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Effects of Reproductive Maturity on Neuroplasticity in Male Bumble Bees (Bombus impatiens)

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EFFECTS OF REPRODUCTIVE MATURITY ON NEUROPLASTICITY IN MALE BUMBLE BEES

(*BOMBUS IMPATIENS*)

by

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Abstract

Within the span of an insect's lifetime, new synaptic connections between neurons are made in response to age and experience. This neuroplasticity leads to anatomical changes within individual brain regions that represent investment in different brain functions. The neuroplasticity of female bumble bees has been well studied, but almost nothing is known about how the male bee brain responds to life experiences. Due to the complex sensory requirements of mate finding and copulation for males, neuroplasticity is likely to be an important component of reproduction. These sensory requirements include olfactory and optic inputs, which are processed in the calyces of insect brains. The aim of my project is to document anatomical changes in brain structures associated with reproductive maturity in male bumble bees (Bombus impatiens). I hypothesized that neurological investment in optic and olfactory function will change as male bees reach reproductive maturity, as they use these senses to locate mates. Because the mushroom body calyces are the structures that receive inputs from optic and antennal lobes, I predicted that the volumes of these mushroom body calyces will increase in volume as the bees age. My work initially consisted of designing a unique protocol, then caring for the microcolonies that produced the males used in the study. Males were collected either promptly after emerging or ten days later, then sacrificed for the purpose of investigating their brains. The brains were dissected, cleaned, and bleached using a protocol specific to this project. Using confocal microscopy, brain images were taken at 5 µm intervals, which were then traced using ImageJ software to create 3D renderings. Volumetric measurements were obtained for individual brain structures, then analyzed to procure the

proportion of half of the whole brain constituted by each structure. I then ran t-tests on the data, looking for a significant increase or decrease in the size of brain structures between newly-emerged and 10-day-old bees. A significant increase in the size of the calyces was observed, confirming this structure's likely involvement in the preparation for reproduction. This study helps to set the stage for future research into the neural workings and associations of these bees.

Acknowledgements

First I would like to thank my capstone mentor, Dr. Karen Kapheim, for the incredible support she has given me throughout this project. I am so grateful for the opportunity she gave me to work in her lab and for the delightful addition it has been to my undergraduate education. Her counseling and insights into my project and the writing process were invaluable and so appreciated.

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In addition, I would like to thank the other members of my committee, Drs. Kim Sullivan and Erin Bobeck, for their willingness to help me write a polished thesis. I would also like to thank the Honors Program at Utah State for the opportunity to complete this capstone project.

I would like to thank the members of the Kapheim lab for being such an exceptional support system through this process. I would specifically like to thank Anna Figgins for helping me care for my microcolonies and for her emotional support as well.

Lastly, I would like to thank my family, especially my dad, for patiently listening to me rant and "nerd out" about bees, and for their constant love and support during this project.

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Introduction

In bees, sensory experiences influence neuronal investment in different brain regions. Changes in this investment over the course of a lifetime are referred to as neuroplasticity (Pyza, 2013). Due to the complex sensory requirements of mate finding and copulation, neuroplasticity is likely to be an important component of reproduction (Ayasse et al., 2001). These sensory requirements include olfactory and optic capabilities, and the aptness of male bumble bees to utilize these senses is crucial to locating and mating with a female (Bastin et al., 2018).

Studies involving neuroplasticity in eusocial insects such as bumble bees almost exclusively focus on females (Lichtenstein et al., 2015), while studies which have explored the subject of neuroplasticity in males are severely lacking. For centuries males have garnered little interest, even going back to the time of Aristotle, who described them as "devoid of sting and lazy" (Thompson, 1907). However, a recent study by Lichtenstein et al. (2015) revealed that males perform just as well as females in basic learning tasks, although workers do significantly better in social tasks. A similar study by Wolf & Chittka (2016) showed that behavioral sex dimorphism does not affect learning ability beyond the mating context in bumble bees. By using similar methods as have been employed by studies on females, insights can be gained into the neuroplasticity of the male bumble bee brain and its role in reproduction.

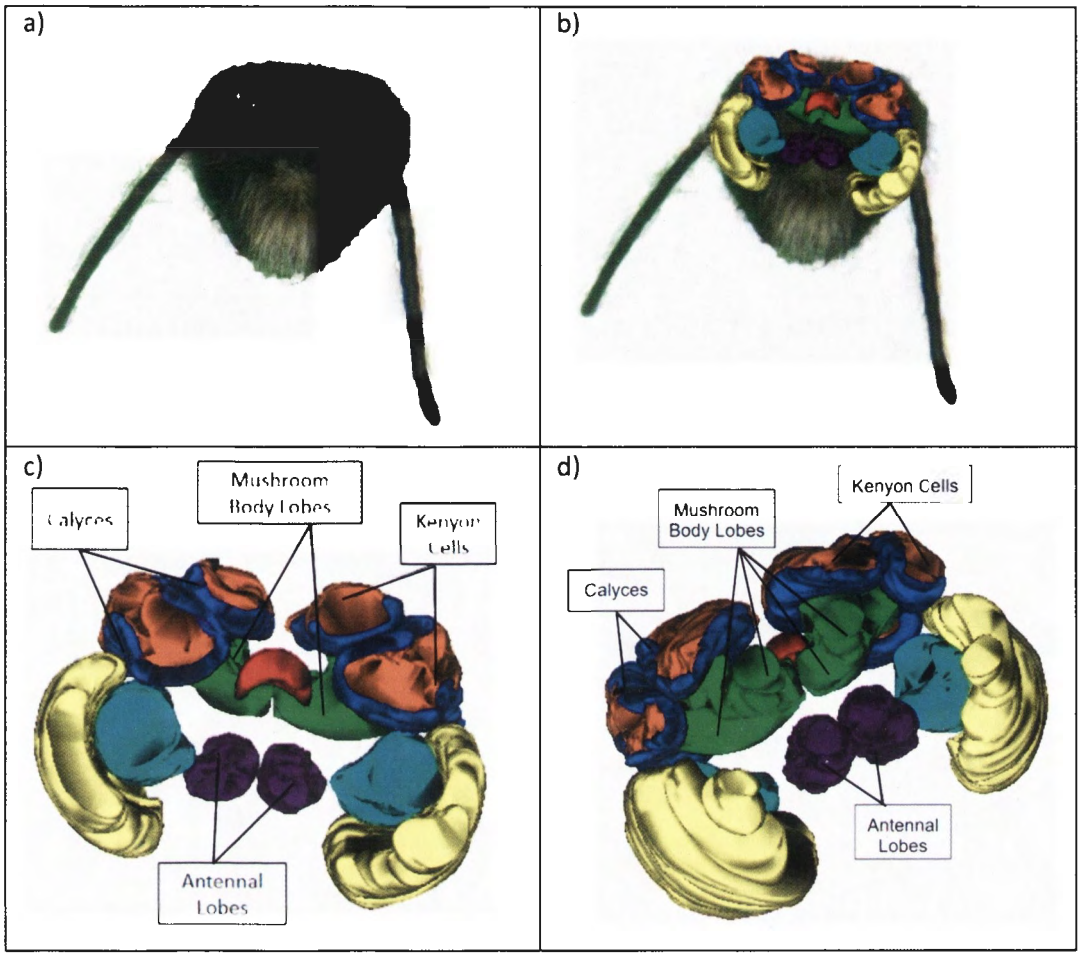

Figure 1. a) Image showing the anterior view of a male bumble bee head capsule, b) image showing the anterior view of a male bumble bee head capsule with an overlaid brain, c) image showing the anterior view of a male bumble bee brain, created using Reconstruct software, with labeled calyces, mushroom body lobes, Kenyon cells, and antennal lobes, and d) image showing the posterior view of a male bumble bee brain, created using Reconstruct software, with labeled structures.

Two major structures within the brains of insects where neuroplasticity may be observed are the mushroom bodies and the antennal lobes. Described in 1853 by biologist Félix Dujardin as the center for *"l'intelligence"*, the mushroom bodies play a critical role in learning and memory (Fahrbach, 2006). These key structures confer the intelligence necessary to

perform complex behaviors such as foraging, navigation, and mate selection. Mushroom bodies are composed of three basic elements: the Kenyon cells, calyces, and lobes (Fig. 1c and d). The Kenyon cells, named after the biologist who first described them, are densely packed neurons located atop the calyces (Kenyon, 1896). Neurites, or cell body fibers, extend from the Kenyon cells towards the other structures within the mushroom body (Strausfeld et al., 1998). A typical Kenyon cell extends a neurite towards the calyces as a dendrite-like branch with arborizations and another neurite towards the lobe as an axon-like branch (Fahrback, 2006).

The calyces are cup-like structures which surround the Kenyon cells. Each calyx is made of three concentric rings: the lip, collar, and basal ring, which receive olfactory, optic, and multisensory information, respectively (Strausfeld et al., 1998). Much of the input to the calyces arrives from axons extending from dendritic arborizations in the antennal lobes. These antennal lobes receive olfactory information from the sensory glomeruli in the antennae (Lihoreau, 2012). The optic lobes, which receive information from the compound eye, also supply substantial input to the calyces in social insect brains (Fahrback, 2006).

In bee brains, the calyces take a double form, and axons extending from the two groupings of Kenyon cells merge to form a single neuropil on each side of the brain (Fahrback, 2006). These axons extend into single mushroom body peduncles, which bifurcate into alpha- and beta-lobes (Heisenberg, 1998). These structures (lobes and peduncles) are collectively referred to as the mushroom body lobes.

The brains of adult insects are notably larger than those of newly-emerged insects. This increase in brain size occurs because during the span of an insect's lifetime, it forms new synaptic connections in response to age and experience (Pyza, 2013). As the number of synaptic

connections between a group of neurons increases, so does the speed and efficiency of communication between those neurons (Abbott & Nelson, 2000). The strength of synaptic connections can also be increased following increased amounts of stimulation at the same synapse, known as long-term potentiation (Menzel & Manz, 2005). As these synaptic changes occur, anatomical changes can be visualized, which represent investment in different brain functions.

Throughout an insect's lifetime, two different types of neuroanatomical changes can occur. The first are experience-expectant changes, which are programmed expansions of the mushroom body neuropil in the first days of adult bee life (Fahrback et al., 1998). Studies have shown that these expansions occur even when bees are deprived of light and socialization. In social Hymenoptera, such as bumble bees, these changes can be observed by increased neuropil to Kenyon cell ratios (N:K) (Withers et al., 1993). This increase in synaptic connections may occur simply because of aging, or in preparation for tasks such as foraging or mating (Fahrback et al., 1998). Research suggests that different structures within the mushroom body may undergo different rates of experience-expectant changes.

The second type of neuroanatomical changes are experience-dependent changes. This type of change is characterized by extensive expansion of the calyces (Fahrback et al., 1998). As the mushroom body's major input region, the calyces expand in response to sensory experience and foraging, so this type of expansion is especially associated with learning (Jones et. al, 2013). Such changes can be observed through changes in brain volume as male bees age.

These experience-dependent changes reflect the neurologically-stimulating events bees encounter during their lives. For the first few days of their lives, newly-emerged males feed on

honey and pollen in their maternal nest (Alford, 1975). Around day three or four, these males begin orientation and mating flights (Frison, 1917), which generally involve the search for mating partners through the detection of sexual pheromonal blends (Bastin et al., 2018). These are odors released by either the male or female to stimulate a behavioral reaction and bring the two sexes together for the purpose of mating (Ayasse et al., 2001). The rapid adaptation of the male olfactory system for recognizing and processing a female's pheromones is critical to the male's likelihood of finding a proper mating partner (Bastin et al., 2018). As previously discussed, these functions are involved with neuroplasticity in the bee brain, specifically in the calyces.

The males reach reproductive maturity by day seven, which is evident by the presence of sperm in the accessory testes. This is accompanied with a noticeable drop in the size of the testes relative to the accessory testes, as sperm moves to the accessory testes in preparation for mating (Herndon, 2019). Male bumble bees generally live for three to four weeks, and in addition to obtaining food requirements from flowers, the primary task of a reproductively mature male is to find an inseminate a young queen (Alford, 1975).

The aim of this research project is to document anatomical changes in brain structures associated with reproductive maturity in male bumble bees. I hypothesized that neurological investment in optic and olfactory function will change as male bees reach reproductive maturity, as they use these senses to locate mates. Because the mushroom body calyces are the structures that receive inputs from optic and antennal lobes, I predicted that the volumes of these mushroom body calyces will increase in volume as the bees age.

Methods

Microcolonies

Males used in the experiment were produced by female *Bombus impatiens* workers. When separated from the queen, bumble bee workers are capable of laying unfertilized eggs. The colonies that produced these females were from Koppert Biological Systems (MI, U.S.A.). I randomly selected workers from three different colonies and placed them into microcolonies within isolated rearing cages. These cages were 173 x 130 x 91 mm food storage containers modified to include an aluminum mesh bottom and hinged plexiglass top. I created fifty-eight microcolonies, each containing five females from the same original colony. I supplied microcolonies with sugar water (cane sugar, citric acid, potassium sorbate, Honey B Healthy Amino Boost, Honey B Healthy Essential Oil, and distilled water) and pollen dough (aforementioned sugar water and pollen) ad libitum. These microcolonies were stored in an incubator to control for temperature and humidity, which were kept at 27°C and ~60-70% respectively. I checked the microcolonies daily to monitor sugar water and pollen consumption, as well as brood development.

Brood cells were checked every 24 hours. During these checks, I removed newly-emerged males from the microcolonies and randomly assigned them to a treatment group. Males in the newly-emerged treatment group were sacrificed immediately. Males in the aged group were placed into isolated rearing cages, given sufficient sugar water and pollen, and kept in the incubator for ten days. On day ten, the males in the aged treatment group were removed and sacrificed.

Preservation and Dissection

Upon sacrificing, I removed the eye capsules and mouth parts, then decapitated the males, placing the heads in 4% Paraformaldehyde (PFA), and storing at 4°C to fix the brains homogeneously. After 18 head capsules were obtained, they were rinsed three times (10 minutes each) in phosphate-buffered saline (1x PBS). Next I dissected the brains from the head capsules while in 1x PBS. They were then placed in 2% glutaraldehyde as a post-fixation step and stored at room temperature.

After 48 hours in the glutaraldehyde, I prepped the brains for imaging. This involved first rinsing three times in 1x PBS for ten minutes, then bleaching in a formamide bleach solution (1x PBS, 3 % formamide, 1 % triton-X, and 20% hydrogen peroxide) (modified recipe from K. Zukor et al., 2010). The brains were left in the formamide bleach solution for between 36 and 45 minutes. Following the bleaching they were again rinsed in PBS three times for ten minutes. A serial dehydration was then conducted through a series of ascending ethanol concentrations (30%, 50%, 70%, 90%, 95%, and 3 x 100%, 10 minutes each). Once the serial dehydration was complete, the brains were cleared and stored in methyl salicylate at -20°C until imaging.

Confocal Microscopy

Visualization of these structures often utilizes confocal microscopy. This technique creates stacks of insect brain images which can then be digitally merged to create a single structure conducive to visualization and volumetric analysis (Fahrbach, 2006). To produce images of the different brain structures, the brains were scanned with a laser scanning confocal microscope (Zeiss LMS 710). Whole brains were mounted in methyl salicylate and imaged at 5 µM intervals (Fig. 2). Individual slices were imaged as 3 x 2 tile scans (2867 x 1946 pixels) and

combined to form image stacks ranging from 760–895 µm. Brains were imaged simultaneously using two lasers. The first had wavelength, laser power, and a range of gains designated as 410–485 nm, 4.0, and between 527–567, respectively. The second laser utilized a wavelength of 495–538 nm, 3.5 laser power, and a gain range including 518–558. Whole brain image stacks were saved as individual jpegs.

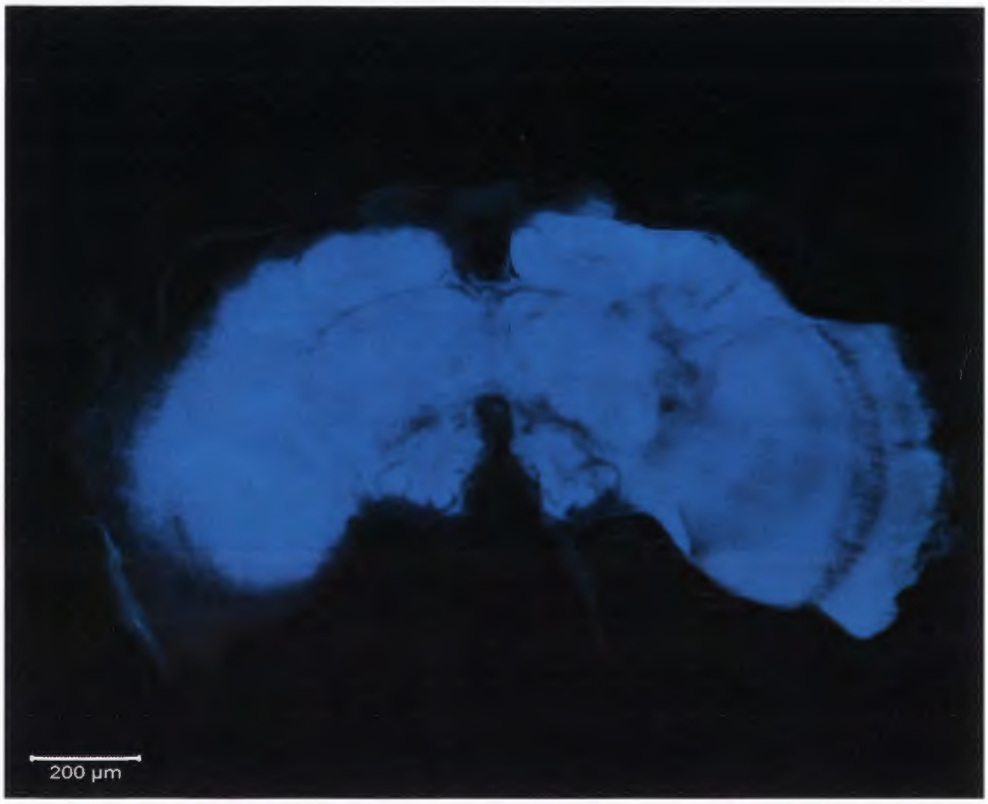

Figure 2. Image of a brain taken using confocal microscopy. Calyces are seen as u-shaped structures in the superior region of the brain.

Structure Tracing and 3D Reconstruction

Images were analyzed using Reconstruct software Version 1.1.0.0 (available at http://synapses.clm.utexas.edu). I traced each structure unilaterally on every other slide (i.e., every 10 µm). The side of the image to be traced was determined randomly. Structures traced

included medial and lateral calyces, medial and lateral Kenyon cells, mushroom body lobes (containing alpha and beta lobes and peduncles), antennal lobes, and half of the whole brain (Fig. 1c and d). 3D renderings were then created of each brain and volumetric measurements for each structure was obtained.

Statistical Analysis

I determined the proportion of the half brain constituted by each brain structure. I then tested for differences in the relative volume of each structure between treatment groups using independent-sample t-tests using SSPS software (IBM Corp., 2017). The dependent variables for the tests were the proportions of the half brain constituted by each structure, while the independent variable was the treatment group (newly-emerged versus aged).

I tested six assumptions before performing the t-test. First, I determined that the dependent variable was on a continuous scale. Proportions of the half brain fell between zero and one on a continuous scale, confirming this assumption. Second, I established that the independent variables were two independent groups. The newly-emerged and aged treatment groups were independent of each other, validating this assumption. Third, I confirmed an interdependence of observations in the study. Each structure in each brain was analyzed once and integrated into a single t-test, confirming this assumption. Fourth, I inspected the data set for outliers, and upon examination a few outliers were discovered. The corresponding bees were identified and no abnormalities, such as different handling during dissection or origin colony, were observed. For these reasons, the outliers were kept in the data. Fifth, I verified a normal distribution of the dependent variables. The Shapiro-Wilk test for was used to test for normality. All but two of the structures (lateral calyces and combined calyces) met the

conditions for normality. These data were then transformed using a square root transformation, and to further assess the normalcy of these two structures, Q-Q plots were analyzed. All data fell within the plots, so it was determined that the structures met the assumption for normality. Sixth, I checked for a homogeneity of variance. This was done using Levene's Test for Equality of Variances. All but two of the structures (lateral Kenyon cells and combined Kenyon cells) met the conditions of this test.

Results

The lateral calyces occupied 3.70% (+/- 0.001) of the newly-emerged bee brains (n=11) and increased to 4.83% (+/- 0.003) by 10 days of age (n=7). Due to this large increase in volume, the lateral calyces were significantly larger in reproductively mature males than in newly-emerged males ($t = -4.123$, $p = 0.001$, n=18).

The medial calyces occupied 2.96% (+/- 0.005) of the newly-emerged bee brains (n=11) and increased to 3.46% (+/- 0.006) by 10 days of age (n=7). Despite this increase in volume, the medial calyces were not significantly larger in reproductively mature males than in newly-emerged males ($t = -1.915$, $p = 0.074$, n=18).

For a more thorough analysis of structures in the brain, the data from lateral and medial calyces were summed to form combined calyces. These combined calyces occupied 7.76% (+/- 0.007) of the newly-emerged bee brains (n=11) and increased to 8.29% (+/- 0.006) by 10 days of age (n=7). Due to this large increase in volume, the combined calyces were significantly larger in reproductively mature males than in newly-emerged males ($t = -4.293$, $p = 0.0006$, n=18; Fig. 3a).

The lateral Kenyon cells occupied 3.33% (+/- 0.008) of the newly-emerged bee brains (n=11) and decreased to 2.81% (+/- 0.004) by 10 days of age (n=7). Despite this decrease in volume, the lateral Kenyon cells were not significantly smaller in reproductively mature males than in newly-emerged males ($t = -1.77$, $p = 0.097$, n=18).

The medial Kenyon cells occupied 2.72% (+/- 0.008) of the newly-emerged bee brains (n=11) and decreased to 2.34% (+/- 0.004) by 10 days of age (n=7). Despite this decrease in

volume, the medial Kenyon cells were not significantly smaller in reproductively mature males than in newly-emerged males ($t = 1.126$, $p = 0.227$, $n=18$).

Similar to the calyces, the data from lateral and medial Kenyon cells were summed to form combined Kenyon cells for a more thorough analysis of the structures of the brain. The combined Kenyon cells occupied 6.05% (+/- 0.015) of the newly-emerged bee brains (n=11) and decreased to 5.15% (+/- 0.006) by 10 days of age (n=7). Despite this decrease in volume, the combined Kenyon cells were not significantly smaller in reproductively mature males than in newly-emerged males ($t = 1.839$, $p = 0.087$, $n=18$; Fig. 3b).

The mushroom body lobes were found to be the largest structures analyzed in this study. They occupied 4.06% (+/- 0.007) of the newly-emerged bee brains (n=11) and increased to 4.52% (+/- 0.006) by 10 days of age (n=7). Despite this increase in volume, the mushroom body lobes were not significantly larger in reproductively mature males than in newly-emerged males ($t = -1.42$, $p = 0.175$, $n=18$; Fig. 3c).

Antennal lobes on average occupied 2.21% (+/- 0.001) of the newly-emerged bee brains (n=11) and increased to 2.60% (+/- 0.002) by 10 days of age (n=7). Despite this increase in volume, the antennal lobes were not significantly larger in reproductively mature males than in newly-emerged males ($t = -1.8$, $p = 0.09$, $n=18$; Fig. 3d).

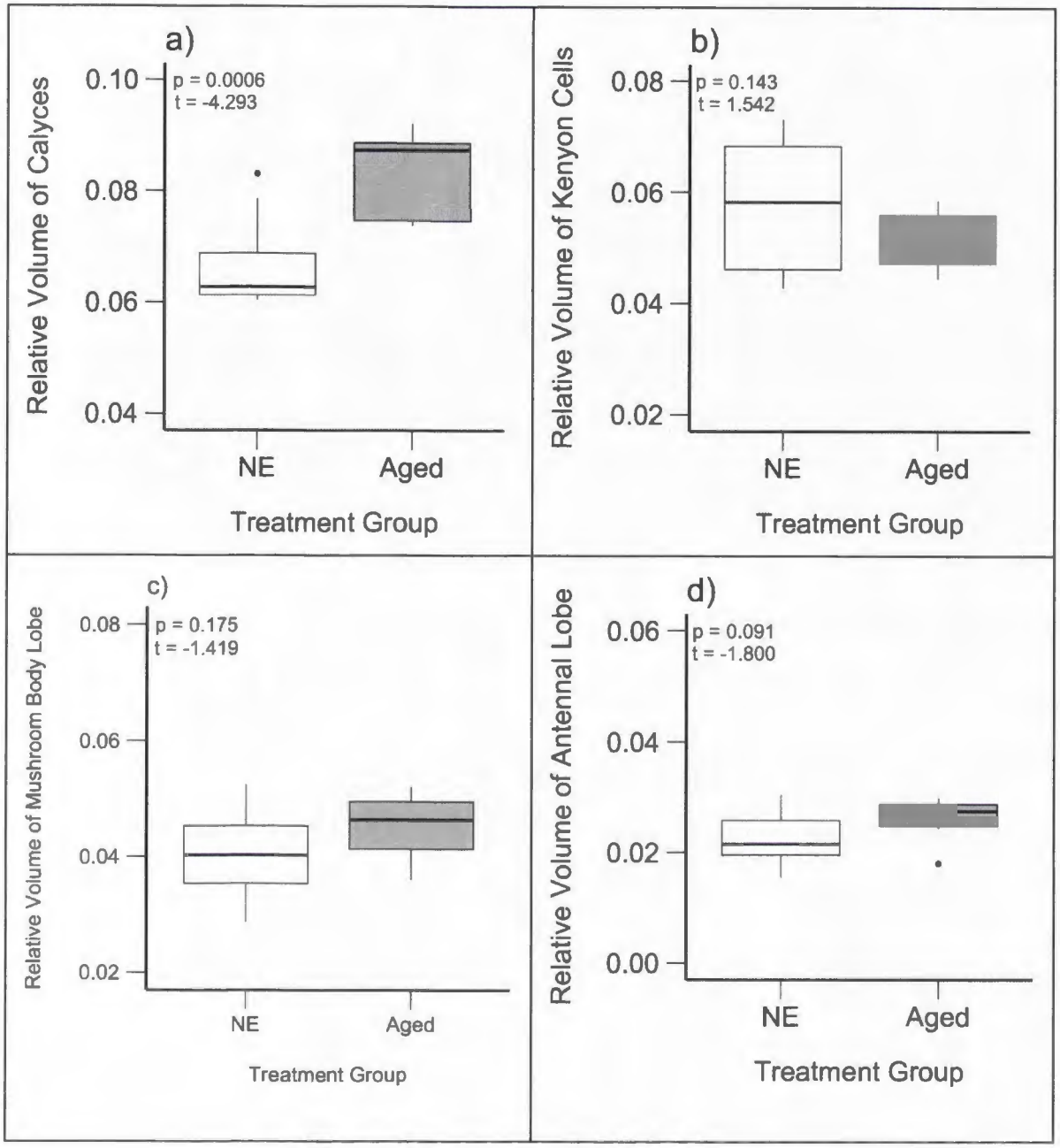

Figure 3. Box plots comparing structure volumes of newly-emerged and aged males for a) combined calyces, b) combined Kenyon cells, c) mushroom body lobe, and d) antennal lobe. Box plots were created using ggplot2 software in R (Wickham, 2016). Thick horizontal lines represent mean proportion of half of the whole brain volume. Box edges represent 25th and 75th interquartile range. Outliers in figures a & d are represented by points.

Discussion

The brains of male bumble bees have not been well studied or documented (Lichtenstein, 2015). A better understanding of the neural workings of these brains begins with an investigation of their neuroanatomy. By examining patterns of neuroplasticity associated with reproductive maturation in male bees, we have established a foundation for further research into this fascinating topic. By rearing males in the laboratory in isolation, I was able to document anatomical changes that occur in the brain as a function of age, independent of relevant ecological experience such as flight or mate searching. This provides insight into experience-expectant neuroplasticity in male bees as a function of reproductive maturation and age (Fahrback et al., 1998).

My results show that the mushroom bodies undergo major synaptic reorganization as male bees age. This was most apparent as an increase in the relative volume of the calyces from newly-emerged bees to those aged to 10 days. These structures are involved in olfactory and optic integration, which are key processes during the search for a mate. It is thus likely that the mushroom body calyces play a vital role in male bumble bee mate finding. The nature of my experimental design (i.e., rearing males in isolation of potential mates) suggests that this increased investment in the calyces occurs in preparation for mate searching, and is not merely a response to mating activity (Fahrback et al., 1998). An interesting topic for future study could involve exploring how taking orientation and mating flights (i.e., experience) affects brain development.

Another finding to note is that all the structures increased in volume as the bees aged, except for the Kenyon cells. This is an expected result because as discussed earlier, the Kenyon cells consist of cell bodies, which send neurites to other structures in the brain (Fahrbach, 2006). These dendrites and axons create new and strengthen existing connections, resulting in an increase in volume (Abbott & Nelson, 2000). Meanwhile, the composition of the cell bodies within the Kenyon cells remains relatively stable, and the volume even decreases as the increasing volume of the surrounding structures intrudes on the space of these Kenyon cells.

Inspection of Figure 3 reveals another intriguing find. The variance in newly-emerged bees is a great deal larger than the variance of aged bees. While caring for the bees used in this study, I checked the microcolonies at the same relative time every day. However, it is quite possible that a male emerged soon after I checked the microcolonies and was not removed from the females until it was checked the next day. During this time, the males likely experienced social interactions with the females, which would have an effect on neuroplasticity (Fahrbach, 1998). The large variance among newly-emerged bees likely reflects this amount of time they were allowed to socialize with females. It also suggests that there is a specific point in time, sometime after emerging, where rapid neural development begins. This would be a compelling topic to investigate in future studies.

One of the most laborious aspects of this study involved rearing males from the microcolonies. Initially, the workers only produced deformed males, and it was determined that the temperature and humidity in the rearing room were not constant enough for ideal offspring production. For this reason, the microcolonies were moved into the incubator to control for these fluctuations. By the time microcolonies were established in incubator, the workers were

rather old. It took much longer for these females to produce offspring than expected, and the development time of these offspring was somewhat drawn out. This resulted in a smaller sample size than would be ideal. Indeed, several other brain regions had substantial changes in volume between newly emerged and aged males, but a marginally insignificant p-value (e.g., Kenyon cells, antennal lobes). This may have been due to the large variance in newly emerged males and small sample sizes. Future projects would benefit from isolating young worker females in controlled conditions, to allow for the production of a more adequate sample size.

This study provides a foundational understanding of the neuroanatomy of male bumble bees. By comparing newly-emerged bees to aged bees, a significant increase in the calycate volume was established. It also revealed what structures are associated with reproductive maturity in these bees. These findings set the stage for subsequent research into the workings and functioning of male bumble bee brains.

Reflective Writing

Completing my honors capstone has been quite a journey. There were plenty of ups and downs, but as my time as an undergraduate is wrapping up and I reflect on my experience, I am so grateful to have had this opportunity. Working in the Kapheim lab and conducting my own research project has been the highlight of my four years at Utah State. I have made numerous friendships, as well as connections with accomplished experts in this field. I was able to explore the fascinating world of bees and develop a fervent desire to learn more. As a student working on a capstone project, I not only observed but participated firsthand in scientific research and I will always reflect on this experience with fondness.

During the 2017 fall semester, utilizing the biology departmental honors advisor, Dr. Kim Sullivan, I searched for a lab to join. She made a few recommendations, and I applied for an opening in a lab that fit my interests and needs. Once accepted into the Kapheim lab, I spent an entire semester and most of the following summer working on current projects being conducted by other students. This benefited me as I gained a foundational understanding of bumble bees and the different techniques used to study them. Although I didn't know what my specific research question would be, I knew it would likely involve dissections, so I conducted practice dissections on close to one hundred bees. This allowed me to develop the fine motor skills essential to my project. Towards the end of the summer, with the help of my mentors Dr. Karen Kapheim and Mal Hagadorn, I decided on a topic that was interesting to me. As a human biology major I have an interest in neuroanatomy, and taking into account the lack of research on male bees, I designed a project regarding neuroplasticity in male bumble bees.

The most time-consuming portion of my project involved rearing healthy males to use in my study. Initially, the males produced by the microcolonies were all deformed, and therefore not viable for the study. I restarted twice with entirely new microcolonies before determining that the problem had to do with fluctuations in the environment that the bees were kept in. For this reason the microcolonies were transferred into incubators, where they finally produced healthy male offspring. Although they were producing viable bees for the study, these bees took much longer to develop than expected, and the brood size was also much smaller. By the time the microcolonies had stopped producing offspring, I was a few months behind on the project and had a much smaller sample size than would be ideal. I was able to work through these problems and still have a successful project, but having more time create new microcolonies with young workers would have been very beneficial.

Taking the aforementioned reasons into account, the best advice I can give to incoming honors students is to get started early, in regards to both working in a lab and to beginning a project. Simply working in a lab is incredibly helpful in regards to understanding the subject matter you will be studying, what the scientific process is actually like, and developing the skills you will use in your project. As a bonus, working in a lab can be a very enjoyable, rewarding experience that will greatly enhance your time at USU.

An especially helpful, albeit occasionally frustrating, aspect of my mentors' advising style is they ensured that this was truly my project. Throughout the entire process they provided me with all the resources and guidance that I needed, then let me figure things out on my own. This led to me acquiring a variety of skills and abilities that have and will benefit me in

a multitude of ways in my academic career. Some of these new abilities include dissections, tracings, statistical analysis, and skills involving the creative representation of data.

Learning how to do dissections was one of my favorite parts of my project. Dissecting a bumble bee brain is extremely tedious and took a tremendous amount of practice. Each person approaches dissections differently, and through trial and error I was able to establish a process that worked well for me. When I began my project I was able to undertake the dissections with confidence and come out with intact brains that were fit for use in the study.

Another enjoyable aspect of this study was learning to trace the different structures of the brain on a tablet. The only way to improve this skill is through practice, and a large amount of my time was spent analyzing brain images, locating the structures, and comparing my tracings to those of other members of the lab. Initially, I was able to trace an entire brain in about eight hours. After many hours of practice and some modifications of the tracing protocol, I was able to trace a brain in around three hours, with confidence in the accuracy of my tracing.

One of the more challenging aspects of this study was the statistical analysis portion. Although I took an introductory statistics class many years ago, I lacked a strong recollection of the material and had to reteach myself many of the things I had learned. My mentors suggested the statistical programs I should use and provided different websites that would help me, and I used these as I researched ways to design error-free methods of analyzing my data. I decided to use a t-test, studied the assumptions that I needed to meet for this test, met the assumptions, and ran the tests using statistical software. These capabilities will benefit me immensely as I continue my academic career.

A final experience and associated skill that I want to mention is that of presenting my findings in an aesthetically pleasing way. I designed two different posters for this study and presented them at research symposiums on campus. Creating these posters involved summarizing my research and explaining it to those unfamiliar with the subject matter in an understandable way. I also created figures and graphs that represented my project and data, and this required creative thinking and an artistic touch that isn't often expected of scientific researchers. With the help of other members of the lab, I was able to design my figures and posters and present my findings in a way that was both visually appealing and fun to discuss.

In conclusion, my experience in research has been immensely gratifying. I developed enjoyable, enlightening relationships with my mentors and other researchers in the lab. I learned so much about the scientific process and the fascinating world of bees, and developed skills that will benefit me in a multitude of academic applications. I proposed a research question that led to a capstone project that I am proud of and hope will inspire other undergraduate researchers in the future. I am truly excited for incoming honors students and the experiences they will have with their own research projects and am grateful for the opportunity to have been a part of this great program at Utah State. Go Aggies!

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Professional Author Bio

Karlee Eck was raised in Smithfield, Utah where she graduated from Sky View High School in 2015 as the school's Science Sterling Scholar and with a 4.0 GPA. While in high school she took a medical anatomy course which led to her being a member, and later president, of Sky View's HOSA—Future Health Professionals—club. Participating in HOSA instilled in her a passion for medicine which was later amplified during her education at Utah State University. She majored in Human Biology, minored in Chemistry, and will graduate in the spring of 2019 with a 3.98 GPA.

During her time at USU, she worked as a Supplemental Instructor, Teaching Assistant, and Research Technician. She also received her CNA license in 2016 and has since spent more than 2000 hours working in a skilled nursing facility, where she realized the joy that results from working with and caring for others in a medical setting.

As a junior, Karlee began working in the Kapheim Lab at Utah State, where research primarily focuses on sociality in bees. She developed an interest in this subject and was able to design a research project that integrated this interest with her background in human biology. This combination of neuroanatomy and bumble bees resulted in an honors capstone experience that she truly enjoyed.

After graduation, Karlee will continue to work in the Kapheim Lab and as a CNA while she pursues her dream of attending school to become a Physician's Assistant.