Nervous System Remodeling in the Drosophila: The Fate of Larval Motor Neurons

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THESIS PROPOSAL:

Nervous System Remodeling in the *Drosophila*: The fate of larval motorneurons

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Summary of Topic:

The remodeling *Drosophila* nervous system is a keystone feature for my project and can easily be used as a model for the extensive human nervous system remodeling. Studying the restructuring of neurons using *Drosophila* is relevant for human health. The human brain is far from static and changes throughout life. As a baby we are born with billions of neurons that modify, redirect and die throughout our life. During early stages, when noticeable development is still occurring we see remodeling of the nervous system involved during mastery of standard locomotor behaviors such as crawling, standing, walking. We also see remodeling when we acquire new motor skills as our neural maps can change based on experience. Remodeling of the Nervous system also occurs in memory formation and loss. When our lives are impacted by trauma, our nervous system also must be altered and remodeled. Finally with aging, our nervous system begins to degenerate and break down. The Drosophila serves as an excellent model to study this complicated and ever present restructuring as it is broken down into stages of drastic remodeling. In the life cycle of the fruit fly, adult flies have behaviors distinctly different from larvae; for example, walking and flying in adults compared to crawling in larvae. Thus, two distinct motor systems are generated to accommodate this behavioral alteration. The formation of the adult motor system involves neurogenesis, apoptosis and remodeling of persistent larval motor neurons, innervating the newly generated muscle fibers. We are studying specifically the abdominal muscle sets as a model for nervous system remodeling and developmental pasticity. 30 motor neurons innervate larval muscles in each hemi-segment and many of them are thought to survive into the adult stage, where it is believed that adult motor neurons are derived from these persistent larval counterparts.

My goal is to identify the fate of abdominal larval motorneurons during metamorphosis: how many persist and respecify, how many die, and whether or not they maintain a general identity. We studied this with three separate approaches. These studies are done in the context of also examining morphological changes in the CNS that occur simultaneously. Three separate approaches are used (1) A fly strain that labels all glutamatergic neurons (mostly motor neurons) OK371. (2) A second marker we use is the BarH1 line. This is another homeodomain protein that plays an important role in specification of laterally located embryonic motorneurons. A smaller subset of cells express in the adult one of these survives and maintains its lateral identity, innervating adult lateral muscle. (3) Lastly we follow individual neurons with MARCM (mosaic analysis with a repressible cell marker). This technique allows us to study individual motor neurons and their targets. With this we work towards cellular identification (cell body position, axonal projection and NMJs) of all larval motor neurons during the third instar stage. The MARCM also grants us the ability to follow neurons during metamorphosis and determine which individual neurons survive from the larva to the adult stage.
I created this figure to illustrate the correlation of the radical restructuring during the *Drosophila* life cycle with the remodeling of the Human nervous system.

**Composition and Pursuit of Thesis:**

I have worked in Dr. Fernandes’s lab for both semesters junior year, and submitted an undergraduate research proposal (pg 23) for this nervous system remodeling project. I was awarded a few thousand dollars from the National Institute of Mental Health that has helped aid me financially for the needs of my research. The past summer I continued and expanded upon the research as part of the Undergraduate Summer Scholars program and presented my initial findings at the Ohio Miami Valley Society for Neuroscience conference. I have reformatted an abstract and a second poster and will be presenting at the Midwest Drosophila Conference in Chicago on October 2. I have presented at the past Undergraduate Research Forum, and plan on attending the 2010 forum as well. I will also be continuing my research this semester as a Dean’s scholar. Dr. Fernandes and I have discussed the Thesis construction and plan on putting together a portfolio-type paper organizing my abstracts, posters, presentation submissions and follow-up papers.
Significance:

Studying the restructuring of neurons and of the brain as an organ using *Drosophila* is relevant for human health. The human brain undergoes restructuring throughout its life— with mastery of standard locomotor behaviors, acquisition of new motor skills, memory formation, trauma and aging. This area of work is also potentially beneficial for cases of neural damage, a better understanding of neural plasticity could lead to alternate nerves being reprogrammed, and directed to new areas for innervation. Reprogramming of cells is a hot new area of stem cell research currently being used to generate motor neurons from skin cells and develop treatments for motor neuron diseases such as Lou Gehrig's. This is something I truly find interesting as my sister suffered a traumatic brain injury my junior year of high school. This was an event that had heavily influenced my life and my choice to pursue a career in medicine. I have had an immense interest in neurology and neural plasticity and my involvement in this research has been a stimulating and fulfilling learning experience.

Completing this thesis also grants me access to a different facet of the Zoology major. While I have already been accepted into medical school, I have thoroughly enjoyed my immersive research experience in a lab. I have learned a multitude of lab skills and procedures such as the use of confocal microscopy, dissections and the use of antibodies, as well as having to effectively utilize the scientific method; formulate hypothesis, observe and collect data and comprehend results. Through this I have learned that research takes a completely different mindset, and a realization that there is not necessarily a clear cut answer to my studies; even no results are still significant results. I have also increased my reading comprehension and my ability to find and effectively utilize peer reviewed journal articles. Lastly, with multiple conferences and presentations, my public speaking and people interaction skills have and will continue to improve.
INTRODUCTION:

Neuroplasticity

The remodeling *Drosophila* nervous system is the keystone feature for our lab and can easily be used as a model for the extensive human nervous system remodeling [1]. Studying the restructuring of neurons and of the brain as an organ using *Drosophila* is relevant for human health. The human brain is far from static and changes throughout life. Humans are born with billions of neurons that modify, redirect and die throughout our life. During early stages, when noticeable development is still occurring we see remodeling of the nervous system involved during mastery of standard locomotor behaviors such as crawling, standing, walking. We also see remodeling when we acquire new motor skills, our neural maps can change based on experience. Remodeling of the Nervous system also occurs in memory formation and loss. When our lives are impacted by trauma, our nervous system also must be altered and remodeled. With aging our nervous system begins to degenerate and break down. Lastly many diseases that plague humans today affect the motor neural system like amyotrophic lateral sclerosis, progressive muscular atrophy and spinal muscular atrophy.

*Drosophila* as a model

*Drosophila melanogaster* has been a popular animal for many genetic experiments and has been imperative in connecting biological processes with their genetic components [2]. It serves as an excellent model organism due to its short generation time, high fecundity, inexpensive maintenance, encoded genome and easy manipulability. Researchers are able to connect the transcription, or lack thereof, of proteins and enzymes that control particular biological processes such as neural pathway development throughout metamorphosis.

The *Drosophila* is an excellent model particularly for neuroplasticity because of its neural remodeling existing throughout the lifecycle [2]. The life cycle is divided into distinct stages: embryo, larvae, pupa, metamorphic changes, and adult. The adult flies have behaviors distinctly different from larvae; for example: walking and flying in adults compared to crawling in larvae. These new behaviors are achieved through the reorganization of the motor system-neurons in the brain, and muscles in the bodywall. During metamorphosis, neuromuscular junctions are severed, muscle fibers die; when these new adult muscles are developed, new NMJs are formed innervating the muscles once again [3].

Background of *Drosophila* Motorneural System

The motor neural system of the *Drosophila* has been studied extensively for the past few decades. A number of researchers have contributed to developing the foundation of our lab’s research including the motor neurons and NMJs. The growing knowledge coupled with the development of new techniques has allowed a wealth of information on this model organism over the years. Highlights of some key researcher’s work is described below:

(1) Studies by Sink and Whitington worked to understand how specific patterns of connections between the motor neurones and muscles are established during the course of embryonic development. They studied the pattern of axon outgrowth from identified *Drosophila* motorneurons from onset of axonogenesis to the time of arborization on target muscles via an intracellular injection of a yellow dye. This dye allowed for a description of the morphology of the neurons and their pathways. Sink and Whitington elaborated on the phases of axon growth, projection from the CNS and extension and innervations onto target muscles [4].

(2) Landgraf in 1997 used a retrograde labeling technique to identify motorneurons in the abdominal hemisegment of stage 16 *Drosophila* embryo. They mapped each of the 30 body wall muscles and described a characteristic cell body position, dendritic arborization and axonal projection. They also established the lack of topographic relationship between cell body positions in the CNS and their innervations in the periphery but motorneurons innervating muscles of similar position often are clustered with overlapping dendritic trees [5].

(3) Chiba’s lab in 2001 next attempted to categorize the cellular identities of the larval neuromuscular synapses. With the use of single-cell dye labeling of individual synaptic boutons Chiba was able to characterize the synaptic partners and bouton differentiation of the 30 motoneuron axons extending from each CNS segment form the six nerve branches (TN, ISN, SNa, SNb, SNc and SNd). The lab also supported the ideas that only one motoneuron axon of a given bouton type innervates a single muscle, while up to four motoneuron axons of different bouton types can innervate the same muscle [6]. The figure below was developed by Chiba and shows the symmetrical bilateral central nervous system (A) and the corresponding body wall muscles for the segment (B). Each CNS segment has 30 muscles innervated by motor neurons from the 6 nerve branches. The lab was able to identify the individual axons in each of these bundles and their corresponding innervated muscles via specific bouton types. Each of these 6 nerve branches has a unique selection of transcriptional regulators. Different combinations of protein transcription factor codes in each of these classes help confer identity for these motor neurons, directing them to the appropriate innervations location. Although it is not fully understood how the identities are translated into specific neuronal properties and how they are implemented in to events of axon pathfinding and connectivity [6].
This shows the Drosophila embryo and larva neuromuscular systems. There is bilateral symmetry and segmental organization of the CNS (A) and periphery body wall muscles (B). This schematic shows a dorsal view after a dorsal midline cut with the anterior at the top. The second abdominal right hemi-segment (A2) is the highlighted CNS region. Each CNS segment has a dorsal midline nerve exit, from which bilaterally symmetric TN nerve branch projects, and a pair of lateral nerves exit, from which ISN, SNa, SNb, SNc and SND nerve branches arise. Thirdy muscles from 1-30 in each hemisegment are innervated by the motorneurons through these six nerve branches. Source: [6]

(4) Garces’s lab studied one particular transcription factor in BarH1. They found in the embryo that it was expressed in a subset of doaminergic neurons forming the segmental nerve. By using the Gal4-UAS system they found five neurons in the SNa branch projecting to lateral muscle in the periphery and were able to visualize their projection and dendritic tree. They also looked at the larval stage and found expression of this transcription factor within a small subpopulation of motor neurons in the SNa branch projecting onto muscles 21-24 in the lateral region as well as muscle 8 and or muscle 5 [7].

**Abdominal Motor Neuron System and Development**

Out of these 30 motor neurons that innervate larval muscles in each hemi-segment, many of them are thought to survive into the adult stage. Here it is believed that adult motor neurons are derived from these persistent larval counterparts. The ultimate goal of our lab is to morphologically analyze the larval motorneural system and identify the persisting larval motor neurons during metamorphosis; which ones persist and respecify, do they maintain a general identity, how many die and how many live?

**Work Done in the Lab:**

The lab has been studying how neuromuscular junctions (NMJs) in the thoracic region are re-organized during metamorphosis [8] given that a new motor function, flight, is controlled by the thorax. Recently, research has begun to focus on the abdomen [9] since it presents an interesting situation- the abdomen becomes indirectly involved in movement, and could be the reason why the size of the abdominal CNS shrinks. In the above studies, the focus has mainly been in the periphery, without much attention to the CNS, where cell bodies of the motor neurons reside and control locomotion. In the last 2 years, instead of only looking at the NMJs in the periphery, the lab is now studying the entire motor pathway, which includes the cell bodies in the CNS, the axons that become bundled and leave the CNS as nerves, and the NMJs on the muscle surface. The adult muscles are organized in 3 groups, ventral lateral and dorsal (Figure 1). Preliminary studies of the role of a transcription factor dHb-9, which controls the identity of motor neurons innervating ventral muscles in the adult abdomen was recently reported [10].
Work done by me:

My contributions in the lab include tissue dissections of all phases of Drosophila: larva, various hours of pupae and adults. I also have learned the process of immunohistochemistry to visualize cell bodies, axons and neuromuscular junctions with BarH1 pattern of expression. I also have used both the fluorescent and confocal microscope to analyze and quantify the expression in the tissue dissections. I have also worked with maintenance of fly lines, picking virgins and selecting flies expressing genetic balancers to expand necessary genetic lines. Lastly I have read current literature published from other labs relating to the field of nervous system remodeling in the Drosophila.

Three approaches to identifying persistent motor neurons:

Our goal is to identify the fate of abdominal larval motorneurons during metamorphosis: how many persist and respecify, how many die, and whether or not they maintain a general identity. We studied this with three separate approaches. These studies are done in the context of also examining morphological changes in the CNS that occur simultaneously. Three separate approaches are used (1) A fly strain that labels all glutamatergic neurons (mostly motor neurons) OK371. (2) A second marker we use is the BarH1 line. This is another homeodomain protein that plays an important role in specification of laterally located embryonic motorneurons. A smaller subset of cells express in the adult one of these survives and maintains its lateral identity, innervating adult lateral muscle. (3) Lastly we follow individual neurons with MARCM (mosaic analysis with a repressible cell marker). This technique allows us to study individual motor neurons and their targets. With this we work towards cellular identification (cell body position, axonal projection and NMJs) of all larval motor neurons during the third instar stage. The MARCM also grants us the ability to follow neurons during metamorphosis and determine which individual neurons survive from the larva to the adult stage.
Tools and Techniques:

(1) **GAL4/UAS system of targeted gene expression:**

This genetic tool allows specific expression of a gene of interest in tissues or cell types of choice. It separates the target gene from the activator in two transgenic lines. The line with the activator protein (GAL4) is under the control of a tissue-specific promoter; in this case, BarH1 is controlling the expression of GAL4. The other line contains DNA sequences to which the activator will bind (UAS) and a target gene. In this case the target gene is Green Fluorescent Protein (mcd8GFP). The target gene is only expressed when the two lines are crossed. Therefore by using this system motor neurons expressing the BarH1 homeodomain protein are visible. Because mcd8GFP is a membrane bound protein, visualization is possible in the cellbodies of the CNS as well as axonal innervations in peripheral muscle fibers [11].

(2) **Immunohistochemistry:**

This is a tool utilized to visually locate proteins in tissue samples. This procedure relies on blood proteins known as antibodies which are produced by lymphocytes of vertebrates. Each lymphocyte produces an antibody type directed against a particular foreign molecule antigens introduced to their systems. The antibody has a specific binding relationship with its corresponding antigen. A given antigen may have several different binding sites and react with different antibodies. Scientists inject the antigen of interest into the laboratory mammal (commonly rabbits or goats) and blood is withdrawn and serum tested for presence of antibodies against the antigen. This creates a primary antibody which binds selectively to the antigen of interest. The primary antibody can then be used as the antigen, and a secondary antibody is prepared. This allows the addition of a fluorescent molecule or some other label.
to the primary antibody to help it to be visualized under the microscope. The secondary antibodies are conjugated with fluorescent dye to amplify the signal [12].

With immunostaining the primary and secondary antibodies are added in sequence to the antigen, binding to the targeted protein in the tissue samples. When viewed under the microscope the fluorescence attached to the secondary antibody shows where the antigen is located assuming the secondary is bound only to the primary antibody which is bound only to the antigen of interest [12].

In our research we most commonly utilized the following

**Primary Antibodies**: Rabbit anti-GFP, Goat anti-HRP

**Secondary Antibodies**: Donkey anti-Rabbit- 488 (Green tag), Donkey anti-Goat-546 (Red tag)

(3) **Confocal Microscopy** is an optical imaging technique used to increase optical resolution and contrast by using a spatial pinhole to eliminate out-of-focus light in a thick specimen. It also enables the reconstruction of three dimensional structures from obtained images and the capability of lasers to activate the different wavelength tags allowing for separate color field visualization [14].

![Diagram of antibody binding and visualization](image)
Works Cited:


USS PROPOSAL:

This proposal was submitted December 2008 for competitive funding. Awarded for work done in Summer of 2009. This Undergraduate Summer Scholars program lasted 9 weeks and I was awarded my 3 Senior Capstone credits (ZOO 419R) as well as 9 credit hours.

Nervous System Remodeling in the Drosophila: The fate of BarH1 motor neurons

Background: Drosophila larva consists of an elongated and uniformly segmented body. Along the length there are 8 abdominal segments of muscles. These muscles allow the larva’s crawling behavior. Every segment can be broken into identical hemisegments, each containing 30 muscles, which can be divided into three general regions: ventral, lateral and dorsal. Each group of muscle is innervated by corresponding motor neurons which have specific identities directed by unique transcription factors. For example, dhb-9 is a transcription factor that regulates and directs projection of motor neurons to ventral muscles. The subject of my study, BarH1, innervates the lateral region of muscles in the larva. A question of general interest is the relationship of the larval motor neurons to their adult counterparts. This is because adult motor neurons are often remodeled larval neurons, innervating newly formed muscles for new behaviors: behaviors changing from larval crawling to walking and flying found in the adult. Larval neurons tend to have two fates: death or a restructuring to innervate new muscles for these new adult behaviors.

My project and its goals: BarH1 is a transcription factor that confers lateral identity. Motor neurons expressing BarH1 will innervate the lateral region of the larva body wall. This project will observe the remodeling of BarH1 expressing neurons during metamorphosis of Drosophila.

Aim 1: I will follow fate of the expression of BarH1 during metamorphosis. This can be followed by using a green fluorescent protein reporter, specifically mCD8GFP. Because BarH1 is a transcription factor that resides in the neuron nucleus, this reporter will target the membrane, thus allowing visualization in all parts of the expressing motor neuron. With this visualization, expression can be followed through all stages of the lifecycle by analyzing animal dissections at different stages. Previous research has observed BarH1 expression in embryo, but no research has followed fate in larvae or adults.

Tools and Techniques: The Gal4/UAS system of targeted gene expression allows specific expression of a gene of interest in tissues or cell types of choice. It separates the target gene from the activator in two transgenic lines. One line has the target gene that is silent without the activator. The second has an activator protein present but no target gene. A crossing of both allows the target gene to be expressed. In this particular experiment, the GFP will be expressed, activated by BarH1. Confocal microscopy will be used to visualize the motor neurons through triple labeling: detecting GFP, an antibody that detects all motor neurons (thus demonstrating the separate subset of BarH1 expression) and a muscle label to help verify post synaptic muscle targets.

Expected Outcomes: The number of BarH1 expressing neurons will be observed in the larval stage as well as in the adult. Fewer expression may be seen in the abdomen because adult abdomen loses
centrality of distinct locomotion. The muscle region targeted will also be followed. The BarH1 expressing neurons will keep their identity and not change location of muscle innervation.

**Aim 2:** I will observe if motor neuron identity can be changed during metamorphosis. I will again utilize the Gal4/UAS system, however this time I will add an additional target gene besides GFP. This will be a transcription factor, distinct from BarH1, that confers motor neuron identity in a different region. In my study I will use dhb9, which regulates ventral identity in larva.

**Tools and Techniques:** Temporal control will be used for this study. This is an extra level of control placed on the Gal4/UAS system so that expression of desired genes can be switched on at specific stages of the *Drosophila* lifecycle. For this study, I will turn on the expression at the onset of the pupal stage and analyze the innervation patterns in the late pupal and adult stages. This will be examined using motor neuron specific markers as in Aim 1.

**Expected Outcomes:** Dhb9 causes ventral identity in lateral motor neurons. With the presence of GFP as well, the location of these neurons can be observed. No change may be seen in muscle region innervation of expressing neurons. Perhaps the identity cannot change from lateral to ventral, even with imposed activation of dhb9 at the pupal stage.

**Educational Benefits:** Through my research I will apply and enrich the previous knowledge I gained from classes such as ZOO 203 (Cell biology), ZOO 305 (Animal Physiology) and PSY 251 (Biopsychology), while also learning new specific techniques in neurobiology. I will also be able to apply the information regarding the *Drosophila* from this research to the remodeling off neurons that also occurs in human physiology. Humans do not develop neural circuits allowing flight, however remodeling occurs in the brain of newborns as they learn many new skills, in young adults while going through puberty, and even adults during aging or recovery from injury. Finally this research will assist with my mastery of the scientific method. By partaking in this project, I will practice all parts of the scientific method. I will strengthen my ability to form hypotheses, develop and conduct experiments, collect data and make observations, and draw conclusions. I believe this will help for future success in later experimental design.
WHAT IS BARH1:

Garces in 2006 worked with the transcription factor BarH1 and its expression in *Drosophila* motor neurons. BarH1 is a homeodomain protein that had previously been found in retinal development and formation of distal leg segments, but Garces’s lab found that in the embryo of the *Drosophila* it was expressed in a subset of dopaminergic neurons forming the segmental nerve. By using the GAL4/UAS system they found five neurons in the SNa branch of the embryo, projecting to lateral muscle in the periphery and were able to visualize their arborization projection and muscle innervation [1].

![Fig. 1. Axons of the BarH1 expressing motorneurons are found in the SNa branch. Stage 16 embryos stained for Fasciclin II (A and C) to highlight the axon projections into periphery. B reveals BarH1 Gal4 positive motorneuron projections, and Phalloidin is used to visualize the body wall muscles in periphery (D and F). CNS is on the left, dorsal muscle field on the right. This shows that BarH1 Gal4 positive motorneurons extend axons in the SNa nerves but not the other branches Snc or ISNd or ISNb. They project onto muscles 21-24 and muscle 8 lateral muscels. Source [1].](image)

Garces looked at the embryo, and found that 5 neurons per hemi-segment express BarH1 transcription factor, with muscle fibers 21-24 and 4 are innervated by these motor neurons. My initial work in the summer of 2009 focused on a continuing this analysis of BarH1 expression in motor neurons by analyzing the expression (cell bodies, arborization, projection and innervations) but instead of the embryo, focusing on the larvae and pupal stages. This focus included cell body counts in the CNS of multiple different pupal stages as well as projection and muscle innervation analysis. I attempted to answer the question if any of these BarH1 expressing motor neurons persisted throughout the stages of remodeling.
RESULTS FROM WORK DONE WITH THE USS PROJECT:

Larvae

I. In the CNS we found 1-2 cell bodies expressing BarH1 per segment with a consistent 1 axon extending out from every segment innervating muscle 23 (n=15).

II. We also examined if the 2nd instar larvae showed more expression but found the same pattern of 1-2 cell bodies and 1 projecting axon. In the periphery the axon is also expressing GFP and innervating the lateral muscle 23. In both stages of larvae we found a similar stereotypy of cell bodies expressing GFP in the CNS.

Recently a new line was developed in hour lab that contains two copies of GFP thus allowing a brighter visualization. Initial analysis of these preps suggest that there is infact 2 axons extending out from every segment with one also innervating muscle 24.

Garces also examined the expression of BarH1 in larval motor neurons and found muscles 21, 22, 23 and 24 all being innervated. Our work did not support this data as we only found 2 nerves in the periphery innervating solely muscles 22 and 23.
Pupa

BarH1 Gal4/GFP expression was analyzed in the 4 and 12 hour pupae. This is because at 4 hours cell death is initiated and at 12 hours cell death has been completed [2].

The expression in the 4, 8 and 12 hour pupae shows a slight decrease, but still see a characteristic 1 to 2 cell bodies persistent per segment. We were also able to follow axons out from the CNS to the projection site in the periphery in each segment. In the 8 hour pupa we were able to follow the axon in to the periphery and see a single innervation on muscle 23 (Figure 5).

We then looked at 24 hour pupa because the motor neurons in the periphery at 24 hours persist to innervate adult muscles that began developing by ~28 hours [3]. We still found 1-2 cell bodies per segment and a single axon projecting through each segmental nerve. We followed this projection out to the periphery and found the location where it stopped, not innervating any muscle. At this time adult muscle development has just started [2].

![Fig 4. Pupal motor neuron expression of BarH1 GAL4/GFP line (n=10) showing CNS cell body expression and a single axon projecting through segmental nerve. (A) 4 hour pupal stage (B) 12 hour pupal stage (C) 24 hour pupal stage](image)

![Fig 5. 1 axon is projecting into the periphery and at 8h it can be seen innervating only muscle 23.](image)
**Adult**

New muscles are developed in the adult for the new behaviors like walking and flying and the loss of larval behaviors like crawling. The adult muscles appear differently and this dissection shows the organization of muscles divided into dorsal lateral and ventral regions.

With analysis in the adult stage, we found that there was in fact a single cell that was maintained during reorganization up through the adult stage. This neuron was also seen to maintain its identity as a motor neuron innervating lateral muscles. The presence of a single neuron projecting in each of the pupal dissections support that this neuron present in the adult is the same cell that was being expressed in the larval stage as it is being expressed throughout metamorphosis.

**Works Cited:**


DEAN’S SCHOLAR PROPOSAL:

This proposal was submitted the first semester 2009-2010 school year for the competitive Dean’s Scholar award, for Senior Undergraduates doing research. Both semesters Senior year I completed 3 credit hours of independent study in the lab (ZOO 477) working towards this proposal.

College of Arts and Science Dean’s Scholar Nomination Form

Department or Program:  **Zoology**  Account # for transfer of funds_____________________

Student Nominee (must be Senior in AY 2009/2010)  **Alex Blair**

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Title of Proposed Project:

**Following the fate of a subset of motor neurons during a period of extensive re-organization in the fruit-fly *Drosophila***.

Brief summary of proposed project for publicity purposes:

In its life cycle, the fruit fly, *Drosophila*, undergoes metamorphosis from larvae to a fully functional adult. Adult flies have behaviors distinctly different from larvae; for example, walking and flying in adults compared to crawling in larvae. These new behaviors are achieved through the reorganization of the motor system-neurons in the brain, and muscles in the bodywall. Although it is believed that adult motor neurons are derived from persistent larval counterparts, this has not been documented for individual motor neurons. Our study will focus on the fate of neurons express BarH1, a transcription factor, which innervate lateral muscles in the larva. Using Green Fluorescent Protein as a reporter of BarH1 gene expression, we will follow the motor neurons from the larval stage into metamorphosis, which is the time period of extensive re-organization in the central nervous system. We have previously discovered a single neuron that has persisted from the larval to the adult stage and now with further research hope to determine (1) If the BarH1 neuron in the adult also innervates lateral muscles as in the larva, (2) The proportion of larval BarH1 expressing neurons that survive into adulthood (3) What proportion of adult segments show BarH1 neuron expression and if there is a stereotypy and (4) If BarH1 is localized to a particular branch in the Adult innervations periphery. Studying the restructuring of neurons and of the brain as an organ using *Drosophila* is relevant for human health. The human brain undergoes restructuring throughout its life- initially after birth, and later during puberty, aging and injury.
RESULTS:

In the summer of 2009 we found that two neurons are expressing the homeodomain protein BarH1 and are innervating lateral muscles 23 and 24 in the periphery. In the pupal stages at 4, 12 and 24 hours we have found only 1 neuron projecting into the periphery. In the adult our initial analysis found this 1 neuron surviving and maintaining its identity, innervating lateral muscle. My work completed in the 2009-10 school year focused on a closer analysis of the BarH1 transcription factor expression in the Adult Drosophila. Using the double copy BarH1/GFP line we were able to obtain a clearer image of the periphery and found a distinct stereotypy of the innervation on the adult lateral muscles (Figure 1). Analysis with fluorescent microscopy found that there was no apparent anterior-posterior regulation as innervations were found in both sides, in every segment from A2-A5 (n=10) We also found greater expression in the CNS with more distinct cell bodies present in the abdomen (Figure 2).

This data supports that out of the 2 neurons expressing BarH1 in the larvae, 1 dies during remodeling, and a single other neuron persists to the adult stage to innervate the new adult muscles.
We also wanted to examine which bifurcation of the peripheral neuron the BarH1 expressing motor neuron took up. To do this we used a double label stain with green GFP for the BarH1 and Red HRP which stains all neurons. Following the neuron projection out of the periphery and to the lateral region of muscle we see 3 bifurcations with branches innervating regions of the lateral muscle. We found that the BarH1 expressing motor neuron was located in the 3rd bifurcation innervating the posterior region of the lateral muscle. This suggests the presence of other motor neuron identity genes that innervate lateral muscles in the adult body wall. Candidates could include any number found and listed in the Landgraf paper: nkx6, even skipped, and others.
CONCLUDING REMARKS:

Statement of Goals:
We wish to determine the role of the transcription factor BarH1 role in the innervation of the motor pathway in lateral muscles during metamorphosis. The main questions that we are concerned with are:

(1) Are the lateral muscles in the adult also innervated by motor neurons that express BarH1 as in the embryo and larva?

Our research has shown that the lateral muscles in the adult are also innervated by motor neurons that express BarH1 in the embryo and larva. Therefore embryo and larval identity is maintained with the BarH1 expressing motor neurons.

Our research also has shown that the neurons projecting to the lateral muscles bifurcate 3 times, and the neuron occupying the 3rd bifurcation is expressing BarH1. Therefore other motor neurons project to the lateral muscles that do not express BarH1, suggesting there are additional lateral identities.

(2) How many larval motor neurons expressing BarH1 survive to the adult stage?

We have found that in the larval stage 2 neurons project into the periphery innervating muscles 22 and 23. By the 8 hour pupal stage the motor neuron innervating 22 is no longer present, and by the adult stage a single BarH1 expressing motor neuron is observe innervating lateral muscels. It is very likely that the adult neuron persists from the larval stage, as is supported by expression in the different pupal stages.

(3) Can the identity of BarH1 motor neurons be changed using alternative transcription factors such as dhb9?

This is a question that has yet to be answered, and is the target of our future research.
**FUTURE DIRECTIONS:**

**Observe effects of targeting the transcription factor dhb9 to lateral neurons:**

Our future research will again utilize the Gal4/UAS system, but add a transcription factor distinct from BarH1, that confers motor neuron identity in a different region. We will determine if the identity of motor neurons can be altered during the process of re-organization by using dHb9. We will have GAL4 controlled by BarH1 bind to the UAS targeting expression of dhb9. By doing this we have induced a gain of hb9 function in BarH1 neurons.

**What is dhb9:**

Dhb9 is a homeodomain protein expressed in some neurons in the *Drosophila*. Previous research in our lab has shown that dhb9 and its analog regulates ventral identity in the larva. Motor neurons that showed expression of this transcription factor were found to be projecting into the periphery and innervating muscles 6, 7, 12, 13, 15, 16, 26 and 28 in the ventral muscle region of the larvae [1]. Thus we are dealing with two transcription factors that confer identity to different muscle regions.

**What is expected from the experiment:**

1. Dhb9 causes ventral identity in the lateral motor neurons. Will GFP be expressed at NMJs of ventral muscles?
2. Dhb9 does not cause ventral identity in the lateral motor neurons. Will GFP be expressed at NMJs of lateral muscles without change in number or stereotypy?

**Significance:**

We know that larval motor neurons that survive metamorphosis undergo a period of restructuring to innervate a different set of new adult muscles and during this process the BarH1 expressing neuron is maintaining its lateral identity. With the force expression of dhb9 we would analyze if reprogramming of motor neurons was possible. Reprogramming of cells is currently at the cutting edge of research on the context of converting one differentiated cell into another functionally different type and has relevance for stem cell research [2] or for neurodegenerative diseases such as ALS.

**Works Cited:**


UNDERGRADUATE RESEARCH PROPOSAL:

This proposal was submitted in 2008 for competitive funding awarded for work done in 2008-2009 school year. An extension was filed in the summer of 2009.

Following the fate of a subset of motor neurons during a period of extensive re-organization in the fruit-fly Drosophila.

Bridget Hartman
Alex Blair

Abstract:

In its life cycle, the fruit fly, Drosophila, undergoes metamorphosis from larvae to a fully functional adult. Adult flies have behaviors distinctly different from larvae; for example, walking and flying in adults compared to crawling in larvae. These new behaviors are achieved through the reorganization of the motor system- neurons in the brain, and muscles in the bodywall. Although it is believed that adult motor neurons are derived from persistent larval counterparts, this has not been documented for individual motor neurons. 30 motor neurons innervate larval muscles in each hemi-segment and many of them are thought to survive into the adult stage, based on studies in a related insect, the moth. Unlike the moth, genetic tools are available in Drosophila to study the genetic basis of biological processes.

Our study will focus on the fate of neurons express BarH1, a transcription factor, which innervate lateral muscles in the larva. Using Green Fluorescent Protein as a reporter of BarH1 gene expression, we will follow the motor neurons from the larval stage into metamorphosis, which is the time period of extensive re-organization in the central nervous system (CNS). We hope to determine (1) If BarH1 neurons in the adult also innervate lateral muscles as in the larva, (2) The proportion of larval BarH1 expressing neurons that survive into adulthood and (3) Determine if the identity of BarH1 neurons can be altered during the process of re-organization using other transcription factors, such as dHb9.

Studying the restructuring of neurons and of the brain as an organ using Drosophila is relevant for human health. The human brain undergoes restructuring throughout its life- initially after birth, and later during puberty, aging and injury. This area of work is also potentially beneficial for cases of neural damage, if neurons can be directed to new areas for re-establishing innervation. Our study will include an experiment on reprogramming, which is a hot new area of stem cell research.
Introduction:
For years, *Drosophila melanogaster* has been a popular animal for many genetic experiments. *Drosophila* is a very good model organism due to its short generation time and high fecundity. *Drosophila* has been imperative in connecting biological processes with their genetic components. Most biological processes are controlled by an array protein and enzymes which are encoded in the genome of an organism. By studying the development of *Drosophila*, researchers are able to connect the proteins and enzymes that control particular biological process to the specific genes that control them. The transcription, or lack thereof, of the proteins and enzymes allows the cell to control the occurrence of these processes, such as the development of neural pathways during metamorphosis.

**Different behaviors in adult and larval stages:** The life cycle of the *Drosophila* are divided into distinct stages: the embryo, the larvae, the pupa undergoing metamorphosis, and finally the adult stage. During the embryonic stage, neurons develop and innervate the muscle fibers. During metamorphosis, these neuromuscular junctions are severed when the muscle fibers die. With the development of new muscle fibers, new NMJs are formed when motor neurons that survive innervate the muscle cells once again. In the larval stage, movement is achieved by crawling. Crawling involves muscles and motor neurons throughout the entire body of the *Drosophila*. After metamorphosis, the new behavior for movement is flight. Flight is controlled by the muscles in the thorax, while the muscles in the abdomen are not directly involved.

**The abdominal motor system and development during metamorphosis:** In order for these new neural circuits to be created, extensive reorganization of the neurons and muscles must occur during metamorphosis (Truman, 1990). During metamorphosis, three events occur for reorganization: the birth of adult specific neurons (neurogenesis), death of larval neurons (apoptosis), and the re-specification of motor neuron innervations of newly developed muscle cells (Truman, 1990; Tissot and Stocker, 2000; Consoulas and Levine, 2000). Also, the shape of the CNS changes. In the larval stage, the segments containing thoracic neurons grow in size while the segments containing abdominal neurons shrink in size. This change in size reflects that change in control of locomotion from both the thorax and abdomen in larvae to mainly the thorax in adults. In the embryo stage, approximately 34 motor neurons extend from each hemi segments of the CNS to innervate ventral, lateral, and dorsal muscles in the periphery. The motor neurons and the specific muscles they innervate have been mapped in previous studies. However, mapping of motor neurons and their muscles innervations has not been done for the adult stage.

**Work performed in the Lab:** The lab has been studying how neuromuscular junctions (NMJs) in the thoracic region are re-organized during metamorphosis (Hebbar and Fernandes, 2004) given that a new motor function, flight, is controlled by the thorax. Recently, research has begun to focus on the abdomen (Hebbar et al 2006), since it presents an interesting situation- the abdomen becomes indirectly involved in movement, and could be the reason why the size of the abdominal CNS shrinks. In the above studies, the focus has mainly been in the periphery, without much attention to the CNS, where cell bodies of the motor neurons reside. and the
control of locomotion. In the last 2 years, instead of only looking at the NMJs in the periphery, the lab is now studying the entire motor pathway, which includes cell bodies in the CNS, the axons that become bundled and leave the CNS as nerves, and the NMJs on the muscle surface. The adult muscles are organized in 3 groups, ventral lateral and dorsal (Figure 2). Preliminary studies of the role of a transcription factor dHb-9, which controls the identity of motor neurons innervating ventral muscles in the adult abdomen was recently reported (Banerjee et al. 2008).

Statement of goals:
We wish to determine the role of BarH1, a transcription factors, role in the innervation of the motor pathway in lateral muscles during metamorphosis. The main questions that we are concerned with are:
1. Are the lateral muscles in the adult also innervated by motor neurons that express BarH1, as in the embryo and larvae?
2. How many larval motor neurons expressing BarH1 survive to the adult stage?
3. Can the identity of BarH1 motor neurons be changed using alternative transcription factors, such as dHb9.

Hypothesis:
#1 A subset of the BarH1 expressing motor neurons survive metamorphosis innervate lateral muscles in the adult abdomen.
   • If this is true, then fewer motor neurons remain to innervate lateral muscles in the adult.
#2 The identity of adult motor neurons is established at the embryo/larval stage.
   • If this is true, then the innervation of muscle fibers will remain the same.
**Work Done By Me:** I have learned tissue dissections and the process of immunochemistry to visualize cell bodies, axons and neuromuscular junctions with BarH1 pattern of expression (Fig 4B,C).

**My Research Project**

**Aim 1:** Follow BarH1 motor neurons through metamorphosis to identify new muscle innervations and determine motor neuron relationship to larval predecessors.

**Rationale:** Is lateral identity maintained at the adult stage, as reflected by BarH1 expression. We want to identify if the motor neurons that innervate adult later muscle fibers express the transcription factor BarH1, which controls the innervation of lateral muscle fibers in embryos. In the embryo/larva 8 neurons per hemi-segment express that transcription factor. Muscles fibers 21 to 24, 4 total, are innervated by motor neurons expressing BarH1.

**Genetic Tool:** The GAL4/UAS system of targeted gene expression allows specific expression of a gene of interest in tissues or cell types of choice (Brand and Perrimon, 1993). It separates the target gene (from the activator in two transgenic lines. One line has an activator protein (Gal4) which is under the control of a tissue-specific promoter. In our case, BarH1 controls the expression of Gal4. The other line contains DNA sequences to which the activator will bind (UAS), and a target gene. The target gene is only expressed when the two lines are crossed. In our case, the target gene is Green Fluorescent Protein (GFP) which allows us to identify the location of motor neurons that express BarH1. Thus, GFP expression is activated by BarH1-Gal4. This will be used for Aim 1.

**Approach and Methods:**
1. Staging of animals at various stages in the life cycle, including the 3rd instar larva, pupa at 12, 24, 48, and 72 hour, and 2 day old adults. Dissection involved cutting open the specimens, pinning, and cleaning them out. We will compare the adult and larval stage to one another. Two specific things we’ll look at is the pattern in the adult stage and when during pupal development is the pattern established.
2. The next step is immunochemistry. We will use primary antibodies to GFP (BarH1 expression) and HRP (all innervation). Fluorescent secondary antibodies will allow us to detect BarH1 (green), all neurons/axons/NMJs (red) and muscle targets (far red-Phalloidin)

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**Figure 4:** (A) Representation of one larval abdominal hemisegment. 34 motor neurons innervate 30 muscle fibers. (B) BarH1$^{\text{GAL4}}$ expression in the larval CNS. Reporter gene expression (GFP) reveals a distinct pattern of motor neuron cell bodies in the CNS. Axons of these neurons exit the CNS and innervate muscles in the periphery (C). Innervation to three lateral muscles is shown (21, 22 and 23).
3. Confocal microscopy is the next step. Triple labeling done in the immunochemistry will be detected using appropriate laser channels.

Data Collection
10 animals for each stage will be analyzed. We will be looking specifically at the CNS and also at the neuromuscular junctions, NMJS, of the neurons’ innervation of muscle fibers. We will count the axons to determine how many cells express BarH1 and follow the axons to their NMJs in order to create maps of the innervations in the larval and adult stage.

Expected Outcomes:
We will consider the following possibilities:
1. The motor neurons that express BarH1 will innervate lateral muscles in the adult abdomen. This means that lateral identity, motor neurons that innervate lateral muscle fibers, is maintained throughout the life cycle by the transcription factor BarH1. There are no new transcription factors that control lateral identity of motor neurons in the adult stage.

2. The number of BarH1 motor neurons that innervate muscles in the abdomen will decrease due to loss of centrality of the abdomen in distinct locomotion. We predict that the number of motor neurons innervating lateral muscles will decrease during metamorphosis. Since in the adult stage, distinct locomotion is centrally controlled in the thorax only, there is no longer a need for an abundance of motor neurons in the abdomen.

3. The innervation of motor neurons can be changed by the use of new transcription factors, such as dHb9 which control expression of motor neurons that innervate ventral muscle cells. We believe that we can cause motor neurons that naturally innervate lateral muscles to innervate different muscle fibers by suppressing the BarH1 transcription factor and activating either the dHb9 or even-skipped transcription factor.

Aim 2: To examine if the identity of BarH1 expressing motor neurons can be changed by forced expression of a different transcription factor

Rationale: BarH1 is a transcription factor that confers lateral identity on a group of 5 motor neurons which is manifested in their innervation of lateral motor neurons. We know that larval motor neurons that survive metamorphosis undergo a period of restructuring to innervate a different set of new adult muscles. The restructuring also allows them to become part of new adult-specific circuits that regulate adult behaviors. During this period, is it possible to reprogram the 5 BarH1 expressing motor neurons from a lateral fate into a ventral fate? If dHb9 (a transcription factor conferring ventral expression) be forced in these five motor neurons, thus reprogramming them? Reprogramming of cells is currently at the cutting edge of research on the context of converting one differentiated cell into another functionally different type and has relevance for stem cell research (Gurdon and Melton, 2008).

Approach: We will force the expression of a transcription factor, dHb9, in BarH1 expressing cells, by using the Gal4/UAS system of targeted expression. An additional level of temporal control will be placed on the Gal4/UAS system so that expression of dHb9 can be “turned on/off
at desired stages of the *Drosophila* lifecycle. In our case we want to allow the animals to develop normally until larval stages and then “switch” on the expression during metamorphosis. Innervation patterns will be examined at 12, 24, 48, 72 hour and adult stages. We will examine motor neuron cell bodies, axons, nerves, neuromuscular junctions and muscles using markers indicated in Aim 1.

**Methods:** We will cross the appropriate lines so that we generate flies containing two desired transgenes- UAS-GFP to follow the BarH1 expressing cells and UAS-dHb9. The expression of these targeted genes will be controlled in time through the use of a third transgene, Gal80. We will use a heat-shock (exposure to 37°C) to induce the onset of dHb9 expression during specific pupal stages: 0hour: onset of metamorphosis, 12hour: onset of neuronal restructuring, 26hour: restructuring ongoing, 48hour: restructuring complete, 72hour: Adult pattern seen. Animals will be dissected at progressively older stages and examined using markers described in Aim 1.

**Data Collection:** We will use ten animals at each stage to observe structures in the Central Nervous system - motor neuron cell bodies, axons, nerves; and in the periphery- neuromuscular junctions and muscles.

**Expected Outcomes:** Two extreme outcomes are possible:

1. There will be no change in identity for the five BarH1 expressing motor neurons, and they will innervate lateral muscles despite forced expression of dHb9. If this is seen, it can be concluded the identity of BarH1 neurons which is first determined in the embryonic stage, cannot be subsequently reprogrammed to change from lateral to ventral through the expression of the ventral identity gene, dHb9.

2. A change in identity occurs such that BarH1 neurons innervate ventral muscles. Therefore conclude that the identity of BarH1 neurons which is first determined in the embryonic stage, can be subsequently reprogrammed to change from lateral to ventral through the expression of the ventral identity gene, dHb9.

**Significance:** *Drosophila* is an optimal model organism to analyze restructuring and motor neuron identity in nervous systems. This is relevant in better understanding neural restructuring that occurs in development and puberty in humans. This is also potentially relevant in the case of neural damage, it could be astoundingly helpful if alternate nerves could be reprogrammed, and directed to new areas for innervation. Also as indicated earlier, reprogramming of cells is a hot new area of stem cell research currently being used to generate motor neurons from skin cells and develop treatments for motor neuron diseases such as Lou Gehrig’s.
BUDGET AND JUSTIFICATION

<table>
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<td>1. Spring Scissors (2/1 per student)</td>
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<td>2. Biologic Tip Forceps (4 pairs/2 per student)</td>
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<td>Purpose: To visualize the entire set of axons and neuromuscular junctions</td>
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References:


Nervous System Remodeling in the *Drosophila*: The fate of larval motorneurons

Alex Blair, Matt Siefert, Soumya Banerjee and Joyce J. Fernandes
Department of Zoology, Miami University, Oxford, Ohio 45056

In holometabolus insects such as *Drosophila* and *Manduca*, radical transformations occur during the transition from larval to adult body forms. Behaviorally, a crawling animal becomes capable of walking and flight. Thus, two distinct motor systems are generated to accommodate this behavioral alteration. The formation of the adult motor system involves neurogenesis, apoptosis and remodeling of persistent larval motor neurons, innervating the newly generated muscle fibers. We are studying specifically the abdominal muscle sets as a model for nervous system remodeling and developmental plasticity, a phenomenon also prevalent in the human brain.

Our goal is to identify the fate of abdominal larval motorneurons during metamorphosis: how many persist and respecify, how many die, and whether or not they maintain a general identity. We studied this with three separate approaches. These studies are done in the context of also examining morphological changes in the CNS that occur simultaneously. Three separate approaches are used (1) A fly strain that labels all glutamatergic neurons (mostly motor neurons) OK371. (2) A second marker we use is the BarH1 line. This is another homeodomain protein that plays an important role in specification of laterally located embryonic motorneurons. A smaller subset of cells express in the adult one of these survives and maintains its lateral identity, innervating adult lateral muscle. (3) Lastly we follow individual neurons with MARCM (mosaic analysis with a repressible cell marker). This technique allows us to study individual motor neurons and their targets. With this we work towards cellular identification (cell body position, axonal projection and NMJs) of all larval motor neurons during the third instar stage. The MARCM also grants us the ability to follow neurons during metamorphosis and determine which individual neurons survive from the larva to the adult stage.
PRESENTATIONS:

Poster 1:


Poster 2:


Poster 3:
Nervous system remodeling: The fate of BarH1 expressing motor neuron

Bridget Hartman, Matt Siefert and Alex Blair
Advisors: Soumya Banerjee and Joyce J. Fernandes
Department of Zoology, Miami University, Oxford OH 45056

1. Introduction
Holometabolous insects, such as fruit fly, Drosophila and moth, Manduca develop through distinct life stages, embryo, larva, pupae and adult. During the pupal phase, the larva is transformed into a morphologically and structurally distinct adult stage, known as metamorphosis. An intriguing feature of remodeling is that new neural circuits are established and consequently new behaviors are manifested. A neural circuit usually consist of larval elements (motor neuron) and adult specific components (interneurons, sensory neurons and muscles).

2. Nervous System Restructuring
During metamorphosis, central motor control shifts from the larval abdominal CNS to the thoracic CNS in the adult. Larval locomotion is coordinated along the body wall by 3 thoracic and 8 abdominal segments., where as in the adult, locomotion is controlled by the thoracic region. This shift in locomotion is reflected in the restructuring of the CNS as the abdominal region shrinks and the thoracic region expands. From this observation we anticipate that fewer abdominal motor neurons will persist into the adult stage.

3. Embryonic/Larval Motor System
In the embryonic ventral nerve cord (VNC), 32-34 motor neurons in each abdominal hemisegment innervate 30 somatic muscles (Landgraf et al., 1997; Schmid et al., 1999). Motor axons from each hemisegment project into the periphery through three principle nerve routes, a) Intersegmental nerve (ISN), b) Segmental nerve (SN) and c) Transverse nerve (TN) These nerve bundles align to dorsal, ventral and lateral general regions and are then further subdivided until singular axons innervate specific muscles. Motor neurons that project to ventral, lateral and dorsal muscle targets can be identified through their expression of specific transcription factors (Landgraf)

4. Goal of this study
In order to follow restructuring of the larval motor system (cell body position and peripheral innervation) we focus on a subset of neurons that express Bar H1 transcription factor. Bar H1 is a homeo-domain protein, which plays an important role in the specification of laterally located motor neurons (Garces and Carroll, 2006).

Our main questions are:
1. Are the lateral muscles in the adult also innervated by motor neurons that express BarH1, as in the embryo and larvae?
2. How many larval motor neurons expressing BarH1 survive to the adult stage, and the fate of the cells that are lost during metamorphosis?

5. Tool (Targeted expression of green fluorescent protein in neurons)
The GAL4/UAS system of targeted gene expression allows specific expression of a gene of interest. The target gene is separated from the activator in two lines. One line has an activator protein (GAL4) under the control of a tissue-specific promoter. In our case, BarH1 controls the expression of Gal4. The other line contains DNA sequences to which the activator will bind (UAS), and a target gene. The target gene is only expressed when the two lines are crossed. The target gene is Green Fluorescent Protein (mcs8GFP) which is membrane bound and allows us to identify the location of motor neurons in cell bodies and axons.

6. Bar H1 Expression in the Larval Motor System

7. Bar H1 expression in the Adult Motor System
Fewer cell bodies are visible in the CNS. Our preliminary data indicates an absence of lateral innervation in periphery

8. BarH1 expression in the Pupal Stage

• In 12 hours pupae, the number of cell bodies expressing Bar H1 in the CNS is reduced to 1-2 cells per hemisegment.

• 3/4th reduction in the larval complement of Bar H1-positive neuron occurs between 0h APF-12h APF

9. Future Directions
Pupa: 8hAPF - Are there more Bar H1-positive neurons and NMIs in the CNS? 12h APF - Are NMIs dismantled?
Adult: Are Bar H1-positive cells motor neuron? Examining projections of the segmental nerves?

10. Acknowledgement
• Funding from the National Institute of Mental Health (R15 077720-01).
• Undergraduate Research Award (2009), Undergraduate Summer Scholarship (2009),
• Matt Duley and Richard Edelman for assistance with Confocal Microscopy and printing facility.
• The Zoology Department for the Williams Award (2009)

11. References
1. Drosophila as a model for neuro-plasticity

The human brain is not static but changes through life with mastery of locomotor behaviors, new motor skills, memory formation, trauma and aging. Drosophila and other holometabolous insects, show drastic restructuring during their life cycle, each stage displaying characteristic behaviors. For example, metamorphosis transforms a crawling larva into an adult stage that displays capabilities such as walking and flight. New behaviors require distinct motor systems to be formed, involving neurogenesis, apoptosis and remodeling of persistent larval neurons (Tissot and Stocker, 2000).

2. Nervous system restructuring

During metamorphosis, abdominal motor control shifts to the thoracic region of the CNS. This is also reflected in the CNS morphology—the thoracic ganglion expands and abdominal region shrinks. Organization of segmental nerves is also altered.

3. Focus of this study: Fate of larval motor neurons

The stereotypic connectivity and location of abdominal motor neurons at the embryonic and larval stages was first described by Sink and Whitington (1991). Thirty-four motor neurons connect to about 30 muscle fibers, and a combinatorial code of transcriptional regulators specifies identities of neurons projecting to Ventral, lateral and dorsal targets (Landgraf et al., 2000).

In this context, the reduced adult abdominal ganglion the shift of locomotor control to the thorax, and a new set of adult muscles not directly involved in locomotion, what is the fate of segmentally repeated larval motor neurons?

In this study, we have followed the fate of subsets of motor neuron. We examined cell body positions in the CNS, axonal projections out the segmental nerves and NMJs on target muscles.

4. Identifying persistent motor neurons: 3 approaches

Approaches:
A: Examining motor neuron specific driver, OK371 during metamorphosis.
B. The expression of BarH1-positive neurons during larval, pupal and adult stage.
C. MARCM (mosaic analysis with a repressible cell marker) to follow individual motor neurons.

5. Bar-H1 expression in the motor system

Pupa: 1 to 2 cell bodies/segment persist in the CNS at 4h and 12h into metamorphosis.

Adult: We have not yet been able to identify the location of cell bodies; however, axons of BarH1 positive motor neurons can be detected in the segmental nerves and NMJs can be observed on lateral muscles in the periphery.

6. MARCM: Mosaic Analysis using a Cell Repressible Marker

Larva: We have identified 15 motor neurons in larvae using D42GAL4.

<table>
<thead>
<tr>
<th>Prep number</th>
<th>Abdominal segment and CNS position</th>
<th>Muscle innervation</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>A5, Dorsal 30</td>
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<td>3</td>
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<td>9</td>
<td>A5, Lateral 12</td>
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<tr>
<td>10</td>
<td>A5, Lateral 15 and 16</td>
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</tr>
<tr>
<td>11</td>
<td>A1, Lateral Lateral Muscles</td>
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</table>

Adult: A labeled cell that persisted into the adult stages. This cell projects to lateral muscles in the adult body wall. We have obtained approximately 5 clones.

7. Future Directions

1. Image MARCM clones in the larva and follow them into the pupal/adult stage. (A) 2nd instar larva showing 2 cells expressing GFP in the CNS. (B): The live imaged larva was aged to adult and immunostained. The 2 cells persisted and maintained a similar CNS location in the adult. Dorsal muscles in A1 were innervated.

2. Follow subsets of motor neurons using additional identity genes such as dHb9, and even-skipped.

8. References


9. Acknowledgements

** The fly community for providing fly stocks and reagents.
** Funding from the National Institute of Mental Health (R15 077720-01).
** Matt Duley and Richard Edelman for assistance with Confocal Microscopy and the use of printing facilities.
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B: The expression of Bar H1: positive neurons during larval, pupa and adult stage.
C: MARCM (mosaic analysis with a repressible cell marker) to follow individual motor neurons.

5. Bar-H1 expression in the Larva and Pupa

Pupa: 1-2 CB segment in CNS. A single axon projecting through segmental nerve to the periphery.

Larva: 2 cell bodies/segment in CNS. With 2 axons extending out into the periphery innervating lateral muscles 22 and 23.

6. Analysis of Bar-H1 expression in Adults

Adult: (A) Expression of cell bodies in the CNS of adult Drosophila abdomen showing a single axon projecting into periphery.

(B) Expression in the periphery showing lateral muscle targets and distinct stereotypy

Bifurcation: BarH1/GFP double copy expression with HRP double label in A3 peripheral segment of Adult Drosophila. The stars mark bifurcation locations, showing 3 bifurcations with the BarH1 located in the third bifurcation innervating the posterior region of lateral muscles.

7. Future Directions

Utilize the Gal4/UAS system, using additional identity genes such as dHb9, that confers motor neuron identity in the ventral region. We will determine if the identity of motor neurons can be altered during the process of re-organization. We will have GAL4 controlled by BarH1 bind to the UAS targeting expression of dHb9. By doing this we have induced a gain of h9 function in BarH1 neurons.

(1) Will GFP be expressed at NMJs of ventral muscles?
(2) Will GFP be expressed at NMJs of lateral muscles without change in number or stereotypy?

8. References


9. Acknowledgements

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