Title
Descending Neurons And Their Role In Visual Cues And Locomotion

Author
Yuen, Nicole

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DESCENDING NEURONS AND THEIR ROLE IN VISUAL CUES AND LOCOMOTION

By

Nicole Erin Yuen

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University of California, Riverside

APPROVED

Dr. Ysabel Giraldo
Department of Entomology

Dr. Richard Cardullo, Howard H Hays Jr. Chair
University Honors

Abstract

An organism's behavior is highly reliant on the stimuli it processes from its surroundings. This is all done via the nervous system and its neurons. One specific type of neuron is the descending neuron, which bridges the communication between the brain and the ventral nerve cord. The ventral nerve cord is analogous to the spinal cord in vertebrates and is responsible for controlling movement and receiving sensory information from the thorax. Neuroanatomical analysis of descending neurons in the model organism *Drosophila melanogaster* demonstrates that a group of descending neurons control leg movements and another group sends signals to the wings. In *Drosophila melanogaster* aspects of flight, walking, and escape behaviors have been linked to specific descending neurons. We are interested in investigating how descending neurons are involved in the connection between visual stimuli and locomotion, such as walking and flying. Here, I will present preliminary data investigating the behavior of wild-type flies (HCS) and their heading direction in response to a sun stimuli. In the future, this data will contribute to the overall understanding of how information is processed in *Drosophila melanogaster* and hopefully lead to further studies concerning the processing of multiple stimuli at once.

Acknowledgments

I would like to thank Dr. Ysabel Giraldo for welcoming me into her lab, as well as for her mentorship, attention, and guidance throughout the completion of my Capstone project. I would also like to thank Haneal Pae and Jingzhu Liao for their constant support, encouragement, and openness to teaching me. I am extremely appreciative of my time in this lab, everything I have learned, and all the connections I was able to make. Finally, I would also like to thank my friends and family for always being there for me while I pursued my educational goals.

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Introduction

Imagine you are in your kitchen and a fly is buzzing around you as you are cooking. You raise your hand to swat it, only for it to fly away and land on the counter on the other side of the kitchen. You take an old newspaper, roll it up, and stealthily walk over to it. You raise your hand with the newspaper to swat it only for the fly to escape its doom again. Flies are everywhere and somehow always find a way to outsmart you. Although flies are very small, they have a very intricately organized nervous system that is responsible for these quick behaviors. They must be able to sense that they are in danger and react to that while avoiding other obstacles around it. From the outside, it seems like a simple process. However, it is a lot more complicated than just walking or flying away from the obstacle. We strive to understand the underlying neural mechanics that cause these reactions to various stimuli.

Arthropods and vertebrate bodies are comprised of a nervous system that has many different neurons and pathways. These neurons and pathways are connected via a brain, located in the head, and either a spinal cord or ventral nerve cord. This central nervous system is connected to the peripheral nervous system, which controls muscles and receives sensory information from the outside world. The nervous system allows the animal or insect to sense its environment and appropriately react or behave to the stimulus that is presented in front of them. The brain, along with the rest of the nervous system, oversees the processing of the external and internal environment of the organism. Sensory signals received in the brain are then sent to the ventral nerve cord or the spinal cord where the activity is coordinated, and the motor neurons directly control the animal’s movements. There are many other neurons involved in this process besides these motor neurons, but a key group of neurons is the descending neurons.

What Are Descending Neurons?

Descending neurons bridge the communication between the brain and the ventral nerve cord (Louis and Simpson, 2018). Just like vertebrates and their spinal cords, the ventral nerve cord receives descending signals coming from the brain and processes them into various coordinated locomotive outputs. Although these neurons are highly complex and the known information about them is limited, almost half of these descending neurons were successfully mapped out in *Drosophila melanogaster* (Namiki et al., 2018). Many of these descending neurons are associated with the maintenance and modulation of locomotion, as well as other behaviors (Namiki et al., 2018). Some other behaviors found to be controlled by descending neurons are courtship and grooming actions that originate from the dorsal and ventral neuropils (Namiki et al., 2018). Each group of descending neurons is associated with the elicitation of a specific coordinated action.

While a detailed description of descending neuron anatomy can be useful for understanding the connectivity of these neurons and generating hypotheses, behavioral and physiological experiments are required to reveal the behaviors that are associated with individual neurons. We want to know how the sensory information is processed in the brain and transmitted to the motor neurons and muscles via the limited number of descending neurons. In some cases, descending neurons function via population coding, where the activity of multiple neurons regulates behavior. Furthermore, they uniquely are representative of a critical bottleneck where a lot of information is condensed into these few descending neurons. My lab tested various lines of descending neurons in *Drosophila melanogaster* in a flight simulator, where they were allowed to fly as if they were orienting with respect to the sun, We did this in order to understand the connection between visual stimuli and flight. I specifically tested wild-type (HCS) flies to study the general behavioral response to visual stimuli, Throughout my research, I found that HCS flies do not change their heading even when their flight is stopped. These experiments will help us to identify connections between sensory cues and how they are translated into behavior in all different types of organisms. Furthermore, we will also gain a more holistic and comprehensive understanding of the roles of the physical organization of descending neurons and how they function with each other.

Descending Neurons Operate Via a Critical Bottleneck

Descending neurons are vital when it comes to connecting the brain to the ventral nerve cord. They play a prominent role in activating, maintaining, and modulating different forms of locomotion, such as walking and flying, and other various behaviors (Louis and Simpson, 2018). Scientists are very interested in studying descending neurons as there is limited knowledge about them. They have many unique characteristics that make them stand apart from other neurons. One of these characteristics is that only a small percentage of neurons are descending neurons. The number of descending neurons is significantly smaller than the number of neurons located in the brain or the posterior ganglia (Namiki et al., 2018). This represents a critical bottleneck that allows information to flow from processing via the sensory information in the brain system to the motor circuits (Namiki et al., 2018). A critical bottleneck occurs because there is a lot of information transferred from the brain to the motor circuit, however, there is a limit on how much information can be processed due to the relatively few neurons responsible for processing and transmitting that information. (Nande et al., 2022). There are only a few neurons that make behavioral choices and many motor units that execute the actions which limit the amount of information that can be processed, hence the critical bottleneck terminology (Fig 1). A study by

Nande and her team strived to study the consequences of the critical bottleneck using a simplified model of descending neurons to represent behavior control. The model was then used to examine the effectiveness and robustness of an information bottleneck in the descending neurons. They discovered that to decrease the size of the bottleneck and to increase the robustness of the signal, the variability that occurs in behavior based on the current state of the fly must be utilized (Nande et al., 2022). In other words, the more actions that the fly is performing when the stimuli are received, the less robust the signal will be due to the influx of information being processed. This is important to know when it comes to understanding how different stimuli are processed via a set of neurons.

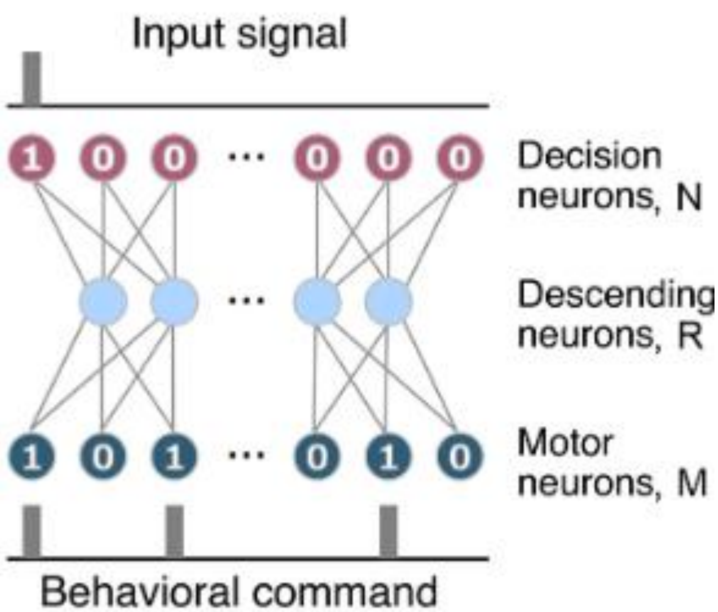

Figure 1: This schematic shows the processing of information via a critical bottleneck. This whole structure represents the ventral nerve cord. The signal starts from the decision neurons (red) and will travel to the descending neurons (light blue) and then to the motor neurons (dark blue). The

bottleneck is seen in light blue where there are fewer descending neurons in comparison to the motor and decision neurons. (Nande, et al., 2022)

Descending Neurons in *Drosophila melanogaster*

Many organisms have very intricate nervous systems, which presents challenges for researchers to identify the function of individual neurons. Finding an organism that has a manageable number of neurons and complex behavioral patterns is important to gain a better understanding and a clearer picture of the workings of descending neurons. *Drosophila melanogaster*, known as the common fruit fly, is a useful model when it comes to studying descending neurons. *Drosophila melanogaster* is a preferred organism for descending neuron research because they have a quick generation time of about 10 days, are reared easily in laboratory conditions, and possess a large number of genetic tools that allow functional manipulations at the single cell level. Genes and sometimes neurons identified in flies can also be often identified in other organisms. In fruit flies, there can be upwards of about 550 bilateral pairs of descending neurons located in their body (Namiki et al., 2018). This is a small number in comparison to the mice who have significantly more descending neurons. In addition to their relatively simple nervous system, flies also have the capacity for complex behaviors. This makes the fly an ideal organism to study the neural basis of behavior. A recently developed library of descending neuron split GAL4-lines (Namiki et al., 2018) allows researchers to target and precisely manipulate individual pairs or small numbers of descending neurons using the combinatorial expression GAL4-UAS systems. The GAL4-UAS system is used as a tool to manipulate gene expression in specific cells of interest, where GAL4 specifies which cells are targeted and UAS directs the expression of the gene of interest, for example, expressing a fluorescent protein in specific neurons.

Descending Neuron Anatomy, Physiology, and Organization

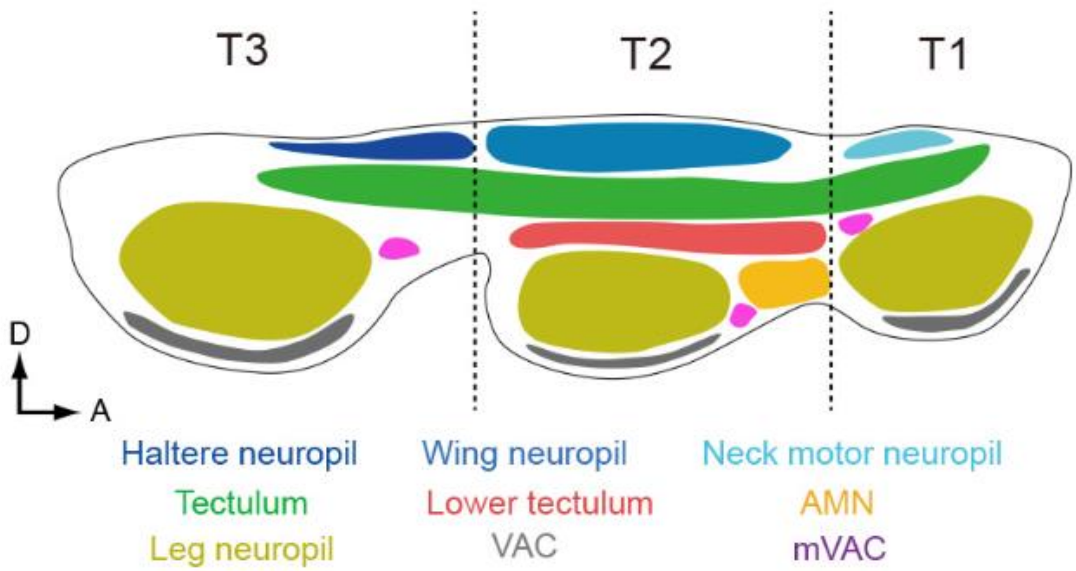

Figure 2: This is neuropil schematic that shows the general place where the descending neurons innervate the ventral nerve cord and the target appendages The ventral nerve cord is organized into many functional regions. Typically, descending neurons project to one or a few of these areas. Since their behavior is dependent on the area they project to, one can infer the function of those specific descending neurons. (Namiki, et al., 2018)

In a study by Namiki and colleagues (2018), they analyzed the physiology, organization, and classification of descending neurons via a combination of different genetic techniques. They were able to pinpoint and target the specific descending neurons in the nervous system of the fly to visualize and manipulate them. From this analysis, the identified descending neurons and a collection of driver lines were used to map the input and output patterns of the chosen population of descending neurons. Two of the pathways were found to control the wings and legs by connecting specific brain regions to particular motor controls (Namiki et al., 2018, Fig. 2). These descending nerves innervate the part of the ventral nerve cord that modulates the wings or the legs (Court et al., 2017). One of the pathways is a convergent pathway (innervates both the wing neuropils and the leg neuropils) and it was found to target an area of neuropils located in the central area between the wing and leg neuropils that control both sets of appendages (Namiki et al., 2018). This specific area is called the tectulum (Court et al., 2017, Fig. 3).

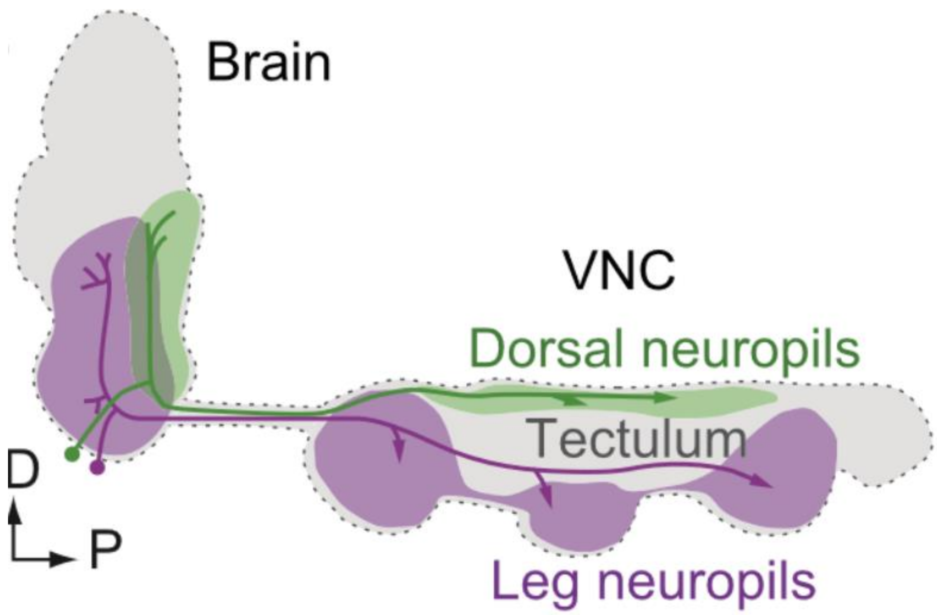

Figure 3: The ventral nerve cord with examples of descending neurons in dark green or purple. The leg neuropils are in purple and the dorsal flight neuropils are in light green. The dorsal neuropils are associated with the neck, wings, and halteres. The tectulum is marked in the middle between the leg and dorsal neuropils. (Namiki, et al., 2022)

Another characteristic of descending neurons is that they often operate via a population code (Namiki et al., 2018). A population code is when multiple neurons simultaneously work together to elicit a function or a response to stimuli. The more neurons that are activated, the greater the magnitude of the behavioral response These neurons are not necessarily working together with the neurons next to them (Louis and Simpson, 2018). Instead of grouping the descending neurons by where they are located in the brain, it is more accurate to categorize or group them based on the areas of the ventral nerve cord that they target (Louis and Simpson, 2018).

An example of population coding is the wing beat amplitude of *Drosophila melanogaster* during flight. The fly controls wing beat amplitude - essentially how high it moves its wings - as it controls power during flight (Namiki et al., 2022). This wing beat amplitude is regulated by a population of descending neurons called DNg02 neurons (Namiki et al., 2022). These neurons descend ipsilaterally down the neck, ie. they do not cross the midline, have a distinct branching pattern in the ventral nerve cord, and form a figure eight shape within the wing tectulum (Namiki et al., 2022). The wingbeat regulation of the wings during flight is highly dependent on the number of DNg02 cells that are activated or recruited. Within a flight stimulator, the flies were presented with a single stripe of light as stimuli. Flies will robustly keep the stripe in front in a well-characterized behavior known as stripe fixation (Namiki et al., 2022). During stripe fixation, the researchers optogenetically activated different populations of descending neurons. Optogenetics is a neuroscience technique in which light is used to activate or silence specific neurons utilizing pulses of light. Regardless of the varying optogenetic activations, the wingbeat amplitude was able to meet approximately the same peak level value for each fly during activation. All of this suggests that the DNg02 neurons are responsible for regulating wing beat amplitude and act via a population code. The more DNg02 neurons activated, the larger the wingbeat amplitude.

Creating a map of the descending neurons has been very helpful in identifying and gaining a better understanding of the physiology, organization, and anatomy of the different descending neurons. However, this does not reveal the functions of descending neurons. In response, this intrigued many scientists and researchers to test these descending neurons and the various behaviors that are associated with them.

Descending Neuron Responses to Visual Stimuli

Descending neurons control many locomotive behaviors in response to visual stimuli. An example of this is the giant fiber system which is composed of three descending neurons. This descending neuron pathway starts in the brain and extends all the way to the thoracic ganglia (Allen et al., 2005). In the thorax, the descending neurons synapse and innervate the wing and leg muscles. The giant fiber system is activated by a sudden visual or mechanosensory stimulus and will cause the fly to react by initiating an escape jump propelled by the extension of their middle legs (Allen et al., 2005). Following the jump, the fly will initiate a wingbeat to maneuver away from the stimuli in an attempt to escape it.

A somewhat unusual behavior of *Drosophila melanogaster* is its so-called moonwalking behavior (Namiki et al., 2018). This is when flies walk backwards in response to visual stimuli such as looming from a predator. This backward walking is controlled and initiated by the activation of the MDN neurons, also known as the medial cluster of descending neurons, which are composed of four descending neurons. These MDN neurons innervate the leg neuropils, specifically the dorsomedial part of it, to control the leg movement of the fly (Namiki et al., 2018). It allows the movement of walking backwards and inhibits forward locomotion. If all four MDN descending neurons are symmetrically activated, the fly will walk backwards straight (Sen et al., 2017). However, asymmetric activation of the MDN neurons will cause the fly to walk backwards turning (Sen et al., 2017). In addition to the MDN neurons, a set of AMB neurons work alongside in the activation of the moonwalking behavior (Omamiuda-Ishikawa et al., 2020). These AMB neurons are a subset of ascending neurons that are cholinergic and have dendrites located in the suboesophageal zone (Omamiuda-Ishikawa et al., 2020).

Another set of descending neurons that control locomotion are the DNp09 neurons. These neurons innervate the leg neuropils and are responsible for regulating walking by eliciting a freezing behavior (Namiki, 2021). When presented with the visual stimuli of looming, the fly will freeze as a defense mechanism. This freezing occurs because the unmoving fly will be harder for the predator to detect since the fly is idle. This freezing behavior is usually followed by a sequence of actions. These actions sequentially are posture adjustment, wing elevation, then jumping (Namiki, 2021). This protects the fly even further as they are able to escape danger or the predator instead of just waiting there for the threat to pass.

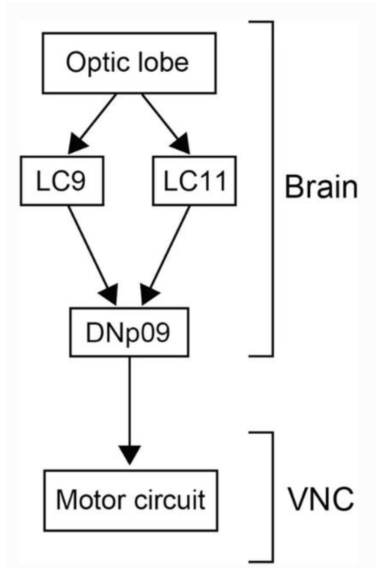

Figure 4: A schematic of how information is processed in the brain and ventral nerve cord using the descending neurons, DNp09. The signal starts in the optic lobe where it is processed via the LC9 and LC11 neurons and then processed via the DNp09 neurons. The message is then sent to the motor circuit for the initiation of the behavior.

These are all impressive studies that have been conducted to identify the functions of different groups of descending neurons. In our lab, we are interested in studying the neurons that are associated with visual stimulation, such as the sun, and their flight behavior in response to it (heading direction). Flies orient towards a sun stimulus by flying straight (Giraldo et al., 2018). Now our lab is more interested in how flies either maintain or change their heading direction, depending on whether they stop flying or encounter a different visual stimuli. I am specifically interested in studying the descending neurons because many of these neurons target the neuropils that are associated with locomotion, such as wing and leg movement. Since flight requires wing movement, the descending neurons may play an important role in flight behavior. I tested whether or not a fly at rest will continue using the same heading direction or change it. With my experiment, I hypothesize that flies do not change their heading direction even after stopping flight for rest.

Flight Heading Experiment Methodology

The goal of our experiments was to see if *Drosophila melanogaster* will change its heading direction if the fly's flight was stopped. Using a tethering machine, flies were tethered by gluing a 0.1-0.15 mm pin on their upper middle thorax. The pin was glued at a 60-degree angle with respect to the fly's body to mimic the natural angle the fly will fly. Once tethered, the flies were placed in a flight simulator where an LED light was used to represent the sun and they were allowed to fly as if orienting using the sun. This LED light position was selected at random and then kept the same throughout the rest of the experiment. The flies were held in place by a magnet located at the top of the arena and the bottom of the arena and were free to rotate about its vertical axis (Fig. 5). During these trials, their heading direction was recorded using a custom-written software (Will Dickson, Fig. 6).

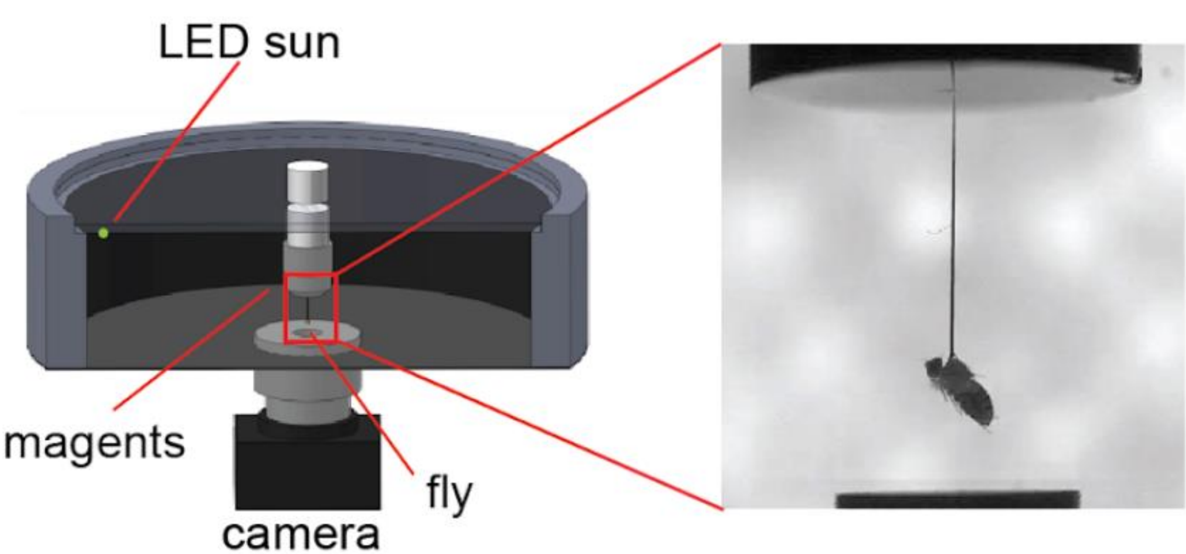

Figure 5: This image showcases the flight simulator set up. The main components of the flight

simulator are the LED light, the magnets, and the camera. The LED light is representative of the sun and will change based on the needs for the specific experiment. There are two magnets: one at the top and one at the bottom of the simulator. These magnets hold the fly in place and allows them to rotate among its vertical axis. The camera was used to track the fly and custom-written software determined the heading direction. The enlarged image of the fly shows the orientation of the fly in the flight arena.

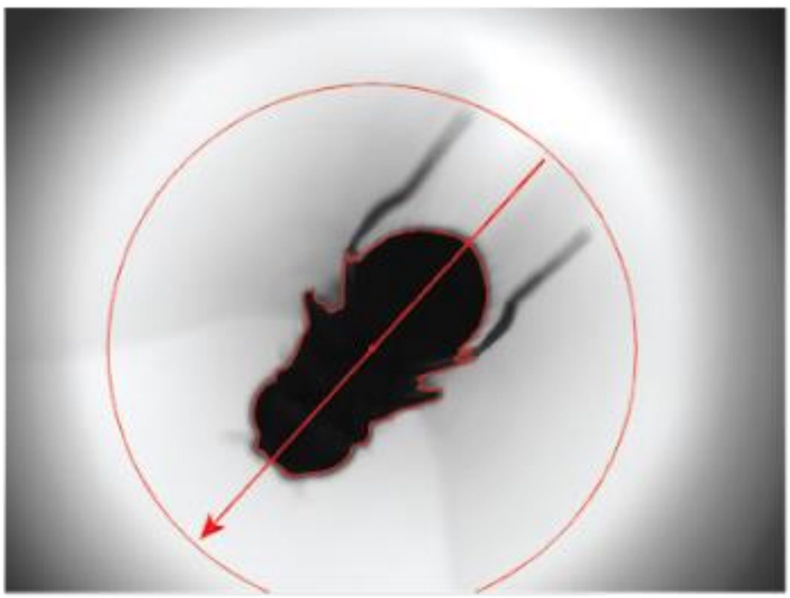

Figure 6: This is a camera image of a fly in flight simulator. The red arrow indicates the fly heading direction.

In my experiment, I tested the wild-type flies (HCS flies) to study the flight orientation to a sun stimulus. After being placed in the flight simulator, flies flew in the dark for 30 seconds to test if they were flying properly. We were looking for the fly to rotate smoothly and rapidly. If the fly did not meet these requirements during this dark period, they were discarded and not used for the experiment. Then they were presented with 5 minutes of the sun where they chose a heading direction during flight. For the next 5 minutes, they were presented with the same sun, but they were inhibited from flying using a small piece of a KimTech wipe on the bottom of their legs. In the final 5 minutes, they were once again presented with the same sun and allowed to fly using the sun to orient (Fig. 7). The data from each flight was collected and compiled into a polar graph, linear graph, and a bootstrap analysis.

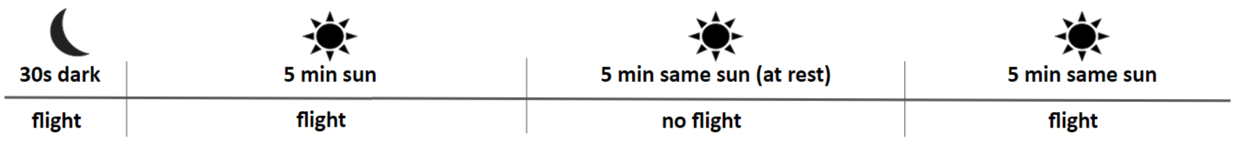

Figure 7: The flight paradigm used to test the role of a rest period on heading direction. The moon indicates the dark period while the sun indicates the sun period. The amount of time they were in each phase and whether or not they were flying or at rest was noted as well.

Results

In my experiments, I was testing whether wild-type flies (HCS flies) maintain their heading, with respect to the sun, even when stopping flight for rest. Figure 8 showcases the flight headings of each fly in A and B trials in a polar graph. Each line represents one fly and the length of each line indicates vector strength, a measure of heading fidelity. Flies with high vector strength maintained the same heading throughout the trial, while those with low vector strength (a shorter line) changed direction during that 5-minute sun presentation. Figure 9 is a polar representation of the heading change between A and B trials. The flies at the 0-degree mark indicate no change in heading. Figure 10 is a linear representation of the heading change between the A and B trials. Each point on this graph represents one fly and the error bars are representative of the variance for each trial. The closer to the line the point lies, the closer to no change in heading it is. Figure 11 is the bootstrap analysis of the A and B trials. This analysis is conducted by taking all the A trials, mixing up the B trials, and randomly assigning a B trial to each A trial. I repeated this analysis 10,000 to generate a distribution of shuffled headings, shown as the grey histogram (Fig. 11). From this graph, I obtained an observed mean heading difference of 47.5°, a bootstrapped mean of 85.8°,, and a p-value of 0. Thus in 10,000 replications, there were no instances where the shuffled headings had a smaller mean heading difference than the observed data. In fact, the bootstrapped mean of 85.8° is remarkably close to a value of 90°, which is exactly what you would expect for two headings selected at random.

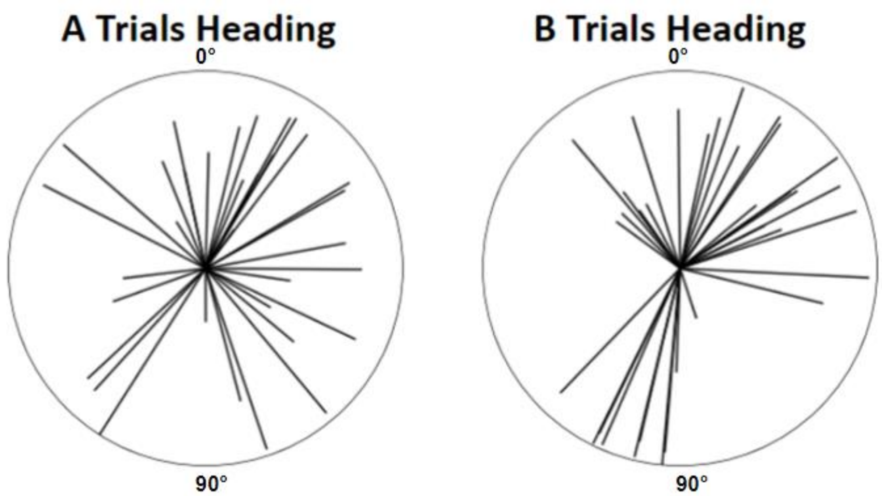

Figure 8: Polar representation of A trials and B trials heading.

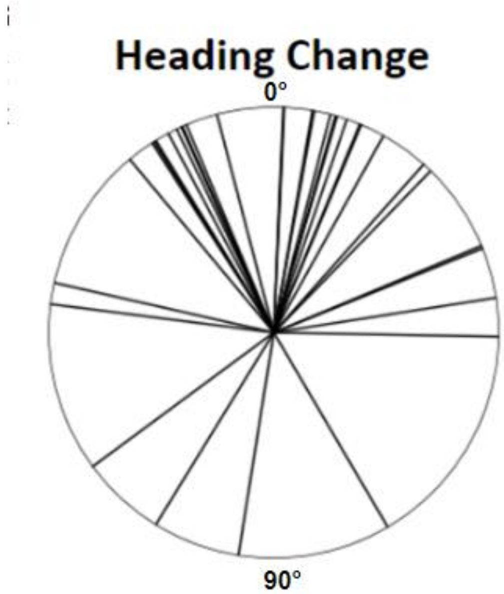

Figure 9: Polar representation of heading change between A trials and B trials

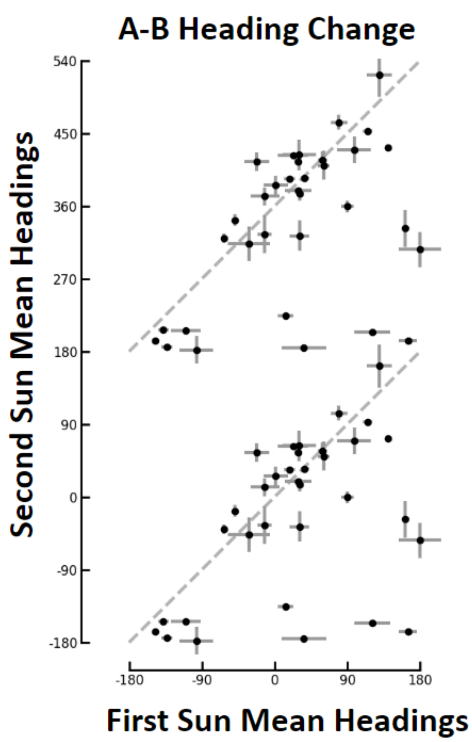

Figure 10: This graph shows a linear representation of heading change between A trials and B trials. Each point is representative of a fly and the error bars show the variance for each trial. The

dashed line represents no heading change. The closer to the line the data point lies, the closer the fly was to no heading change.

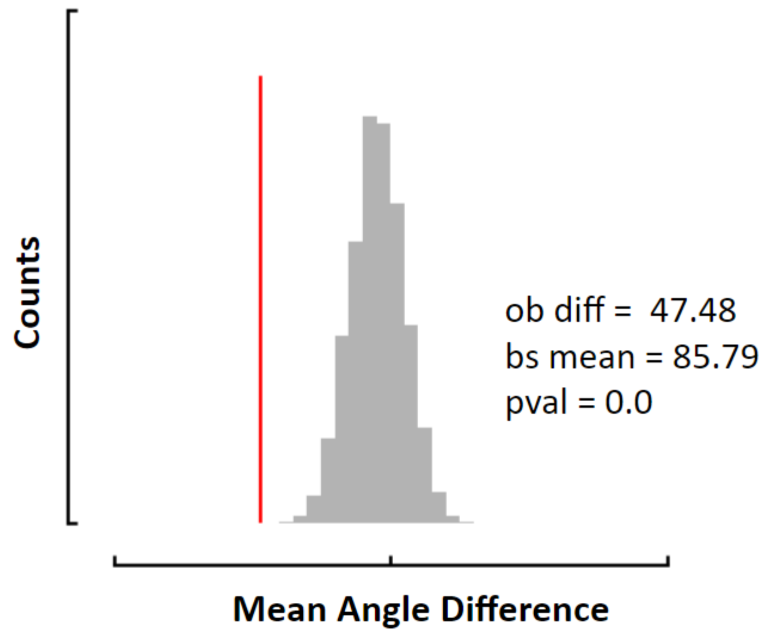

Figure 11: This graph is a bootstrap analysis of the data. The x-axis represents the mean angle difference, and the y-axis represents the count. All the B trial headings were randomly assigned to an A trial heading, and this was done 10,000 times to create this graph. The observed difference is 47.48, the bootstrapped mean is 85.79, and the p-value is 0. The red line represents the mean heading of the original data.

Conclusions

Overall, in conjunction with known studies, my results indicate that flies maintain their heading direction even after stopping flight. These data are consistent with other data collected in the laboratory, which indicates that when placed in the dark or at rest, flies maintain the same heading direction. However, when the sun position is moved, the fly changed its heading with respect to the new sun. These studies provide a general understanding of flight behavior (heading behavior) to sun stimuli and lay the foundation for experiments in which we manipulate the function of specific neurons, such as descending neurons that project to flight neuropils. This research adds to a growing body of research on descending neurons, such as DNg02, DNp09, MDN, and the giant fiber system, identifying their responses to various visual stimuli and their role in controlling behavior. There is a plethora of research that has shown that descending neurons are highly associated with the maintenance or initiation of locomotive behaviors. However, more research needs to be conducted to gain a more thorough understanding of the functionality of descending neurons.

Future experiments could include a genetic screen to identify the neurons responsible for heading behavior in *Drosophila melanogaster* under a variety of conditions. In the future, I would like to dive deeper into the critical bottleneck concept and introduce more stimuli at once to these experiments. It would be interesting to see how flies respond to multiple stimuli as that is what happens in their natural environment. In nature, flies are constantly exposed to multiple stimuli at once. For example, when trying to evade being swatted away by someone's hand, they would still have to maintain the ability to take into account the wind when they move. It would be interesting to see if there was a system of prioritization with certain stimuli, a reaction that encompasses all stimuli, or if too many stimuli would overwhelm the fly. To conduct these experiments, I would continue using the LED light to mimic the sun, but I would also include a source of wind and/or heat. Overall, this will help us gain a better understanding of the behavioral responses of flies, how descending neurons respond under different condition, and how they function within the body. These topics can be compared to other animals and insects and see how their DNs function and whether or not they have similar processes, operate via the same pathways, or elicit the same type of responses.

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