Peripheral Glial Cell Layers are Differentially Remodeled during Adult Nerve Formation in *Drosophila*

Bridget Hartman

Advisor: Joyce Fernandes

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Department of Zoology
Oxford, Ohio
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Peripheral Glial Cell Layers are Differentially Remodeled during Adult Nerve Formation in Drosophila

By Bridget A. Hartman

Approved by:

__________________________, Advisor
Dr. Joyce Fernandes

__________________________, Reader
Soumya Banerjee

__________________________, Reader
Dr. Jack Vaughn

Accepted by:

__________________________, Director
University Honors Program
Contents

Chapter 1: Proposals: Dean’s Scholar Award, Honors Thesis ............... 4
Chapter 2: An Introduction to Glia ................................................. 9
Chapter 3: Project Background ..................................................... 12
Chapter 4: Results ........................................................................ 18
Chapter 5: Future Direction of Research ....................................... 27
Appendix ......................................................................................... 29

Proposal for Undergraduate Research Award
Posters and Presentations
Paper on Neurological Diseases
Chapter 1

Proposals

A. Proposal for Dean’s Scholar Award, submitted fall 2009 for the 2009-2010 academic year.

**Background:** During the life cycle of the fruit fly, the nervous system undergoes extensive reorganization during metamorphosis. The fruit fly nervous system is composed of a brain and ventral ganglion containing 3 thoracic segments and 8 abdominal segments. From each of the segments a pair of nerves projects to the body wall. Thus, there are 3 pairs of thoracic and 8 pairs of abdominal nerves. As the animal progresses through the life cycle, the abdominal portion of the central nervous system (CNS) decreases in size and the thoracic portion increases reflecting changes in locomotor control. As part of the reorganization, nerves A4 to A8 fuse into a single terminal nerve trunk (TNT). Each nerve is surrounded by four layers of glial cells: wraping glia, subperineurial, perineurial, and neural lamella. Glial cells have been shown to influence neuronal development, such as guiding axon pathfinding and eliminating excess axonal projections in the periphery (Freeman and Doherty 2006). We are concerned with the presence and prevalence of glial cells during metamorphosis and TNT fusion.
The goal of my research is to examine and determine in the context of fusion of abdominal neurons to form the TNT the presence and prevalence of the glial subtypes during metamorphosis.

Hypothesis: Some glial layers will unsheath during metamorphosis and during formation of the TNT.

Experimental Design: The 2 glial subtypes neural lamella and wrapping glia will be identified using cell-specific markers. The target genes for these layers are Viking and Nervana, which label the specific cell layers as visualized with Green Fluorescence Protein (GFP). These strains allow the cytoplasmic extent of the glial cells to be visualized and wrap around nerves. The technique I will use is a gene trap, in which the fluorescence gene is inserted into the target gene. The GFP gene is inserted into these genes to create promoter fusion. Promoter fusion means that the promoter for the target gene now acts as a promoter for the expression of GFP as well. Also, I will use the Gal4/UAS system which uses a tissue specific gene to drive the expression of GFP. Highlighting the cells with GFP in this way allow us to visualize the degree of ensheathment of the nerves.

Expected Outcomes: I expect that some degree of unsheathing of the glia subtypes will occur to allow the fusion of the TNT to occur. I also expect that each glial subtype will be remodeled to different extents.
B. Honors Thesis Proposal, submitted fall 2009

During metamorphosis of the fruit fly *Drosophila melanogaster*, extensive reorganization of the nervous system occurs. This metamorphosis occurs during the fly’s transition from the larval to the adult stage. Before the transition, the abdominal portion of the central nervous system (CNS) is larger than the thoracic proportion. However, during metamorphosis, the thoracic ganglion become larger and the abdominal ganglion shrink. In the larval stage, the peripheral nervous system (PNS) is organized into 8 pairs of nerves and after metamorphosis, in the adult stage, the PNS is organized into 3 pairs of nerves plus a terminal trunk.

Through the studies on the nervous system remodeling of the fruit fly, we are able to gain a better understanding of the genetic basis of the process. The knowledge gained from the studies of fruit fly can be applied to human health. The human nervous system undergoes remodeling throughout life. If scientists succeed in understanding the genes and proteins involved in remodeling as well as applying this knowledge to alter the remodeling process of neurons, they may unlock cures and treatments to many neurological disorders. One day, doctors may be able to use this knowledge to stimulate neural connections after brain damage or cell proliferation of nerve cells.

My project will focus on the glial cells that encase segmental nerves. Glial cells provide neurons with insulation from the outside environment as well as forming the important blood-brain barrier. Glial cells and nerve cells are constantly interacting with
each other therefore it is believed that glial cells play a role in the process of nerve fusion occurring in metamorphosis. This study will focus on the different layers of glial cells: perineurial, subperineurial, and wrapping glia. My focus will be on the presence and prevalence of these different subtypes of glia at the different stages of metamorphosis in order to determine their specific roles in the fusion of the terminal trunk. Our prediction is that the glia must actively unwrap and then ensheath the newly developed terminal trunk. We will determine their roles by either killing off cells at specific times of development or preventing their re-ensheathing to determine how this affects the formation of the terminal nerve trunk. The transition from the pairs of nerves to the terminal nerve trunk occurs between the 24th to 36th hours of metamorphosis.

In our first approach, we will cause the expression of cell death inducing gene called reaper in the glial cells during this time period. The complication with this method is that it can kill the animal if it is expressed for too long, so we must determine how long the gene can be expressed without killing the animal. After determining this, we will express the gene in overlapping periods to determine the effects this has on the developing terminal nerve trunk. We will observe the nervous system organization before and after the expression of reaper.

Our second approach will be to slow down development of the glia in order to prevent glial cells from ensheathing the terminal nerve trunk. In order to do this, we will express a mutant gene that prevents cell membrane turnover. This gene will be expressed during the 24 to 36 hour period of metamorphosis. The expression of this mutant gene is not as
drastic or effective as killing of the cells, so it is considered a milder form of “disruption” of the remodeling process. Again, we will examine the nervous system before and after the expression of this mutant gene.

This thesis is very significant to me and my area of research. The fruit fly and their nervous system is a very popular area of research. My research in particular could play a very important role in the research of others because it could unlock some of the driving factors involved in the remodeling of the nervous system. These factors could help other scientists to understand their research on the nervous system. The potential knowledge that can be gained from studying the fruit fly nervous system is limitless. All of the studies on the fruit fly, including mine, could help unlock the key to treating many neurological disorders. Over the summer, my research has become even more important to me due to personal reasons. A very close friend of my family has been diagnosed with Lou Gehrig’s disease, which is only one of many neurological disorders that have no cure or even treatments. It is heart breaking for me to know that there is nothing that can be done to help his condition at this point in time, but my research and the research of others studying the fruit fly give me hope that in the future, people like my friend will be able to get help and even survive their disorders.
Chapter 2

An Introduction to Glia

In the last 10-15 years, research in neuroscience has provided insight into the role of glial cells in the nervous system, specifically their interaction with neural counterparts. Glia cells are the most plentiful cell type in the nervous system, ten times more than neurons, but were previously viewed as accessory cells that have little influence on the neurons they ensheath (Edenfield et al. 2005). However, scientists have discovered that glia play a very important role in the development and sculpting of the nervous system. It has been established that glia play a vital role in neuronal pathfinding, axosome trimming, cell death engulfment, and neuron target selection (Freeman 2006). If these functions are interrupted, the development of the nervous system could be severely derailed. This change in the view of glial cells from being passive cells to active cells involved in nervous system development has led to a greater focus of studies on glial cells and their functions.

Invertebrate models have been very useful in unraveling basic cellular process; however, invertebrate glia were commonly dismissed because they were viewed as being too different developmentally from vertebrates to be a useful source of information. This has changed in the recent past; studies focusing on the development of NMJs have shown similar roles for glia in both vertebrates and invertebrates. Invertebrate glia play a role in axonal NMJ sculpting through a draper-mediated clearance of projections which is similar to the function of Schwann cells in vertebrate NMJs (Fuentes-Medel et al. 2009).
It has become more evident that invertebrate glia are very similar to their vertebrate counterparts and thus can serve as a good model organism for studies.

There are four basic categories of glial types in *Drosophila*: cortex, neuropil, surface, and peripheral glia (Freeman and Doherty 2006). Each of the four glial types has a counterpart in vertebrates that performs similar functions (Table 1). The glial type we are focusing on are the peripheral glia, which consists of 4 subtypes: wrapping glia, subperineurial, perineurial, and neural lamella (Stork et al 2008).

**Table 1**: Comparison of glial cells and their vertebrate counterparts. Source: Freeman and Doherty 2006.

<table>
<thead>
<tr>
<th><em>Drosophila</em> glial type</th>
<th>Vertebrate Counterpart</th>
<th>Function</th>
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<tbody>
<tr>
<td>Cortex glia</td>
<td>Astrocytes</td>
<td>Cellular conduits for gas and nutrients, encase neurons in cell cortex, some immune function</td>
</tr>
<tr>
<td>Neuropil glia</td>
<td>Oligodendrocytes</td>
<td>Aid is fasciculation of nerves, insulate neurons to improve firing, trophic support, some immune function</td>
</tr>
<tr>
<td>Surface glia</td>
<td>Immune function and cell engulfment</td>
<td>Microglia</td>
</tr>
<tr>
<td>Peripheral glia</td>
<td>Schwann Cells</td>
<td>Ensheath and support PNS sensory and motor neurons</td>
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It is believed that impairment of these glial cell types can lead to some mental illnesses because the neuron-glial interactions cannot occur or occurs improperly. Mental illnesses in which impaired glial function has been implicated are Schizophrenia, Huntington’s disease, depression, and Alzheimer’s (Bernard et al 2010) (Bradford et al 2009) (Ting et al 2009). In Schizophrenia, disruption of astrocytic cells expression of chondroitin
sulfate proteoglycans (CSPG) has been observed, implicating the CSPGs-glial interactions are important to this disease (Pantazopoulos et al 2010). This disruption in glial activity leads to difficulty in neuronal pathfinding, synaptic formation, and neuronal miscommunication which could cause the symptoms of Schizophrenia.

The funding for our research in the lab come from the National Institute of Mental Health (NIMH), whose mission statement says one of their goals is to “transform understanding and treatment of mental illnesses through basic and clinical research, paving the way for prevention, recovery, and cure” (www.nimh.nih.gov). Our research on glial cells can be one of the avenues for achieving this goal. By studying the role of glial cells in fruit flies, we can determine their role in nervous system maturation and formation of neural connections. By gaining knowledge into the function of glial cells and neuronal-glia interactions, we can gain a better understanding of these diseases characterized by glial interruption and eventually find a way to treat and even cure many of these diseases. The study of glial cells in fruit fly can have far reaching implications, not just in mental illnesses, but also in many other neurodegenerative diseases and even memory formation and loss.
Chapter 3

Project Background

**Introduction:** The fruit fly, *Drosophila melanogaster*, has served as a model organism for biological research since the early 1900s. The high fecundity, short life span, and small genome make it an ideal organism for study. The studies are also beneficial for vertebrates, and more specifically humans, because of the compatibility of its genome to that of vertebrates. *Drosophila* has been imperative in discovering and understanding biological processes, the function of genes, and morphological changes and development. By studying the fruit fly, scientists have been able to study genes that are homologous to vertebrate genes. The *Drosophila Hox* genes are very similar to *homeotic (hox)* genes in vertebrates that are involved in patterning of the hindbrain (Bellen et al 2010). Cells communicate and influence one another through cell to cell communication, use of chemical messengers, cell surface receptors, and direct contact.

**Different behaviors in adult and larval stages:** The life cycle of the *Drosophila* is divided into distinct stages: the embryo, the larvae, the pupa undergoing metamorphosis, and finally the adult stage. The fruit fly starts as an embryo, which involves the formation of the nervous system. During the larval stage, expansion of the nervous
system continues. The larva’s main locomotion is crawling, which involved the entire body length. The larva then enters the pupal stage, in which extensive reorganization of most tissues occur. After the larval stage, the animal has completely matured. The locomotion of the adult fly has changed from crawling to flight, which involves only the thoracic portion of the body. Some of the most drastic reorganization occurring involves the nervous system and muscles. During the embryonic stage, neurons develop and innervate the muscle fibers. During metamorphosis, these neuromuscular junctions (NMJs) are severed when the muscle fibers die. With the development of new muscle fibers, new neuromuscular junctions are formed when motor neurons that survive innervate the muscle cells once again. Neuromuscular junctions are the junction at which the neuronal dendrites innervate and make connections with muscle fibers. NMJs are the site at which communication by neurotransmitters between neuron and muscle occurs. The changes in locomotor function are at the center of the extensive reorganization that occurs during metamorphosis. Neurons are not the only cells that change during metamorphosis. Their glial counterparts undergo extensive reorganization (Hebbar and Fernandes 2010).

**The nervous system of the fruit fly and developmental changes:** During metamorphosis, the fruit fly nervous system undergoes many morphological changes. In the embryo, the central nervous system (CNS) lies along the entire length of the embryo and projects 11 nerves outward on each side. The nerves are approximately the same length. In the larval stage, the CNS is made up of 3 thoracic hemisegment and 8 abdominal hemisegment. One nerve projects out of each hemisegment into the periphery
for a total of 16 abdominal nerves (Fig.3). The nerves are labeled A1 to A8 starting with
the most anterior projecting nerve. After metamorphosis, the thoracic portion of the CNS
has enlarged in size and the abdominal portion has shrunk in size (Fig.2). These changes
can be explained by the move in the central control of movement from both the thoracic
and abdominal regions to only the thoracic region. Rearrangements of the nerves occur
as well. The nerves A4 to A8 no longer project separately from hemisegments but are
now fused into the TNT, giving the abdominal portion of the CNS a 3 + 5 confirmation
(Fig.3).

**Figure 2**: Central Nervous System at the different stages of the cell cycle: embryo, larva, 
pupa, and adult. The abdominal portion of the CNS is outlined by dotted lines. 
Courtesy: Soumya Banerjee.
Figure 3: The abdominal nerves in the larval stage and the adult stage. The larval stage has 8 pairs of nerves and the adult stage has 3 pairs of nerves and a terminal nerve trunk. Courtesy: Camillo Molina.

Glial cells and glial subtypes: Drosophila peripheral glia are divided into 4 subtypes: wrapping glia, subperineurial, perineurial, and neural lamella. The innermost layer of glial cells is the wrapping glia (red), which express the tissue specific gene Nervana. These cells ensheath the neurons and project inside the bundle in between the individual neurons. The second innermost layer is the subperineurial (purple), which expresses the tissue specific gene Moody. The third layer of glia is the perineurial layer (blue), which expresses the tissue specific gene C527. The outermost layer is the neural lamella (black), which expresses the tissue specific gene Nervana. This layer of cells has a very

Figure 4: Cross-section of Drosophila nerve depicted the glial layers: wrapping glia, subperineurial, perineurial, and neural lamella. Ref: Stork, et al 2008.
uniform appearance and surrounds the entire bundle of cells.

**Work performed in the Lab:** Previous research in the lab has shown two things: that glial cells retract during the life cycle and are later reproduced (Hebbar and Fernandes 2010).

**Statement of Goals:** There are two goals pertaining to the presence of the 4 glial subtypes during remodeling of the nervous system at metamorphosis. The Questions we wish to address in this experiment are:

1. What subtypes are present or absent at the different stages of metamorphosis?
2. To what extent are the different layers differentially remodeled?

**Tools and Techniques:** For this experiment, we will be using a gene trap technique and the Gal4/UAS system with target genes to drive the expression of GFP. A gene trap involved the insertion of the GFP gene into the target gene. Our two target genes are Viking and Nervana for the neural lamella and wrapping glia. The GFP gene is inserted in these genes so that their expression is tied with their expression in a promoter fusion. This technique allows use to visualize the individual layers so that the degree of ensheathment can be determined. Along with GFP, we utilized the Gal4/UAS system and Repo to label all types of glial cells. The Gal4/UAS system is a tissue specific technique that uses a target gene to drive the expression of GFP. The Gal4 gene expression is driven by the promoter for the target, in this case Repo. The products of the Gal4 expression bind with the upstream activating sequence (UAS) of the GFP gene and
drive its expression. In this way, we are able to express GFP in a tissue specific manner. After the lines were created, we dissected animals at the various stages. We performed dissections for the larval stage, the pupal stage at various hours, and the adult stage. Each stage involved a different type of dissection to expose the nervous system. To study the various preps, we will use immunostaining to visualize GFP and HRP. We will first treat the preps with the primary antibodies goatαGFP and mouseαHRP along with blocking. After 24 hours, the primary antibodies are replaced with a solution of secondary antibodies donkeyαgoat and donkeyαmouse along with blocking. Once the preps have been prepared with primary and secondary antibodies, pictures are taken using the confocal microscope, which allows us to view both the GFP and HRP staining, which are characterized by different wavelengths, at the same time. Using the pictures made on the Confocal Microscope, we will determine the degree of ensheathment of the cell subtypes at different times during pupal development.
Chapter 4

Results

My project addresses two questions: (1) Does glial unsheathing occur during metamorphosis, and (2) Are the glial subtypes remodeled to different extents. We used two lines to examine the neural lamella and wrapping glia layers: VikingGFP and NervanaGFP. We looked at the fruit flies at the larval, 12h, 24h, 72h, and Adult stages to determine the presence of the layers. The flies were dissected and fixed to prepare them for immunostaining. For all of the preps, we used both primary and secondary antibodies for the immunostaining. The preps were then mounted on slides and viewed using the confocal microscope, which produced the photos in the following section.

Respective Contributions: Pupal dissections to view the neural lamella and wrapping glia were done by Matthew Siefert and myself. Immunostaining was performed by Soumya Banerjee and Matthew Siefert with some help from me. All work done with the confocal microscope to obtain images was performed by Soumya Banerjee.

Glial Ensheathment of the Nervous System at various pupal stages

The peripheral glia are completely ensheathed at the larval stage (Fig.5A). At 12h APF, glial ensheathment has decreased as seen by the reduced intensity of GFP and increased intensity of red on nerves (Fig.5B). At 24h APF, GFP intensity has slightly increased (Fig.5C). At 28h APF, ensheathment of peripheral nerves has increased significantly, though not to the levels observed in the larval stage (Fig.5D). Glial unsheathing is occurring during metamorphosis, though it is not completely unsheathed. Therefore,
some glial layers are not present during metamorphosis, while others are still present and being reorganized. This raises the question of which layers are present or absence and to what extent is each of the layers being remodeled. We therefore looked at the individual cell layers to answer these questions.

**Figure 5:** Glial cells are labeled with GFP shown in green and neurons are labeled with HRP shown in red. The line used was Repo-Gal4/mcD8GFP. A. Larva/0h APF  B. 12h APF  C. 24h APF  D. 28h APF

**The expression of GFP in peripheral glia driven by Viking in Viking-Gal4/mcD8GFP to indicate the presence of the neural lamella.** The neural lamella is the outermost layer of glial cells ensheathing axons. This layer is an extracellular matrix that is secreted by the layer below it, the perineurial layer. In the larval stage, the neural lamella is present along the axons of the PNS (Fig.6A). At 12h APF, degradation of the neural lamella has begun (Fig.6B). At 24h APF, the neural lamella cannot be detected (Fig.6C). By 72h APF, the neural lamella is still not ensheathing the axons and the newly formed TNT (Fig.6D). In the adult PNS, the neural lamella is completely ensheathing the nerves (Fig.6E). Therefore, the neural lamella is not present between at all times but disappears around 24h APF and does not ensheathe the axons once again until 72h APF of pupal development. This degradation occurs because the layer producing it, the perineurial, may be remodeled during metamorphosis. When the neural lamella is once
again detected, sometime after 72h, it may mean that the perineurial layer is finished being remodeled.

**Figure 6**: The neural lamella is labeled by GFP in green and all neurons are labeled with HRP in red except in D. which is in blue. A. Larva/0h APF B. 12h APF C. 24h APF D. 72h APF E. Adult

**The expression of GFP in the peripheral glia driven by Nervana gene in Nervana-Gal4/mcD8GFP.** Wrapping glia are the innermost layer of the 4 glial subtypes and these cells are in direct contact with the axons of the nerve. The wrapping glia may be responsible for the bundling of axons into nerves. The wrapping glia are present in the larval stage, ensheathing the peripheral nerves (Fig.7A). At 24h APF, the wrapping glia are not present along the nerves (Fig.7B). At 72h APF, the wrapping glia begin to
ensheath the axons once again as indicated by the presence of GFP (Fig. 7C). In the adult stage, the wrapping glia completely ensheath all of the axons and the newly formed TNT (Fig. 7D). Like the neural lamella, the wrapping glia cannot be detected during a large portion of metamorphosis. This may occur to allow the death and remodeling of neurons and the fusion of the TNT.

**Figure 7**: The wrapping glia is labeled with GFP in green and all neurons are labeled by HRP in red. A. Larva/0h APF  B. 24h APF  C. 72h APF  D. Adult
Comparison of presence of the 4 peripheral glial subtypes before fusion of the TNT in metamorphosis. The lines used are Viking-Gal4/mcD8GFP, C527-Gal4/nlsGFP, Moody-Gal4/mcD8GFP, and Nervana-Gal4/mcD8GFP. The Neural Lamella (Fig.8A) and wrapping glia (Fig.8D) are not present on the axons before the fusions of nerves A4-A8. The Perineurial and Subperineurial are both present before terminal nerve trunk fusion as indicated by the GFP positive cells along the axons (Fig.8B and 8C). Each layer experiences a different outcome during metamorphosis. The wrapping glia are not detected for most of metamorphosis. Normally they are in direct contact with the axons and connect to them. However, the absence of wrapping glia may aid in the fusion of the TNT and the rebundling of the nerves. The neural lamella is degraded during metamorphosis and cannot be detected, this may also aid in the fusion and rebundling of the nerves. Also, the perineurial layer which produces the neural lamella may be undergoing reorganization and may cause this absence. The Perineurial and Subperineurial layers are both present during metamorphosis and might be extensively reorganized during this time. The glia layers may also be involved in the neuron cell death seen in metamorphosis, either triggering cell death or simply aiding in degradation of deal cells (Freeman 2006).
**Figure 8:** The glial layers are labeled with GFP in green and all neurons are labeled with HRP in red. A. Viking-Gal4/mcD8GFP  B. C527-Gal4/nlsGFP  C. Moody-Gal4/mcD8GFP  D. Nervana-Gal4/mcD8GFP

<table>
<thead>
<tr>
<th>Stages</th>
<th>Larva</th>
<th>12h</th>
<th>24h</th>
<th>48h</th>
<th>72h</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neural Lamella</strong></td>
<td>Present</td>
<td>Present</td>
<td>Reduced</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Perineurial</strong></td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Subperineurial</strong></td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Wrapping Glia</strong></td>
<td>Present</td>
<td>Present</td>
<td>Reduced</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>
Of the 4 subtypes of the glial cells, the Neural Lamella and Wrapping Glia are not present through all of metamorphosis. They are absent between 24h and 72h of metamorphosis. The perineurial and subperineurial layers are always present throughout the entire life cycle. Therefore, glial unsheathing occurs during metamorphosis and the glial cell types are remodeled to different extents.

**Summary of Results**

Our results supported both parts of our hypothesis: that some glial unsheathing would occur during metamorphosis and that glial layers would be remodeled to different extents. During metamorphosis, the wrapping glia and neural lamella were not detected. However, when Repo was used as a driver of GFP, there was reduced expression which indicated glial unsheathing occurred though not completely. This means that some glial layers are present and others are absent during metamorphosis. Since the wrapping glia and neural lamella could not be detected, then the perineurial and subperineurial layers must be present throughout metamorphosis. Also, unlike the neural lamella and wrapping glia, which are completely degraded or unsheathed, the perineurial and subperineurial are remodeled to a different extent.
Works Cited


Chapter 5

Future Directions

The next step that can be taken with the information we have discovered in this study is to manipulate the 4 glial subtypes through the use of the Reaper(UAS)-Gal80 system. Using this technique, individual can be killed off individually to observe how reorganization of the nervous system and TNT fusion are disrupted. The Reaper(UAS)-GAL80 system is a temperature sensitive system, which we can exploit to selectively kill the layers at a certain times in the life cycle. By changing the temperature of incubation, we will kill the layers between 0h and 24h, which is the time range in which most cell proliferation occurs during metamorphosis.

The way this system works is that Gal4 is a driver for both reaper and GFP. At 18°C, Gal80 is active and acts as a competitive inhibitor of Gal4 so that the Reaper and GFP are not expressed. If the temperature is raised to 28°C, Gal80 becomes inactivated and the products of Gal4 can now bind to the upstream activators sequences (UAS) for both Reaper and Gal4. Once Reaper is expressed in a cell, then the cells will undergo apoptosis, induced death of a cell. We can make this system tissue specific by using the cell specific genes C527 or Moody as the upstream activators of Gal4.

To achieve the apoptosis of the specific layers, we will increase the temperature of incubation at 0h and leave them at 28°C until the 24th hour of metamorphosis. At this
point we will return the pupae to a temperature of 18˚C for the remainder of metamorphosis.

<table>
<thead>
<tr>
<th></th>
<th>C527Gal4 ; Gal80 ; UASReaper</th>
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<tr>
<td>At 18˚C</td>
<td>Gal80 ON</td>
</tr>
<tr>
<td></td>
<td>Gal4 Blocked</td>
</tr>
<tr>
<td></td>
<td>UASReaper non expressed</td>
</tr>
<tr>
<td>At 28˚C</td>
<td>Gal80 OFF</td>
</tr>
<tr>
<td></td>
<td>Gal4 Active</td>
</tr>
<tr>
<td></td>
<td>UASReaper expressed</td>
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We anticipate that as a result of this programmed cell death, the reorganization of the nervous system and the fusion of the TNT will be interrupted and incomplete.
Appendix

A. Proposal for Undergraduate Research Award

B. Posters and Presentations
   a. 2009 Undergraduate Research Forum Poster
   b. 2010 Undergraduate Research Forum Poster
   c. 2010 Undergraduate Research Forum Oral Presentation

C. Paper on Neurological Disorders
Proposal for Undergraduate Research Award

I collaborated on this proposal with Alex Blair, and it was submitted in fall 2008 for the 2008-2009 school year. We were awarded an Undergraduate Research Award for a total of $750. In addition, we received the Williams Research Award for $200. This work was reported at the 2009 Undergraduate Research Forum.
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 body wall. 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

![Image](image_url)

**Fig 2:** Left Panel: The Drosophila embryo (not to scale) has a uniform pattern of muscles in the head thorax and abdomen. Many of the motor neurons innervating these muscles are thought to survive and innervate corresponding segments of the adult. The abdominal bodywall musculature has 3 sets of muscles as seen in a dissected preparation: ventral (v) lateral (l) and dorsal (d). Right Panel: Nerve terminals on each muscle group

Source: (Hebbar and Fernandes, 2006).
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:**

![Fig 3: Schematic represents the central theme of our investigations: to determine which larval motor neurons survive to innervate adult muscle targets in the context of the development of an adult motor system (muscles and nervous system).]
#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).

![Figure 4:](image)

**Figure 4:** (A) Representation of one larval abdominal hemisegment. 34 motor neurons innervate 30 muscle fibers. (B) BarH1^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).

**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)

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? Can 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>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
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<tbody>
<tr>
<td><strong>Tools</strong></td>
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<tr>
<td>1. Spring Scissors (2/1 per student)</td>
<td>$367.50</td>
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<tr>
<td>Product # 15000-08. Fine Science Tools Inc., CA</td>
<td>Purpose: for Tissue dissections</td>
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<tr>
<td>2. Biologic Tip Forceps (4 pairs/2 per student)</td>
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<td><strong>Primary Antibodies</strong></td>
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<tr>
<td>3. Rabbit Anti-Green Fluorescent Protein (GFP)</td>
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<tr>
<td>Product # ab6556. Abcam, Inc. Cambridge MA</td>
<td>Purpose: For visualizing GFP reporter in transgenic fly strains</td>
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<tr>
<td>4. Goat anti-HRP (2ml)</td>
<td>$125.00</td>
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<tr>
<td>Product # 55970; ICN Pharmaceuticals, OH</td>
<td>Purpose: To visualize the entire set of axons and neuromuscular junctions</td>
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<td><strong>Secondary antibodies</strong></td>
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<td>5. Alexa Flour 488 (green) Donkey anti-Rabbit</td>
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<tr>
<td>Product #A-21206 0.5 ml. Molecular Probes., Eugene, OR</td>
<td>Purpose: For visualizing anti-GFP</td>
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<td>6. Alexa Flour 660 (far red) Donkey anti-goat</td>
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<td>Product #A-21083 0.5 ml. Molecular Probes, Eugene. OR</td>
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<td>7. Alexa Phalloidin 555 (red)</td>
<td>$330.00</td>
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<tr>
<td>Product # A-34055. Molecular Probes., Eugene, Oregon</td>
<td>Purpose: For visualizing muscle fibers</td>
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TOTAL: $1432.75
References:


Report For Undergraduate Research Award, Spring 2009

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

Our study focused on the fate of the neurons expressing BarH1, a transcription factor, which innervated lateral muscles in the larva. We use Green Fluorescent Protein (GFP) as a reporter of BarH1 gene expression to 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).

The first aim of our project was to follow BarH1 motor neurons through metamorphosis to identify new muscle innervations and determine motor neuron relationship to larval predecessors.

Results: In the larval stage, there are 4 to 5 motor neurons expressing BarH1 present in each hemisegment of the CNS. These motor neurons innervate the lateral muscles 21 to 24. In 12 hour pupae, the number of cell bodies in the CNS expressing BarH1 is reduced to 1 to 2 cells per hemisegment. This 3/4th reduction in the larval complement of BarH1 occurs between the 0h APF to 12h APF. In the adult stage, there are fewer cell bodies present in each hemisegment of the CNS expressing BarH1. Our preliminary data indicates an absence of lateral innervation in the adult periphery. The flies used in this experiment contained one copy of the gene coding for GFP, whose expression is driven by the UAS/Gal4 system. We are currently creating a cross that will result in a line
containing two copies of the GFP gene. We will use this line to determine if the absence of innervation in the adult stage is a result of not enough GFP expressed in the axons of motor neurons or if there is in fact no expression of BarH1 in lateral innervating motor neurons. We also plan to look at pupae in stages of metamorphosis earlier than 12 hours to determine when reduction of BarH1 expressing cells occurs.

The second aim of our project is to examine if the identity of BarH1 expressing motor neurons can be changed by a forced mis-expression of a different transcription factor.

Results: A series of crosses are required with our end fly resulting from the required crossing of two doubly homozygous, five generation lines. These crosses have started and are still in early stages. The use of balancers such as cyo (curly wings), Tm6Tb (Tubby body) and Dr (dropped eyes) are necessary to verify a homozygous chromosome. Each cross takes ten days and another four to set up the following cross. Therefore 66 days are required to complete these 5 generation crosses. We currently are in the 2nd generation stage. When we complete the cross, one line will have a chromosome homozygous for BarH1 and another Gal80, and the second line will be homozygous for UASdhb9 and UASmcD8GFP. Therefore when these lines are crossed we will result in heterozygotes allowing for the possibility of heat controlled mis-expression of BarH1 by dhb9. We will be able to determine if this mis-expression occurred by observing if there is now visible peripheral innervation in the adult.
Posters and Presentations

I have presented two posters at three forums and given one oral presentation of my research in the past two years. The first poster was presented at the 2009 Undergraduate Research Forum. The second poster was presented at the Poster Session for Undergraduate Research and Mentoring on March 30th 2010 and at the 2010 Undergraduate Research Forum. I gave an oral presentation on April 14th at the 2010 Undergraduate Research Forum on my current research as well.
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 consists 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, whereas 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 hemisegements innervate 30 somatic muscles (Landgraf et al., 1997; Schmidt et al., 1999). Motor axons from each hemisegment project into the periphery through three principle nerve routes, a) Intersegmental nerve (ISN), b) Segmental nerve (SNk) 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 (mcdBGFP) 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

A: BarH1GFP 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. We see approximately 4-5 Bar H1-positive neurons per abdominal hemisegment.

B: BarH1 expressing motor neurons are present in each SNk bundle. As seen here in preliminary data only 2 are bright enough to visualize, innervating muscles 21 through 24.

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:
What 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

Glial remodeling during abdominal segmental nerve fusion in pupa
Soumya Banerjee, Matthew Siebert, Camilo Molina, Bridget Hartman, Meredith Dorr and Joyce J. Fernandes
Department of Zoology, Miami University, Oxford OH 45056

1. Organization of segmental nerves is distinct in larva and adult
Segmental nerves:
• Larva: 8 pairs of abdominal nerves exit the ventral ganglion.
• Adult: 3 pairs of nerves directly exit the ventral ganglion. The rest are bundled in a terminal nerve trunk (TNT).

2. When does this Transition occur?
• 28h APF: 5 nerves (A1-A5) exit the CNS. The posterior three nerves (A6-A8) are in the process of bundling. Transition of the larval CNS to adult morphology,
• 48h APF: By the second day of metamorphosis, 3 nerves (white arrows) exit the CNS, the remaining 5 exit via the terminal trunk (red arrows). Adult CNS morphology can be observed as early as the second day of the lifecycle The adult pattern of segmental nerve organization is evident. Adult CNS morphology can be observed as early as the second day of pupation.

Hypothesis: During terminal nerve trunk fusion a transient axonal unshethment occurs along the segmental nerves. We predict that unshethment will be seen in all glial cell layers or it will only be seen in specific glial layers.

3. Goals of this study
A. Examine glial nuclei along segmental nerves during larval and pupal stage.
Approach: Anti-repo antibody.
B. Monitor glial wrapping during metamorphosis.
Approach: Repo-Gal4::UASmCD8GFP
C. Examine glial subsets during metamorphosis.
Approach: Glial cell layer specific Gal4.

A. Glial number in segmental nerves during TNT fusion
Larva: Work done in the embryo has identified 6-12 glial cells are present in each segment of muscle field (Sepp et al., 2000; Hilchen et al., 2008). Our study of repo+ve cells along larval segmental nerves shows that the number of glia in the muscle field remain the same (5/8/segment). The length of segmental nerves increases in size during the larval stage up to 3mm in length (Leiserson et al., 2000). Our data indicates that 13-24 additional glial nuclei are found along this nerves prior to entering muscle field (A2-A4 have 13-18; A5-A7 have 20-24).
Banerjee et al in 2006 have shown the distribution of glial nuclei along the nerves during larval stage.

Pupa (prior to terminal nerve trunk (TNT) formation):
12h APF (prior to fusion): More than two fold increase of glial nuclei can be observed along the abdominal segmental nerves.
24h APF (during fusion): More than three fold increase of glial nuclei can be observed in the nerve.

B. Glial wrapping of abdominal segmental nerve during metamorphosis

Prior to and during terminal nerve trunk fusion (12-28h APF) glial ensheathment as detected by repoGal4::UAS-GFP along segmental nerves. Are glial subsets differentially affected/alted/remodelled?

B’. Examining Subset of glial wrapping during metamorphosis
Several glial cell layers wrap the Drosophila nervous system; neural lamella, perineurial, subperineurial and wrapping glia (Stork et al., 2008). To study individual layers we used layer specific enhancer and gene trap lines.

Wrapping glia: study the wrapping glia layer we used nerva2GFP line. During larval and adult stage wrapping glia can be observed along the segmental nerves. At 24h APF, wrapping glia cannot be detected, suggesting that this layer is remodelled during metamorphosis.

Neural lamella: To study neural lamella we used viking-GFP line. During larval and adult stage neural lamella can be observed along the segmental nerves. At 24h APF, it cannot be detected, suggesting that this layer is remodelled during metamorphosis.

Conclusion: At 24h APF, the neural lamella and wrapping glia layers are absent but the perineural and subperineural layers are present (data not shown). Neural lamella and wrapping layers return at 72 APF after previously missing in early time stages (24h APF).

C. Remodeling of perineurial glial layer prior to TNT formation

Larva: Out of 15 glial nuclei (±0.80), 10 (±0.65) of them are CS27-positive (perineural) along A3 segmental nerve.

Gial nuclei: Anti-repo
CS27 nuclei: CS27-Gal4 X UAS nls-GFP

Pupa (12h APF): Out of 35 glial nuclei (±2.81), 31(±3.75) of them are CS27-positive (perineural) along A3 segmental nerve.

Gial nuclei: Anti-repo
CS27 nuclei: CS27-Gal4 X UAS nls-GFP

4. Future Directions
• Glial layers will be manipulated using reaper prior to terminal nerve trunk formation.
• Phospho-histone staining will be used to examine glial proliferation.

5. Acknowledgements
• The fly community for providing fly stocks and reagents.
• Funding from the National Institute of Mental Health (R15 077720-01) and NSF.
• Matt Duley and Richard Edelman for assistance with Confocal Microscopy and the use of printing facilities.

6. References
Glial Cell Layers are Differentially Remodeled During Adult Nerve Formation in *Drosophila*

Bridget Hartman
Department of Zoology
Advisors: Joyce Fernandes and Soumya Banerjee

Drosophila as Model for Neuralplasticity

Changing Shape of the CNS

- Changes are needed to bring about new locomotive behaviors
Organization of Adult Nerve Formation

Larva = 8 pairs
Adult = 3 pairs + TNT

What is the Role of Glia in Terminal Nerve Trunk?

Glial Cells

- Glia ensheath nerves
- Most abundant cell type in Nervous System

Allen and Barres, 2009
Glial Cells are Present in Layers
- Cross section
- Glial Layers:
  - wrapping glia
  - subperineurial
  - perineurial
  - neural lamella

Hypothesis
- Some degree of glia unsheathment occurs. Glial sub-type will reorganize to different extents.

Repo Positive Glia
- Present
- Decrease
- Increase
- Present
Conclusions

- **Hypothesis:** Some degree of glia unsheathment occurs. Glial sub-type will reorganize to different extents.
  - Yes, Glial unsheathing occurred
  - Yes, Glial subtypes were reorganized to a different extent.
  - Wrapping Glia and Neural Lamella are not present but other glial layers are.
Neurological Diseases

This section is a compilation of human neurological disorders. This work was one of the first projects I conducted in Dr. Fernandes’ lab in the fall of 2007. Funding for the research conducted in the lab comes from the National Institute of Health. In order to gain a better understanding of the relevance of *Drosophila* as a model organism for the nervous system development, I examined the website of the Nation Institute of Neurological Disorders and Stroke, which has a listing of a large number of neurological diseases. I focused on the diseases where motor neurons were affected. This helped me to understand that the knowledge we gain from the experiments can be applied to human diseases. *Drosophila melanogaster* has many homologous genes to vertebrates and therefore can allow a better understand of the genetic and molecular bases of cellular processes that occur in normal and diseased conditions.
Canavan’s Disease

- Gene linked neurological birth disorder
- White matter of the brain is degenerated into spongy tissue with microscopic fluid filled spaces
- One of the genetic disorders known as Leukodystrophies
- Disease results in imperfect completion or growth of myelin sheath
- Myelin sheath comprises the white matter of the brain and is made of ten different chemical components
- Canavan’s disease is caused by mutation in the gene for enzyme called aspartoacylase
- Recessive gene disorder

Characteristics

- CNS
- Affects myelin sheath of motor neurons
- Rare disorder
- Manifestation: Appears in infancy and progresses rapidly. Results in mental retardation, loss of previously acquired motor skills, feeding difficulties, abnormal muscle tone, and abnormally large, poorly controlled head. Can lead to paralysis, blindness, or hearing loss. Children with Canavan’s disease are characteristically quiet and apathetic.
- Genetic: Recessive
- No cure and no set treatment
Central Pontine Myelinolysis

• Neurological disorder

• Shows up after a too rapid of medical correction of Sodium deficiency

• Usually shows up after two to three days

• Rapid rise in sodium causes movement of small molecules and water from brain cells

• Leads to destruction of myelin sheath and neurons may be damaged

• Mechanisms that cause destruction are unknown

• Area of brain that is most susceptible is the pons

• Can also have extrapontine myelinolysis, which are other parts of the brain that are affected

• Many affected people recover, but many have permanent disabilities

• Death is possible, but chances decrease with treatment

Characteristic

• CNS

• Interneuron affected

• Manifestation: Decreased level of awareness, difficulty speaking and swallowing, impaired thinking, weakness or paralysis in arms and legs, stiffness, impaired sensation, and difficulty with coordination.

• Genetic Disorder: no

• Cure: yes, degree of cure depends on treatment
**O’Sullivan-McLeod Syndrome**

- Also known as Monomelic Amyotrohpy (MMA) and Hirayama Syndrome
- Progressive degeneration and loss of motor neurons in the brain and spinal cord that control voluntary movement
- Occurs in males between the age of 15 and 25
- Onset and progression are slow
- No known cause, though some suggest traumatic or radiation injury
- There are some familial forms of MMA

**Characteristics**

- CNS
- Motor neurons
- Rare in North America, more common in Asia
- Manifestation: weakness and wasting in a single limb, most likely an arm and hand rather than a foot and leg. There is no pain associated with MMA.
- Genetic: no
- Cure: no

**Neuromyotonia**

- Also known as Isaacs Syndrome
- Rare neuromuscular disorder
- Result of continuous signaling of the end regions of peripheral nerve fibers that activate muscle fibers (motor neurons)
• Age of onset is between 15 and 60 and most experience symptoms before 40
• Hereditary and acquired forms
• Acquired may develop in association with peripheral neuropathies or as an autoimmune condition.

**Characteristic**

- PNS
- Motor neurons affected
- Rare: yes
- Manifestation: Progressive muscle stiffness, continuous vibrating or twitching muscles, cramping, increased sweating, delayed muscle reaction, weakened reflexes and muscle pain, but numbness is relatively uncommon. Usually limited to cranial muscles and in most patients, stiffness is most prominent in limb and trunk muscles. Speech and breathing may be affected.
- Genetic: some forms
- Cure: no but different forms of treatment

**Motor Neuron Diseases**

- Group of progressive neurological disorders that destroy cells that control essential muscle activity
- Disruptions in signals between upper motor neurons (in brain) to lower motor neurons (in brain stem and spinal cord) can cause gradual muscle weakening, wasting away, and uncontrollable twitching
• Ability to control voluntary movement is lost
• Inherited or acquired
• Causes are not known

**Characteristics**

• CNS
• Motor
• Rare: some forms
• Manifestation: gradual muscle weakening, wasting away, and uncontrollable twitching
• Genetic: some forms, others acquired
• Cure: none, no standard treatment

**Lambert-Eaton Myasthenic Syndrome**

• Disorder of neuromuscular junctions
• Disruption of electrical impulses between nerve and muscle cells
• Autoimmune disorder; immune system mistakenly attacks the body’s own tissue
• Disruption associated with antibodies
• LEMS associated with cancer, especially small cell lung cancer

**Characteristics**

• PNS
• Motor/muscle cells affected (neuromuscular junctions)
• Rare: yes
- Manifestation: Muscle weakness, tingling sensation in the affected areas, fatigue, and dry mouth.
- Genetic: no, however, some have higher affinity (for autoimmune diseases) due to genes
- Cure: no, however some treatment

**Pelizaeus-Merzbacher Disease**

- Rare, progressive, degenerative CNS disorder
- Coordination, motor abilities, and intellectual function deteriorate
- One of the Leukodystrophies disorders (gene-linked)
- Affects growth of myelin sheath
- Caused by defect in gene for production of proteolipid protein (PLP), which controls production of myelin sheath
- X-link recessive trait, males affected and mother’s carrier
- Severity and onset depend on type of PLP mutation
- Changes in myelination can be detected by MRI

**Characteristics**

- CNS (brain)
- Motor neurons affected
- Rare: yes
• Manifestations: Nystagmus, spastic paraparesis, and limb ataxia. Symptoms can also include slow growth, tremor, and failure to develop normal control of head movement, and deterioration speech and mental function.

• Genetic: yes, recessive and sex-linked

• Cure: no

**Globoidal Cell Leukodystrophy**

• Also known as Krabbe Disease

• Rare, inherited degenerative disorder

• CNS and PNS

• Characterized by presence of globoid cells, which have more than one nucleus

• Breakdown of myelin covering and death of brain cells

• Leukodystrophies

• Caused by deficiency in galactocerebrosidase, which is essential for myelin metabolism

**Characteristics**

• PNS and CNS

• Motor neurons affected

• Rare: yes

• Manifestation: Irritability, unexplained fever, limb stiffness, feeding difficulties, vomiting, and slowing of mental and motor development. Muscle weakness, spasticity, deafness, and blindness also occur.
• Genetic: yes
• Cure: no, some treatment

Familial Periodic Paralyses

• Inherited neurological disorders

• Cause by mutations in genes that regulated sodium and calcium channels in nerve cells

• Two common types:
  o Hipokalemic periodic paralyses
    ▪ Fall in potassium levels in the blood
    ▪ Begins in adolescence
    ▪ Triggered by strenuous activity or high carbohydrate meals
  o Hyperkalemic periodic paralyses
    ▪ Rise in potassium levels in the blood
    ▪ Attacks begin in infancy or childhood
    ▪ Caused by rest after exercise or fasting
    ▪ Attacks are usually shorter, more frequent, and less severe than the hypokalemic form

Characteristics

• Motor neurons affected
• Rare: no
• Manifestation: Affected muscles become slack, weak, and unable to contract. Between attacks, muscles function normally

• Genetic: yes, mutation

• Cure: no, some treatment

**Hallervorder-Spatz Disease**

• Also known as neurodegeneration with brain iron accumulation

• Rare, inherited, neurological movement disorder

• Progressive degeneration of nervous system

• Symptoms may vary and usually develop during childhood

**Characteristics**

• CNS

• Motor neurons affected

• Rare: yes

• Manifestation: Writhing, distorting muscle contractions of the limbs, face, or trunk, choreoathetosis, muscle rigidity, spasticity, ataxia, confusion, disorientation, seizures, stupor, and dementia. Less common symptoms are painful muscle spasm, dysphasia, mental retardation, facial grimacing, dysarthria, and visual impairment.

• Genetic: yes

• Cure: no
Hereditary Neuropathies

- Inherited disorder of PNS
- 4 Subcategories
  - Hereditary motor and sensory neuropathy
  - Hereditary sensory neuropathy
  - Hereditary motor neuropathy
  - Hereditary sensory and autonomic neuropathy
- Charcot-Marie-Tooth disorder accounts for the majority of all hereditary neuropathies

Characteristics

- PNS
- Motor and Interneuron
- Rare: no
- Manifestation: numbness and tingling in the face and hands, muscle weakness (especially distal muscles), scoliosis, thin lower legs, foot deformities, insensitivity to pain, and autonomic symptoms such as impaired sweating, postural hypotension, and skin blotching.
- Genetic: yes
- Cure: no

Huntington’s Disease

- Results from genetically programmed degeneration of brain cells (neurons)
• Causes uncontrolled movements, loss of intellectual faculties, and emotional disturbance
• Familial disease
• Mutation in normal disease
• 50-50 chance of inheriting gene
• If a child does not have HD, it cannot pass it on

Characteristics
• CNS
• Interneuron affected
• Rare: yes
• Manifestation: Mood swings, depression, irritability or trouble driving, learning new things, remembering a fact, or making a decision. As disease progresses, concentration or intellectual tasks become increasingly difficult and the patient may have difficulty feeding himself or herself and swallowing
• Genetic: yes, dominant
• Cure: no, some treatment

Hypertonia
• Increase in muscle tension and a reduced ability of a muscle to stretch
• Caused by injury to motor pathways in CNS
• Cerebral Palsy in children under age of 2
• Untreated hypertonia can lead to loss of function and deformity
May result from injury, disease, or conditions such as spasticity, dystonia, rigidity, or a combination of factors

**Characteristics**

- CNS
- Motor neurons affected
- Rare: no
- Manifestation: Spastic hypertonia involves uncontrollable muscle spasms, stiffening, or straightening out of muscles, shock-like contractions of all or part of a group of muscles and abnormal muscle tone. Dystonia hypertonia involves muscle resistance to passive stretching and a tendency of a limb to return to a fixed, involuntary posture following movement

**Hypotonia**

- Decreased muscle tone (muscle weakness)
- Two conditions
- Caused by trauma, environmental factors, or by genetic, muscle, or CNS disorders such as Down Syndrome, Muscle Dystrophy, Cerebral Palsy, Prader-Willis Syndrome, Myotonic Dystrophy, and Tay-Sachs Disease
- Not always possible to find cause
- With infants, their arms and legs hang by their sides and have little or no head control, giving them a rag-doll appearance
• Does not affect intellect; however, some children may take longer to develop social, language, and reasoning skills

• When hypotonia develops in adults it is associated with cerebellar degeneration, in which neurons in the cerebellum deteriorate and die

Characteristics

• CNS

• Motors neurons affected

• Rare: no

• Manifestation: problems with mobility and posture, breathing and speech difficulties, lethargy, ligament and joint laxity, and poor reflexes

• Genetic: no

• Cure: no, some treatment

Kennedy’s Disease

• Inherited motor neuron disease that affects males

• One of the Spinal Muscular Atrophy (AMP) group

• X-linked recessive disease

• Mother must carry gene on one of her chromosomes

• Daughters can be carriers and have one in two chance of passing it onto a son

Characteristics

• CNS (spine)

• Motor neurons affected
- Rare: no
- Manifestation: Tremor of outstretched hands, muscle cramps with exertion, and falsifications. Individuals develop limb weakness usually begins in pelvic and shoulder regions. Weakness of facial and tongues muscles may occur later. Can lead to dysphagia, dysarthria, and recurrent aspiration pneumonia. Some develop gynecomastia and low sperm count of infertility, while others develop non-insulin-dependent diabetes mellitus.
- Genetic: yes, recessive
- Cure: no

**Kugelberg-Welander Disease**

- Also known as Spinal Muscular Atrophy and Werdnig-Hoffman Disease
- Genetic, motor neuron disease
- Results of progressive degeneration of motor neurons in the spinal cord
- Childhood SMAs are all autosomal recessive diseases
- Progressive spinobulbar muscular atrophy
- Occurs between ages 15 and 60
- Slowly progressive
- X-linked recessive disorder, women are carriers and disease occurs in males

**Characteristics**

- CNS
- Motor neurons affected
- Rare: no
- Manifestation: Weakness of muscles in the tongue and face, difficulty swallowing, speech impairment, and excessive development of the mammary gland in males
- Genetic: yes, recessive sex linked
- Cure: no, treatment symptomatic

**Terms**
- Ataxia: Inability to coordinate movements
- Choreoathetosis: involuntary, purposeless jerky muscle movement
- Dysphasia: difficulty speaking
- Dysarthria: poorly, articulated speech
- Dysphagia: difficulty in swallowing
- Gynecomastia: excessive enlargement of male breasts
- Limb ataxia: lack of coordination in arms and legs
- Muscle Rigidity: uncontrolled tightness of the muscles
- Nystagmus: involuntary, jerky movements of head and eyes
- Spastic Paraparesis: Paralysis of legs with hyperactive tendon flexes
- Spasticity: Sudden, involuntary muscle spasms