SCIRF#2015 P-04

Grant Title: *DigiGait Analysis, a Valuable Correlate between Functional Recovery and Molecular Markers of Repair following Experimental Spinal Cord Injury*

Aim 1: Status (Planned, In Progress)

**Specific Aim:** *To determine the time-dependent alterations in multi-parametric DigiGait analysis following experimental SCI as a predictor of functional recovery.*

Significant progress was made in the first six-month period of the grant. Experimental spinal cord injury (SCI) was induced in adult male Sprague-Dawley rats using Perot’s weight-drop method with modifications, and adequate post-injury care was provided to the SCI rats. On post-SCI day 7, exercise was started in one subset of the SCI rats utilizing the built-in treadmill in the DigiGait system. Sham rats were not exercised. Based on the previous preliminary data from the Lab, a longer exercise duration including two sessions each of 15 min duration per day for exercise was adopted in the current experimental study. All the rats were sacrificed on day 28 post-SCI and biochemical and molecular analyses were performed to study the molecular markers of repair and recovery induced by exercise.

Rats with inadequate injury were excluded from the study in both the experimental SCI groups.

Listed below are the preliminary data generated till date.

**Early start of exercise (7 days post-SCI) failed to improve functionality in chronic SCI rats:** As one of the main focuses of any SCI therapy is to ultimately improve motor function, chronic SCI experiments were used to determine if rats recover following injury until sacrifice under anesthesia on day 28 post-SCI (Figure 1). SCI rats were graded based on the Basso, Beattie, and Bresnahan (BBB) scale 24 h after surgery and weekly thereafter for 4 weeks. BBB scores were compared in SCI rats with / without exercise. There was a steep rise of scores on days 7-28 compared to day 1 in both the SCI groups. Unexpectedly, BBB scores were higher and better in the SCI groups compared to SCI + exercise group. A significant difference (*p < 0.05) was noted between the SCI versus SCI + Exercise groups at 28 days post-SCI only (Figure 1).

**Figure 1. Lower BBB score with early on exercise for SCI rats on 28-day post SCI.**

SCI rats with / without exercise were followed for 28 days post injury. Rats were clinically graded weekly using the Basso, Beattie, and Bresnahan (BBB) scale to examine motor function following injury. *p = 0.0345 at day 28 post SCI compared with SCI + Exercise group (n = 3-4) in each group.
Next, we tested whether there was any significant metabolic arrest in the SCI alone groups compared to SCI + Exercise group of rats which received two sessions of exercise daily, each for 15 minutes duration.

### Table 1. Arrest in weight gain in experimental rats 28 days post-SCI

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight Gain (g) ± SD</th>
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<tbody>
<tr>
<td>SCI group</td>
<td>93.67 ± 12.12</td>
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<tr>
<td>SCI + Exercise group</td>
<td>84.53 ± 22.24</td>
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Sham group of rats that had received laminectomy alone gained weight in a range of 101.4 – 116.5 g over the 28 days period. SCI rats showed an arrest in weight gain, this effect was more prominent in the SCI + Exercise group, however not significant at p = 0.05 probably due to the low n (3-4).

As per the proposed experiments in the grant, we next compared the profiles of a battery of molecular markers including the axonal intermediary filament proteins (neurofilament – light, medium and heavy) and the microtubule associated axonal motor proteins (dynein and kinesin), which are responsible for the retrograde and anterograde axonal transport, respectively. Overall, dynein and kinesin play an integral part in axonal transport, and their structural-functional profiles are thus, important in repair and recovery following SCI. We wanted to investigate if exercise had any effect on these axonal markers post SCI.

We examined all the three neuronal intermediate proteins in 28 days post SCI rats and compared the two groups with and without exercise in relation to sham. SCI causes significant axonal degeneration that prevails through the chronic phase and is a major hurdle in repair and recovery post SCI.

![Figure 2. Degeneration of axonal intermediate filaments, neurofilament light (NF-light) following chronic SCI.](image)

Spinal cord homogenates from caudal penumbra were compared for the extent of degeneration in axonal NF-light. Unlike sham spinal cord, both the SCI and SCI + Exercise groups had significantly (*p < 0.01) elevated levels of degenerated fragments of NF-light (48 % and 54% respectively compared to sham). There was however no difference between the two SCI groups indicating that early on exercise failed to benefit the axonal preservation.

To further investigate the extent of axonal degeneration and the effects of exercise on post-SCI rats, we assessed the profiles of the retrograde axonal motor protein, dynein and the anterograde axonal motor protein kinesin and compared amongst sham, SCI and SCI + Exercise rats as shown in Figure 3.
Figure 3(A, B). Degeneration of axonal motor proteins in chronic SCI rats. In both the SCI and SCI + Exercise groups, there was a significant loss of dynein (3A) and kinesin (3B), *p < 0.05. GAPDH and β-actin was used as loading controls for the dynein and kinesin blots respectively. There was no significant difference between the SCI, and SCI + Exercise groups for either of the axonal motor proteins suggesting early on exercise had no influence on these molecular markers of repair or recovery.

List all articles, publications, presentations, grant applications or grant awards related to the SCIRF award.

The award helped in successful publication of the following scientific article and SCIRF#2015 P-04 grant award has been duly acknowledged in this peer-reviewed publication: