FINAL NARRATIVE REPORT
(An original and 15 copies of the Final Narrative Report must be submitted and sent to the New Jersey Commission on Spinal Cord Research, P.O. Box 360, Trenton, New Jersey 08625.-- NJCSCR office at 609-292-4055, or e-mail at NJCSCR@doh.state.nj.us.)

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2. Name of Organization/Institution

Department of Neuroscience and Cell Biology
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3. Grant Title

Cell Proliferation in the CNS after Spinal Cord Injury

4. Grant Number

NJCSCR grant # 02-3015-SCR-E-0

5. Grant Period Covered by the Report

July 1, 2002 through June 30, 2004. (no cost extension through June 30, 2005).

6. Date of Submission of the Report

February 22, 2007

BODY OF REPORT

The report should cover the following information in 2 - 5 pages, in addition to photographs, figures, charts, etc. Use language suitable for lay readers.

1. Original aims of the project.

The goal of this project was to characterize the cell proliferation that occurs after a traumatic injury to the spinal cord. We hypothesized that: 1) cell proliferation occurs both in areas adjacent to the injury and also in areas distant from the lesion, e.g., dorsal column nuclei, thalamus, motor areas of the cerebral cortex, etc.; 2) cell proliferation in each area occurs for a specific period of time and for a limited number of cell cycles; and 3) different classes of cells (e.g., astrocytes, microglia, etc.) will proliferate in different areas or at different times after the injury. To test these hypotheses cell proliferation was to be characterized after an experimentally produced spinal cord transection at a mid-thoracic level in three Specific Aims. In Specific Aim 1, the location (i.e., in areas close to the injury or in areas which receive or send connections from/to the injured area) and the time course of the cell proliferation was to be determined using bromodeoxyuridine (BUdR) immunohistochemistry. In Specific Aim 2, the length and number of cell cycles was to be determined for each area in which cell proliferation is found (in Specific Aim 1) using double labeling with BUdR in combination with tritiated thymidine (^{3}H-TdR; double S-phase labeling) and with the proliferative marker Ki67. In Specific Aim 3, the cell type of the proliferating cells in each area was to be determined using double labeling with BUdR and cell class specific antibodies.

2. Project successes.

The project was successful in it primary aim, i.e. we showed that cell proliferation occurs both in areas adjacent to the injury as well as in areas distant to the injury. We also showed that this proliferation distant to the injury is widespread, occurring in both the origin of the descending brainstem inputs to the spinal cord and also in the ascending tracts that carry information from the spinal cord. Also, this proliferation at a distance especially in the structures that are the origin descending input to the spinal cord occurs much more rapidly than we expected, increasing to a significant degree within 48 hours of the injury. To be frank, the proliferation in the structures that originated the descending inputs to the spinal cord was an unexpected and delightful finding. It is novel and previously unknown finding. We believe that this proliferation reflects the existence of synaptic reorganization in these structures in a time frame and to a degree previously unrecognized.

Overall, the experiments performed during this project were very successful and have formed the nucleus of a continued and expanded research program on spinal cord injury. In addition, the experiments performed during this project were seminal in terms of expanding the horizons of this laboratory. Previously, we had focused exclusively on cell proliferation in the developing brain. With the support from this project we have expanded our focus to include specifically the issue of cell proliferation after spinal cord and other brain injuries. This expanded focus has been beneficial to the lab and we believe it will be beneficial to the field. This expanded focus continues today through other support and collaborations – see below.

3. Project challenges.

Unfortunately, the project faced major challenges during its funding period. These major challenges were of two types: scientific and non-scientific.

The scientific challenges were actually quite simple. We found that work on the spinal cord injury is more time consuming than we expected! A major reason for this is that for many years we have worked on mouse embryos and the much larger size of the adult nervous system was daunting to us at first. We have since adapted to this through some simple changes in our protocols. In short, the scientific challenges have been conquered.

The non-scientific challenges that presented themselves during the funding period for this project were related to personnel issues. Three specific sets of circumstances combined to inhibit our progress. First, part way through the project, the lab technician, (Mr. Giovanni Sarmiento) that we had hired for this project unexpectedly left, and we were not able to find a suitable replacement for him for several months. Second, Dr. Huaying Li, the postdoc who performed many of the spinal cord lesions, had "health issues" involving a close family member which have required him to travel to China for some time. Later, we discovered that these "health issues" were not real; we also discovered that he was blithely mixing contusion and hemisection spinal cord injuries. The main issue here was language; Dr. Li seemed to be linguistically challenged and there were clear communication problems, even when other Chinese speaking scientists were involved. In the end, we had Dr. Li leave the laboratory. The additional downside is that we had to sort through his experiments and discard some of his data. Third, the PI (Richard Nowakowski) suffered some serious health issues himself during the second year and no-cost extension third year of the project. These health issues were serious enough to force him to cut back significantly on the time that he spent in the lab and office and seriously impacted his productivity for this period. These health issues have since been resolved.

The combined effect of these issues on this project were significant in that they limited the collection of data. As a result, we finished the project with considerable and exciting preliminary data, but to our disappointment insufficient data to publish in a reputable journal. We have decided, however, to continue the project initiated during this period and have obtained additional funding to do, based to a great extend on the preliminary data collected.

4. Implications for future research and/or clinical treatment.

The potential for future research and clinical impacts of this project are great. Cell proliferation in the CNS after an injury is a complex and poorly understood process. The continued research in this area is important as a "dissection" of the complexities involved is necessary. There are no other labs anywhere working on these issues. With respect to clinical therapies, we believe that the prospects are enormous. Cell proliferation in the CNS after an injury is the response to the many aspects of the injury. In addition, the proliferating cells themselves may play a major role in the "healing" process. The proliferating cells are the likely source of many growth factors, cytokines, etc. The complexity at the lesion site is enormous as the blood brain barrier is damaged and cells from the periphery pour into the lesion site. Some of the resulting interactions are no doubt deleterious to the spinal cord; whereas others are likely beneficial. As a result of the funding for this project, this theme has now become a major focus in this laboratory. We expect that this will continue for many years.

With respect to clinical impacts, the sky is literally the limit. Why do I say this? To date, virtually all of the proliferation after a spinal cord injury is glial in nature, mostly microglial, but some oligodendroglial and some astroglial. The first is certainly involved in "clean-up" operations, but the

microglia may also be the source of damaging diffusible "factors". The oligodendroglial proliferation needs to be understood and promoted to increase the re-myelination of axons denuded of myelin after the injury. The astroglia proliferation may represent the source of an endogenous stem cell to provide new neurons to the injury site. Future research in this area, therefore, could lead quite directly to new clinical treatments that address specific avenues not addressed by the currently available therapies.s

5. Plans to continue this research, including applications submitted to other sources for ongoing support.

This project continues with the funding of a Senior Professorship award to the PI from the NJ Commission on Spinal Cord Research. Spin-offs from this Senior Professorship award are in progress. An additional grant for a multi-investigator award from the New Jersey Commission on Traumatic Brain Injury is pending. Additional NIH, etc. grants are planned for the future. The theme of the "dissection" of cell proliferation as originated in this small grant from the NJ Commission on Spinal Cord Research will reverberate considerably in the coming years in this lab.

Pending and Planned Grant Support
Co-PI for : "Regional and Genetic Analysis of Traumatic Brain Injury" (PI, NL Hayes), Subproject 3 of "Multi-Investigator Analysis of Traumatic Brain Injury Models" (PI, M. Schachner). Agency: NJ Commission on Traumatic Brain Injury. This grant will provide support for an analysis of cell proliferation in mice of different strains in the brain after a traumatic brain injury. This grant is for a total of $1,000,000/year for 2 years; the budgeted amount for this subproject is $200,000/year.

6. List and include a copy of all publications emerging from this research, including those in preparation.

As described above no publications from the spinal cord work done during this project period were achieved. Publications from the continuation of this work from the Senior Professorship Award to the PI will be reported separately. There was, however, two collaborative project initiated during this project period that resulted in publications. The citations are below the and reprints are appended.

Sekiguchi, M., Y. Sugiyama, K. Takagi, N. Takagi, S. Takeo, O. Tanaka, I. Yamato, K. Torigoe, **R.S, Nowakowski**. (2003) Rapid appearance of pathological changes of neurons and glia cells in the cerebellum of microsphere-embolized rats. Brain Res. 978(1-2): 228-32.

Sekiguchi, M., Takagi, K, Takagi, N., Date, I., Takeo, S., Tanaka, O., Yamato, I., Kobashikawa, S., Kojun Torigoe, K. and **Nowakowski**, **R.S.** 2005. Time course and sequence of pathological changes in the cerebellum of microsphere-embolized rats. Exp. Neurol., 191: 266-275.