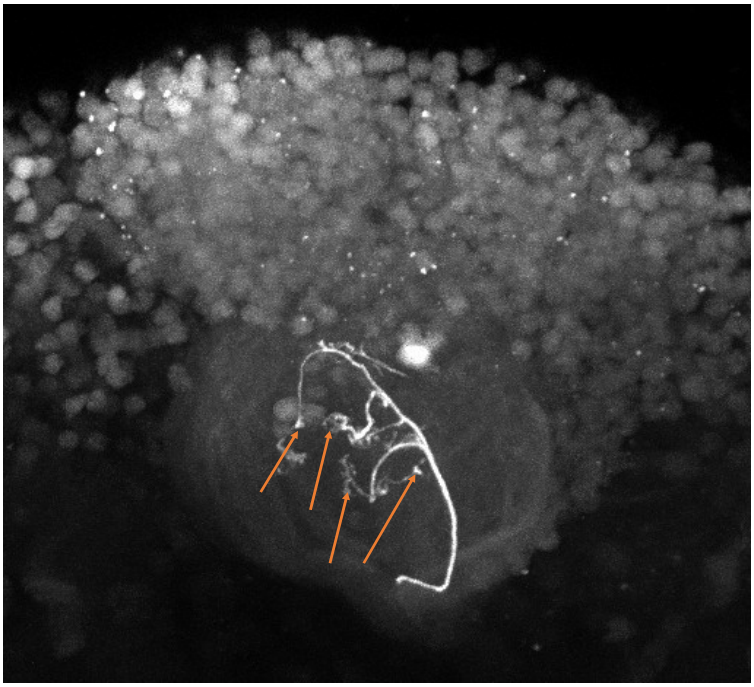


Figure 4. Alpha'Beta' Control

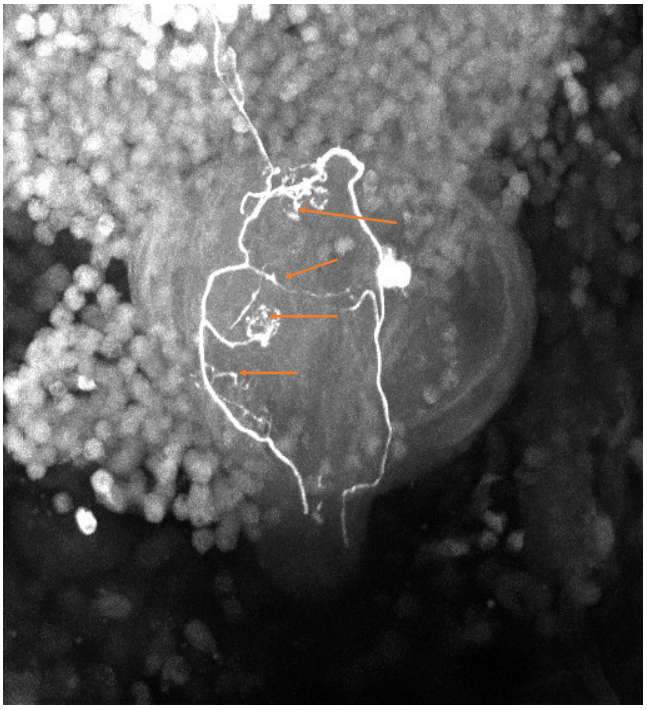


Figure 5. Alpha'Beta' *Orco -/-*

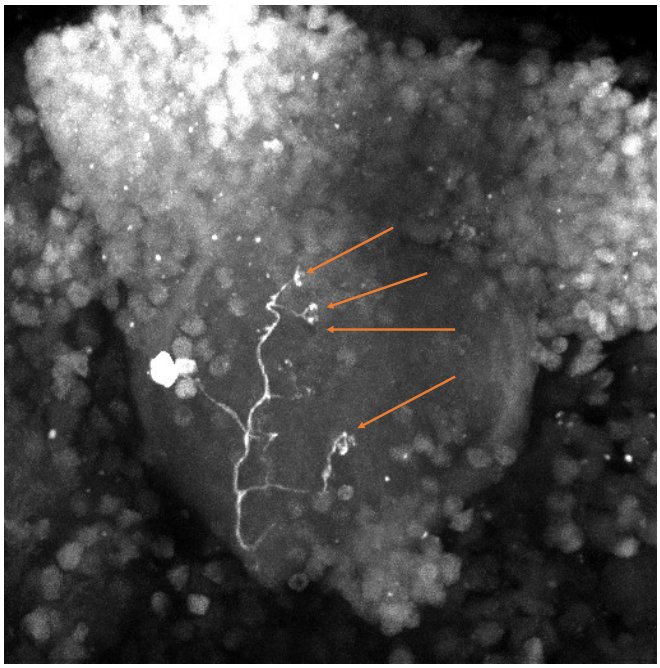


Figure 6. Gamma Control

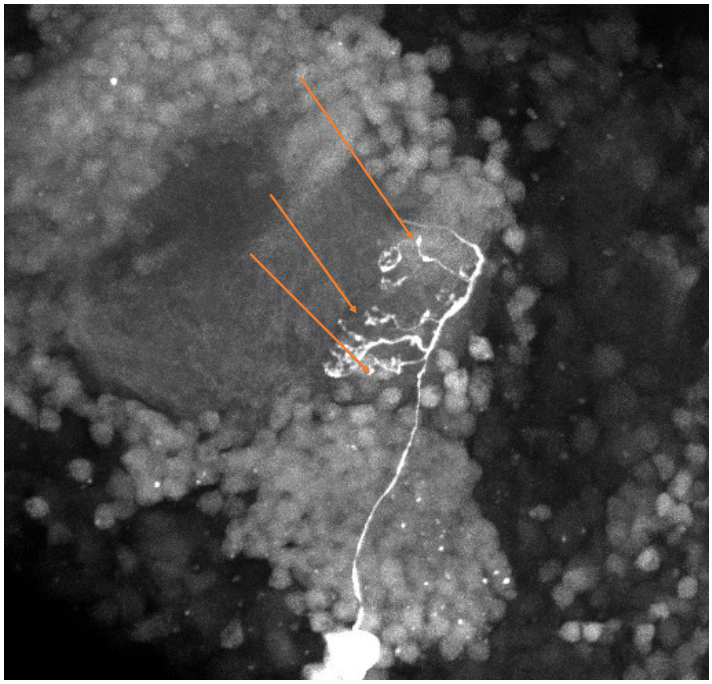


Figure 7. Gamma *Orco -/-*

Methods

Flies

Flies were fed ad libitum with standard cornmeal food, raised in the humidified incubator at on a25C° and 12h:12h light:dark cycle. Below are genotypes used in this study: Control: *w;[nSyb-GAL4]$^{2.2}$, [10xUAS-IVS-Syn21-mC3PA-GFP-p10]^{attP40};;*, *Orco -/-*: *w;[nSyb-GAL4]$^{2.2}$, [10xUAS-IVS-Syn21-mC3PA-GFP-p10]^{attP40};Orco -/-^{2};*. Both flies express photoactivatable GFP in all neurons. To enable visualization of a single or handful of neurons, this photoactivatable GFP has three features (Patterson and Lippincott-Schwartz, 2002; Ruta et al., 2010). 1) The fluorescence intensity in basal state is lower than conventional eGFP; 2) Upon exposure to ultraviolet laser, this photoactivatable GFP increases fluorescence level by 100-fold; 3) It diffuses through cytosol of a cell. If you hit that cell with the ultraviolet laser, diffusing photoactivated GFP will visualize the whole neuron.

Photoactivatable-GFP cell imaging and labeling

2-3-day old females underwent dissection of brains in saline (2 mM $CaCl_2$, 4 mM $MgCl_2$, 1 mM $NaHPO_4$, 4 mM $NaHCO_3$, 10 mM sucrose, 5 mM trehalose, 5 mM HEPES, 5 mM KCl, 108 mM NaCl). Photoactivation and imaging were performed using Ultima, a 2-photon laser scanning scope (Bruker) with an ultrafast Chameleon Ti:S laser (Coherent), that was modulated by Pockels Cells (Conotpics). We used a water immersive objective (60x/1.0 NA, Olympus). A GaAsP detector (Hamamatsu Photonics) was used to detect photons for green fluorescence, and a PMT for red fluorescence. The laser was tuned to 925nm (1 - 14 mW laser power measured after the objective) for imaging, and to 710 nm (5 - 30 mW) for photoactivation. Both imaging and photoactivation was performed with a pixel size of 0.4 μm, the pixel dwell time of 2 μs, and image resolution of 512 x 512. For photolabeling Kenyon cells, the brains were mounted

posterior side up in a Sylgard-coated Petri dish. We defined ~ 1.0 x 1.0 μm region-of-interest square in a cell body of a single Kenyon cell, and averaging-frame number was set to 2 in the microscope GUI software (PrairieView, Prairie Technologies) to scan each pixel 2 times repeatedly. Then single photoactivatable scans were repeated 4-5 times. After photolabeling Kenyon cells, we imaged the calyx of the mushroom body with 2 x zoom. For photolabeling projection neurons, the brains were mounted anterior side up. We defined region-of-interest square in the glomerulus of target, and averaging-frame number was set to 4. This photoactivation scan was repeated 25 times with 10-second interval between each. After photolabeling projection neurons, the brains were turned over, so that the posterior side faced up to see the calyx of the mushroom body. Then we imaged the calyx of the mushroom body with 2 x zoom. We photolabeled two types of projection neurons: DL4 and VA2, both of which receive inputs from OR-type receptors that were eliminated in *Orco* -/-.

Image Analysis

Postsynaptic claw numbers were then analyzed using Fiji. (ImageJ) Each sample of fly brain was analyzed using Fiji by first uploading the file to the software. Then subsequent steps included the conversion of the file to RGB and viewing as a Z stack. It is best to view it as a stack because each file from imaging includes up to 15-60 images, and viewing all as a stack allows for fluid viewing between all images. Usually, the first 10 or so images as well as the last 10 or so images are blank and simply there for diagnostic imaging purposes. Once the brain is clearly visible, I progress through the entire Z stack and pinpoint the location where Kenyon cell connection branching occurs. This is done using the ROI manager in Fiji as well as the tracker tool. Depending on the image type, the amounts of markings will most likely vary. However, in order to properly mark branching patterns and avoid bias, I am blinded to knowing the genotype

of each sample. This way the most accurate comparison can be made between AlphaBeta, Alpha'Beta', and Gamma Kenyon cells. I have an excel sheet where I track all data and am able to import files based on the file name and not genotype. I counted the claw number for each of these samples and rechecked every single one to make sure no mistakes were made, and the most accurate data could be obtained.

I analyzed the presynaptic boutons in the samples where projection neurons were labeled with the photoconverted form of photoactivatable GFP. Using FIJI, I quantified the number of boutons, bouton clusters, and primary branches. Bouton is defined as an individual particle of presynaptic sites, the structure that resembles raisin. Bouton clusters are defined as an aggregate of boutons that looks like a grape that is at least 1 micron far from another cluster. A primary branch is defined as the branch that directly stems from the main shaft of the projection neuron. The main shaft is the single, thick tract that comes from the antennal lobe to end in the lateral horn of *Drosophila* brain. After analyzing each data set, I created a graph detailing the comparisons between genotypes with a program in the lab called Prism version 8. Prism allows for very detailed graphs to be made with statistical input, including t-test, *p* values, and error bar markings to ensure the most accurate analysis of completed data collection. The graph was made by extracting the data I analyzed from excel and creating a specific column for each genotype in Prism. Then, using a t-test with *p* values to check for statistical reliability and validity. Once this was done, I created dot plots with error bars. These graphs can be seen in the results section of this paper.

Results

The results of my work studying presynaptic and postsynaptic connectivity throughout the olfactory circuit of *Drosophila* shows that there is little to no difference in pre and postsynaptic morphology between control fruit flies and *Orco* -/- mutant fruit flies. Figures 8-18 illustrate the comparisons between control and *Orco* -/- mutants for several different categories. Presynaptic boutons were studied for two different projection neurons; VA2 and DL4.

Presynaptic Bouton and Projection Neuron Figures (VA2 and DL4)

These images (Figures 8-11) are important in seeing the different presynaptic boutons with projection neurons. The images portray a stark difference in projection neuron number for VA2 versus DL4 neurons, with VA2 projection neurons highlighting more photolabled presynaptic boutons. This is important as we study the olfactory circuitry with differences in genes.

Figures 12 and 13 show comparisons between the control flies and *Orco* -/- mutant flies with regard to presynaptic boutons. Figure 12 illustrates the number of clusters of boutons for a DL4 projection neuron between the two genotypes and shows a significant difference ($p<.01$) with more clusters of boutons in the control flies than the *Orco* -/- mutant flies. Figure 13 gives an illustration of the number of boutons themselves for DL4 projection neurons as well for each genotype. This figure does not show a significant difference of bouton number between the control and *Orco* -/- mutant genotypes of *Drosophila* fruit flies.

Figure 14 and Figure 15 continue looking at presynaptic plasticity by comparing control flies to *Orco* -/- mutants once again, but are now looking at VA2 projection neurons. I also measured the number of primary branches that stem from the main tract of projection neurons.

Figure 16 is analyzing a DL4 projection neuron, and Figure 17 is analyzing a VA2 projection neuron. I did not observe the difference in the number of primary branches between genotypes.

Kenyon Cell Claw Figures

Next, I analyzed the postsynaptic claws in Kenyon cells. Claw numbers were compared between claw numbers of AlphaBeta, Alpha'Beta', and Gamma Kenyon cells in each genotype. It also shows that for specific Kenyon cell types, there is a similarity of claw numbers across genotypes. The AlphaBeta Kenyon cell claw number is roughly equal to that of Alpha'Beta' Kenyon cell claw number while Gamma Kenyon cell claw number proves to be lesser. This proves to be true for both the control and *Orco* -/- mutant flies. However, in *Orco* -/- mutants, the average claw number per Kenyon cell is higher at 6.7 than that of the control genotype at 5.4 claws per Kenyon cell. This can be seen in Figure 18. The differences between Kenyon cell claw number can be recognized between genotypes. AlphaBeta Kenyon cells and Alpha'Beta' Kenyon cells both exhibit similar numerical quantities within the mushroom body calyx, however, the interesting conclusion that the data points to is that Gamma Kenyon cells behave differently in that they show fewer claws within the mushroom body calyx of *Drosophila*. Figure 11 depicts the important postsynaptic claw numbers per Kenyon cell between control flies and *Orco* -/- mutant flies for each Kenyon cell type. As can be seen in the figure, there is no significant difference between the genotypes for each Kenyon cell type. Postsynaptic claw number is unaffected by the *Orco* -/- knockout flies in this instance. Therefore, the morphology of the presynaptic and postsynaptic sensory olfactory circuitry is not significantly different between the control and *Orco* -/- mutant fruit flies. These results suggest that sensory activity does not exert a large influence on the connectivity development in the calyx of the mushroom body.

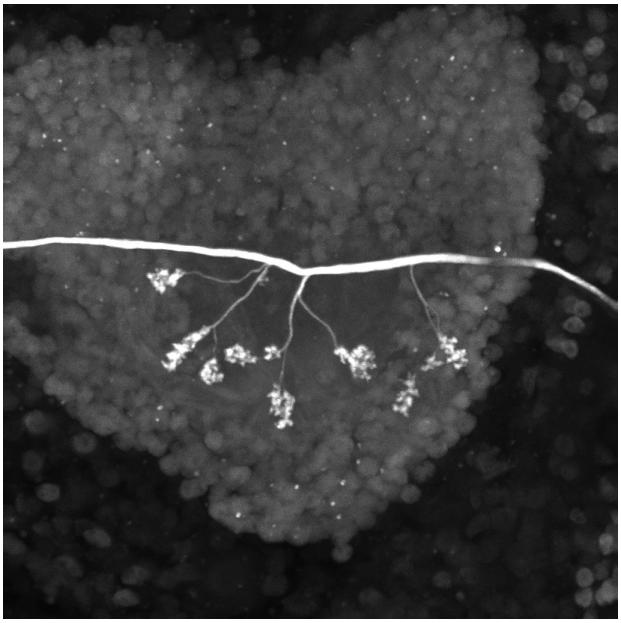


Figure 8. A photo labeled VA2 projection neuron highlighting the presynaptic boutons within the mushroom body calyx of a control*Drosophila* genotype.

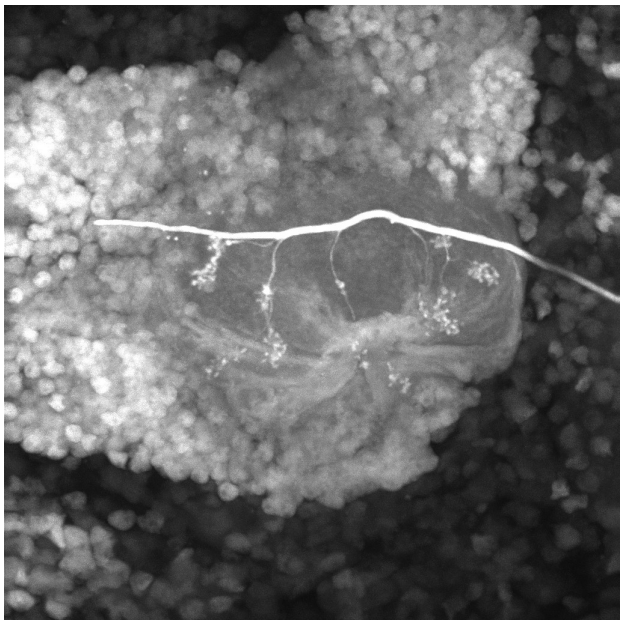


Figure 9. A photo labeled VA2 projection neuron of an *Orco -/-* mutant *Drosophila* genotype.

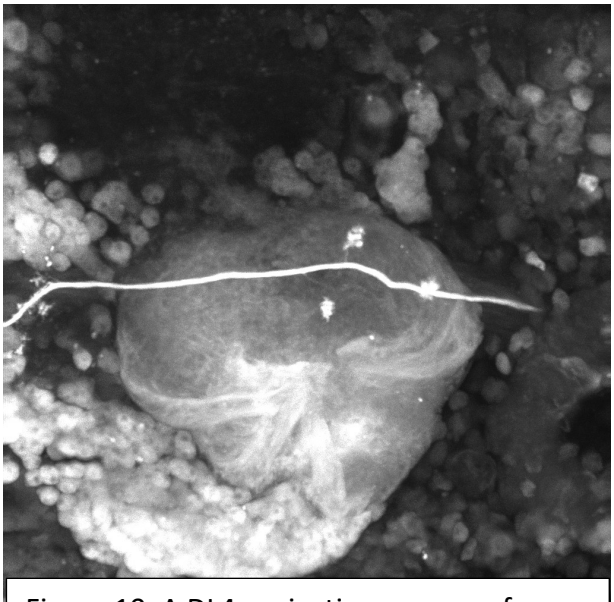


Figure 10. A DL4 projection neuron of an *Orco -/-* mutant *Drosophila* genotype

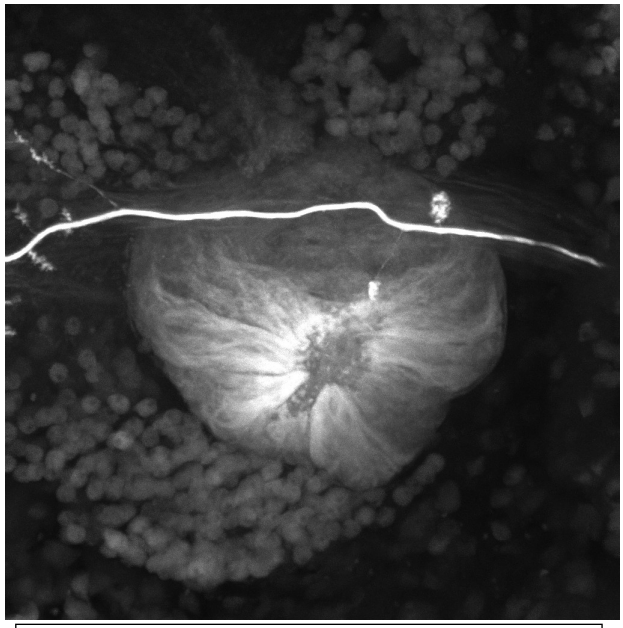


Figure 11. A DL4 projection neuron of a control *Drosophila* genotype within the calyx of the mushroom body.

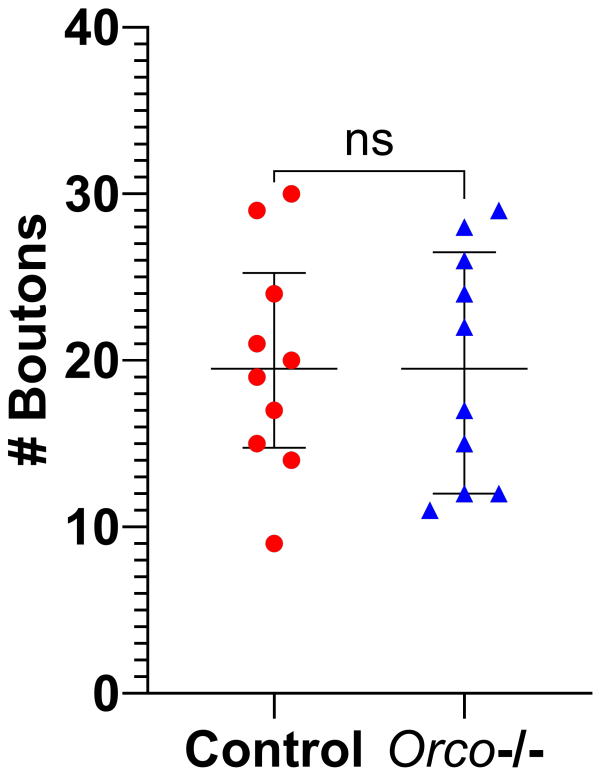


Figure 12. Clusters of boutons for a DL4 projection neuron between the Control and Orco -/- flies.

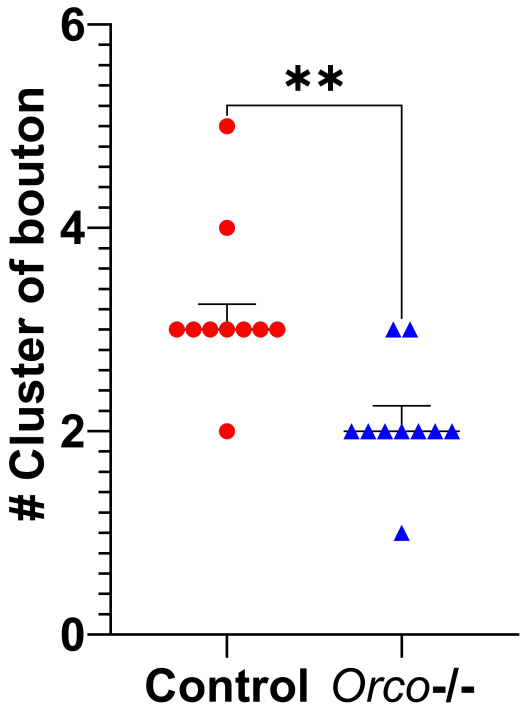


Figure 13. Number of boutons themselves for DL4 projection neurons as well for the Control and Orco -/- flies.

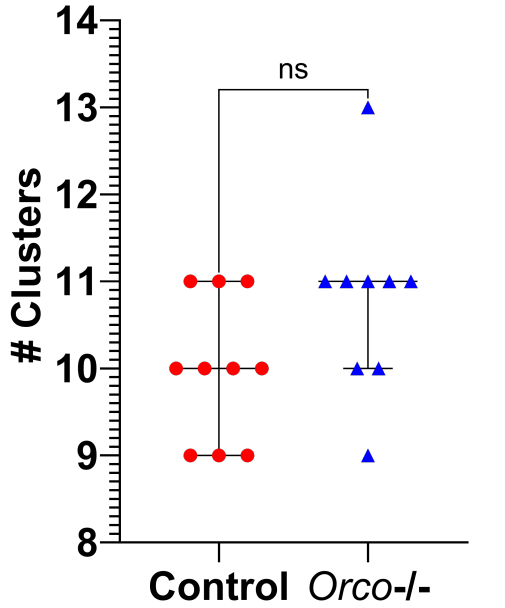


Figure 14. Comparison of the number of VA2 Clusters between Control and Orco -/- flies.

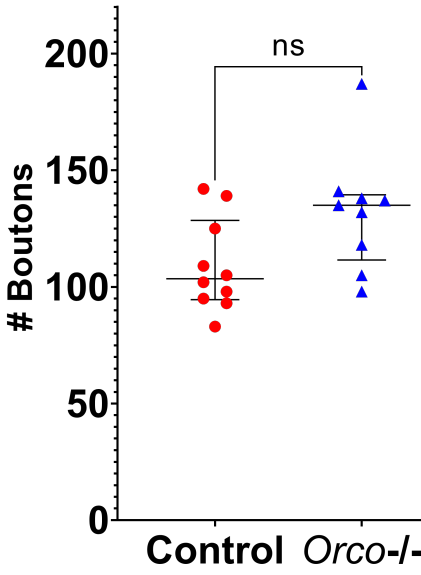


Figure 15. Comparison of the number of VA2 Boutons between the Control and Orco -/- flies.

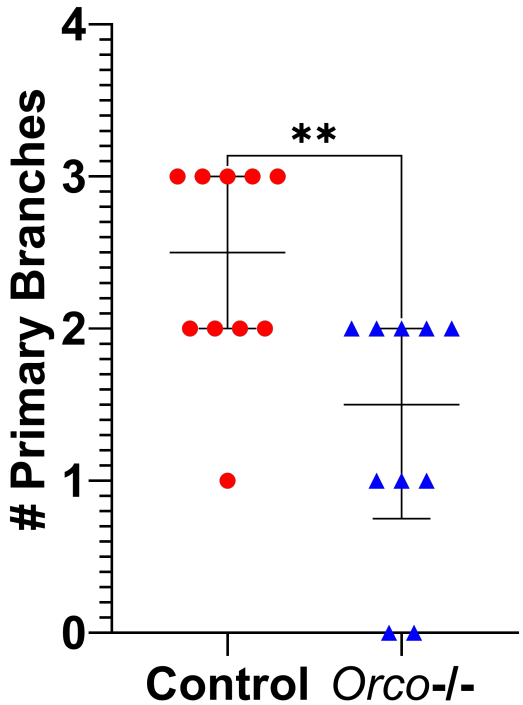


Figure 16. Comparison of the number of Primary DL4 Branches between the Control and Orco -/- flies.

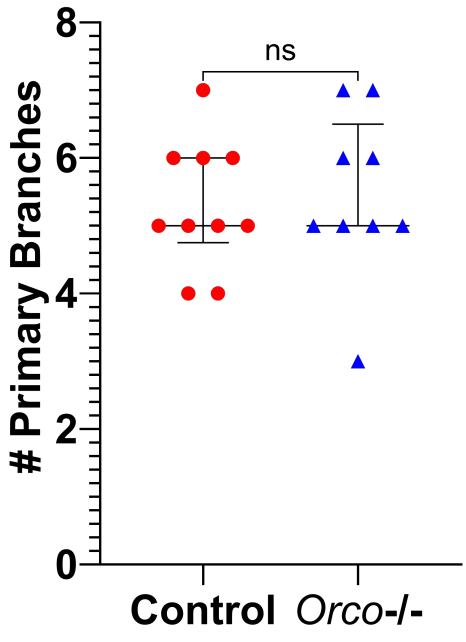


Figure 17. Comparison of the number of VA2 Primary Branches between the Control and Orco -/- flies.

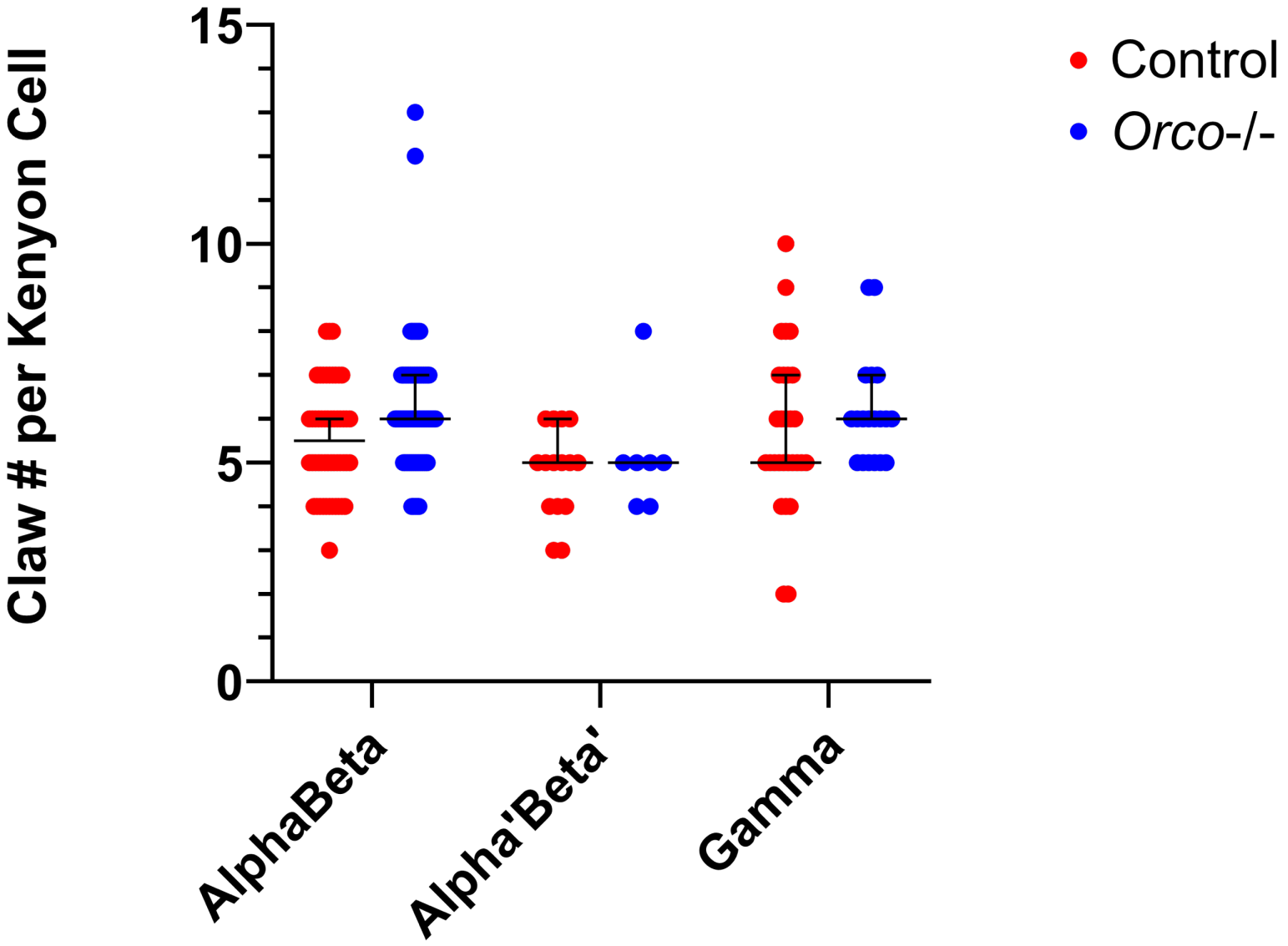


Figure 18. Comparison of Claw numbers for each Kenyon cell type for the Control and Orco -/- flies.

Discussion

My results show that there is no significant difference between *Orco* -/- and wildtype genotypes with regard to presynaptic bouton number and postsynaptic claw number. The main conclusion of this study is that there are subtle to no morphological changes in Projection Neurons and no significant morphological changes to Kenyon cells when comparing between the two genotypes. This means that receptor-driven activity is not a major factor contributing to the synaptic plasticity in the calyx of the mushroom body. There must be another way of forging synaptic plasticity in the olfactory circuit, and we are closer to understanding what is required for doing so. In contrast, we found the reduction of bouton cluster and primary branches in DL4 projection neurons. The interpretation of this result is not simple as VA2 projection neurons did not show statistically significant differences. It is possible that the activity-dependent plasticity exists in a glomerulus-type-specific manner. However, we cannot conclude that the change is caused by the receptor removal; the genetic background segregated by *Orco* -/- knockout may contribute to the change in *Orco* -/-. To test this possibility, we need to manipulate activity in a manner independent of genotype difference, such as chronic odor exposure or optogenetic activation of the olfactory circuit.

As the results point out, it is important to recognize the differences between genotypic ratios of Kenyon cell claw numbers because they are not the same in each genotype. This finding implicates important functional and genetic considerations. The olfactory circuit can be somewhat variable between genotypes of *Drosophila*; however, Kenyon cell types can play a role in synaptic plasticity and olfaction memory enhancement as well[3]. Recent studies have shown how synaptic plasticity is the crutch to learning and possible behavioral variabilities. However, now we see more into the presynaptic and postsynaptic connectivity matrices and their

importance in relaying information to the mushroom body calyx dependent on a specific olfactory sensation. This information allows us to dive deeper into the manner of specific Kenyon cell responses and more.

To further enhance studies considering the mushroom body calyx in the context of olfaction with presynaptic and postsynaptic plasticity, Kenyon cell branching lengths will be studied to determine if there is any significant difference between genotypes and/or Kenyon cell types. This will allow for more insight into how these connections aid in memory formation are made. Future studies will continue researching this complex mechanism to try to recognize unique patterns within the fly brain for memory formation and retention.

This study has shown that there is little to no difference between these genotypes of *Drosophila*, and that another animal must be studied as well to determine the mechanism behind olfactory synaptic plasticity within the mushroom body calyx. We know that sensory circuits in mammals exhibit the plasticity induced by sensory activity – that is the striking contrast to the connectivity of the *Drosophila* olfactory circuit, as we did not detect the change in *Orco* -/- morphology. Once we determine what this mechanism of connectivity development is, we can compare and contrast it to mammals, especially humans. Such comparative studies will contribute to better understanding what factors confer activity-dependent plasticity within the sensory circuit in general. This will also lead to a better understanding of memory and brain functioning in human beings. Now that this study has shown no difference between these genes, we are closer to making that connection and reaching that understanding.

In conclusion, synaptic plasticity is incredibly important and relevant to us as human beings because our brains form memories and retain knowledge learned by creating and fortifying new connections. It is these connections that allow us to live the very lives that we do,

and they allow us to form hopes and dreams for the future. Our brains work in such a remarkable way that we are only beginning to understand, but what we do know is that synaptic plasticity is paramount to our very survival as a species. If we can create new connections, we can propel our species forward through advanced understanding and learning. *Drosophila* fruit flies are a great microcosm of our experience in that their brains work in the same way, only on a simpler scale.

Acknowledgements

I would like to extend great thanks to Tatsuya Hayashi for mentoring me on this project and guiding me throughout. I would like to especially thank Dr. Sophie Caron for allowing me the opportunity to work in her lab for many years. I would also like to thank all of the other Caron lab members who have helped me along the way, especially Dane Larsen, for mentoring me over the past several years. The Sophie Caron Laboratory has been a research haven for me, dating back 3 years now, and I am extremely grateful for the opportunity I have had to participate in neurobiological research there as an undergraduate student. My research has allowed me to view how different genes can affect morphology and synaptic plasticity in the olfactory circuit. Moreover, it has shown me how cellular morphology can contribute to the complexity of the olfactory circuitry, and how we must continue studying different genes to garner a deeper understanding of how the circuitry is developed. Understanding how claw numbers are different in unique genotypes of *Drosophila* allows us to take a step towards a greater understanding of ourselves. The olfactory circuit has proven to be an example of how our brain can potentiate our subjective realities, and what are we without that? Although small, my research gives further insight into how our amazing brains do what they do and will hopefully allow for deeper investigation and discovery to take place in the future.

Literature Cited

1. Akalal DB, Wilson CF, Zong L, Tanaka NK, Ito K, Davis RL. 2006. Roles for Drosophila mushroom body neurons in olfactory learning and memory. Learn Mem 13:659 – 668.
2. Ashraf SI, McLoon AL, Sclarsic SM, Kunes S. 2006. Synaptic protein synthesis associated with memory is regulated by the RISC pathway in Drosophila. Cell 124:191–205.
3. Aso Y, Grubel K, Busch S, Friedrich AB, Siwanowicz I, Tanimoto H. 2009. The mushroom body of adult Drosophila characterized by GAL4 drivers. J Neurogenet 12:1–17.
4. Benton, R., Vannice, K.S., Gomez-Diaz, C., Vosshall, L.B. (2009). Supplemental Experimental Procedures. Cell 136(1): .
5. Bonhoeffer T, Yuste R. 2002. Spine motility. Phenomenology, mechanisms, and function. Neuron 35:1019 –1027. Connolly JB, Roberts IJ, Armstrong JD, Kaiser K, Forte M, Tully T, O'Kane
6. Caron, Sophie J. C. "Brains Don't Play Dice-or Do They?" *Science*, American Association for the Advancement of Science, 1 Nov. 2013, science.sciencemag.org/content/342/6158/574.2.long.
7. Couto, A., Alenius, M. & Dickson, B. J. Molecular, anatomical, and functional organization of the Drosophila olfactory system. Curr. Biol. 15,1535–1547 (2005).

8. Doll, S., Dreßen, M., Geyer, P.E. *et al.* Region and cell-type resolved quantitative proteomic map of the human heart. *Nat Commun* **8,** 1469 (2017). https://doi.org/10.1038/s41467-017- 01747-2

9. Fishilevich, E. & Vosshall, L. B. Genetic and functional subdivision of the Drosophila antennal lobe. Curr. Biol. 15, 1548–1553 (2005).

10. Jefferis, G. S., Marin, E. C., Stocker, R. F. & Luo, L. Target neuron prespecification in the olfactory map of Drosophila. Nature 414, 204–208 (2001).

11. Galizia CG, Sachse S. Odor Coding in Insects. In: Menini A, editor. The Neurobiology of Olfaction. Boca Raton (FL): CRC Press/Taylor & Francis; 2010. Chapter 2. Available from: https://www.ncbi.nlm.nih.gov/books/NBK55975/

12. Gomez-Diaz, Carolina et al. "The Two Main Olfactory Receptor Families in Drosophila, ORs and IRs: A Comparative Approach." Frontiers in cellular neuroscience vol. 12 253. 30 Aug. 2018, doi:10.3389/fncel.2018.00253

13. Jones, W.D., Cayirlioglu, P., Kadow, I.G., Vosshall, L.B. (2007). Two chemosensory receptors together mediate carbon dioxide detection in Drosophila. Nature 445(7123): 86--90.

14. Kremer, M.C., Christiansen, F., Leiss, F., Paehler, M., Knapek, S., Andlauer, T.F., Förstner, F., Kloppenburg, P., Sigrist, S.J., Tavosanis, G. (2010). Structural long-term changes at mushroom body input synapses. Curr. Biol. 20(21): 1938--1944.

15. Kwon, J.Y., Dahanukar, A., Weiss, L.A., Carlson, J.R. (2007). The molecular basis of CO2 reception in Drosophila. Proc. Natl. Acad. Sci. U.S.A. 104(9): 3574—3578

16. Marin, E. C., Jefferis, G. S., Komiyama, T., Zhu, H. & Luo, L. Representation of the glomerular olfactory map in the Drosophila brain. Cell 109, 243–255 (2002).

17. Peterson, Mark D et al. “Resistance exercise for muscular strength in older adults: a meta- analysis.” Ageing research reviews vol. 9,3 (2010): 226-37. D oi:10.1016/j.arr.2010.03.004

18. Saberi, M., Seyed-allaei, H. Odorant receptors of *Drosophila* are sensitive to the molecular volume of odorants. *Sci Rep* 6, 25103 (2016). https://doi.org/10.1038/srep25103

19. Shang, Y., Claridge-Chang, A., Sjulson, L., Pypaert, M. & Miesenbock, G. Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe. Cell 128, 601–612 (2007).

20. Stocker, R. F., Heimbeck, G., Gendre, N. & de Belle. J. S. Neuroblast ablation in Drosophila P[GAL4] lines reveal origins of olfactory interneurons. J. Neurobiol. 32, 443–456 (1997). Stocker, R. F. The organization of the chemosensory

21. Tanaka, N.K., Awasaki, T., Shimada, T., Ito, K. (2004). Integration of chemosensory pathways in the Drosophila second-order olfactory centers. Curr. Biol. 14(6): 449--457.

22. Wilson, R. I. & Laurent, G. Role of GABAergic inhibition in shaping odor-evoked spatiotemporal patterns in the Drosophila antennal lobe. J. Neurosci. 25, 9069–9079 (2005)