Overview
During this project, I will be trying to accurately count the number of stems that survive after implantation in injured and non-injured spinal cords using a method of quantifying data called stereology.

Introduction
Advancing knowledge in the area of stem cell implantation has been increasingly important in helping to understand diseases like Parkinson’s and diabetes, as well as spinal cord injury. Advancement has helped to develop possible means of treatment, however none have been successful yet. Stem cells are undifferentiated cells that can divide and renew themselves for long periods of time as well as give rise to specialized cell types under the appropriate physiological or environmental conditions (Cao, et al 2002). For example, a stem cell properly implanted in an injured spinal cord could theoretically produce mature, functioning neurons. The process of turning into one cell type or another is called differentiation. Because of these special properties, neural stem cells may be used to replace dead or dying cells such as neurons in a contused spinal cord or dopamine-producing neurons in a Parkinson’s patient’s brain. Through implantation, stem cells may be induced to create healthy, productive cells, although the mechanism by which this occurs is unknown. Previous studies have indicated that differentiation and survival of stem cells may be strongly affected by the host environment. In culture, stem cells can turn into any type of cell found in the brain. In areas of severe injury, it has been found that stem cell differentiation may be inhibited (Cao et al 2002) possibly due to the toxic nature of the injury site. One of the purposes if this study is to gather information about the survival rates of implanted stem cells in injured spinal cord versus non-injured spinal cord. I will also determine what kind of an effect the injury has on their differentiation.

Recovery from spinal cord injury requires the regeneration of functioning nerve cells: glia and neurons. Neurons conduct and inhibit messages sent throughout the body while glia can be described as non-excitable cells supporting nerve cells. Astrocytes, oligodendrocytes and microglial cells make up the three types of glial cells. Astrocytes maintain the appropriate chemical environment for signaling. Oligodendrocytes are responsible for laying down insulating tissue, or myelin, along neuron axons. Microglial cells can be considered scavenger cells that remove debris from injury sites or due to cell turnover and will not be tracked in this study. During traumatic brain injury, demyelination frequently occurs and neural messages cannot be properly conducted. Replacing damaged glial cells, as well as neurons, is vital for spinal cord regeneration and to promote recovery of limb movement. It has been shown that when oligodendrocytes are produced in culture and then transplanted in Shiverer mice (Shiverer mice have very little myelin due to a mutation), myelination of axons occurs (Liu et al 2000). During this project, undifferentiated stem cells will be implanted into the spinal cords of injured and non-injured mice in order to determine what kind of nerve cells they produce and whether these nerve cells help to reduce the extent of the injury. In a parallel study, others in the lab will be tracking the behavioral recovery of these animals. Improvements in behavior will then be compared to the quantification of stem cell survival and differentiation that I perform. Human skin cells, or fibroblasts, will be implanted as a control so that any improvement in the injury site can be related to improved neural functioning due to the physical connection of stem cells with the host versus chemicals such as growth factors that are released by all cells.

Samples taken from the spinal cords will be stained using immunocytochemistry. Antibodies that react only with human cytoplasmic protein will introduced to the samples and stain only cells that are human in origin. This will allow us to differentiate between mouse nerve cells and human nerve cells.

In order to determine changes in the volume of the contusion in the spinal cord, a special method of quantifying data will be used. Stereology is a systematic yet random method of determining, for example, whether the volume of an injury site has changed or how many human stem cells are in a given area of spinal
cord. Using stereology, spinal cord injury volume and stem cell survival and proliferation can be determined within greater than 95% accuracy (Olympus 2000).

**Methods**

*Preparation of Animals*

In this study, 40-48 female NOD-Scid mice will be used. These mice have a compromised immune system and thus will not reject the human cells grafted into them. There will be four different groups tested consisting of 10-12 members per group (Fig 1.1). Group A will consist of uninjured mice that were injected with human stem cells (hCNS-SC), Group B will consist of uninjured mice that were injected with human fibroblasts, Group C will consist of injured mice injected with human CNS stem cells, and Group D will consist of injured mice injected with human fibroblasts. All of the mice will undergo a laminectomy, and mice in Groups C and D will be injured by technicians in the Christopher Reeve Paralysis Foundation core facility. Injection of either 100,000 human fibroblasts or 100,000 human CNS stem cells will follow nine days later. There will be four injection sites to the T9 section of the spinal cord (Fig 1.2). Two will be rostral to the injury, to the left and right of the midline. Two will be caudal to the injury and to the left and right of the midline. Each injection site will contain 25,000 injected cells. Uninjured mice will be injected as if they were injured in the same area as the injured mice.

<table>
<thead>
<tr>
<th>Figure 1.1</th>
<th>Non-injured</th>
<th>Injured</th>
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<tbody>
<tr>
<td>Human CNS-Stem Cells</td>
<td>Group A = 12</td>
<td>Group C = 12</td>
</tr>
<tr>
<td>Human Fibroblasts</td>
<td>Group B = 12</td>
<td>Group D = 12</td>
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*Tissue Preparation and Staining*

The mice will be kept alive for 60 days while kinematic behavioral testing will be done to evaluate their physical improvement or degeneration. At the end of the two months, the mice will be sacrificed according to protocol and perfused with 4% para-formaldehyde. The spinal cords will be removed and frozen before being cut with a cryostat at 25 microns. Every tenth slide will be stained with SC-121, an antibody which reacts with a protein in the cytoplasm of human cells only. Slides from injured spinal cords will also be stained with fibronectin, a protein that accumulates in the injury sites. The slides will then be cover-slipped and analyzed in a microscope using CAST-Grid stereology software. The observer (me) will be blind as to what group the animal is in during the microscopic analysis.

**Stereology Analysis** In comparing groups A and C, we will be able to determine whether injury has a negative or positive effect on proliferation of cells. In comparing groups C and D, we will be able to determine whether any reduction in the injury is due to improved neural functioning because of the engrafted stem cells or is due to the release of chemicals such as growth factors. Comparisons between groups A and B and B and D will serve as controls.

**Level of Preparation**

I have been involved in behavior-related spinal cord research for the past year. During this project, however, behavior testing will be done by another group in the lab and my focus will be in answering questions such as how many stem cells survive after implantation, what do they turn in to, and do they have any effect on the
area of the injury site. Throughout the next quarter, I will be trained in animal care, tissue staining and cutting, and in using stereology software to analyze spinal cord slides in the microscope.

**Timeline**

Because of the length of time needed to complete this project, the experiment will begin in mid-May. The animals will be injured and nine days later the cells will be injected. Two months after that, the animals will be sacrificed and perfused. Their spinal cords will be removed and frozen. This should take about two weeks. At the end of spring quarter, the tissue will be cut over a period of two weeks and then stained over a period of two weeks. Stereology and counting of cells will take a final two months over the summer.

**Works Cited**


