Electrical-stimulation-assisted standing in our miniaturized pre-clinical standing frame, reduces the muscle atrophy and negative muscle protein changes caused by SCI.

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Introduction

Spinal cord injury (SCI), is a life-altering event for over 80,000 Canadians. In addition to the motor paralysis and loss of sensation below the level of injury, there is a rapid and permanent loss of bone mineral and muscle mass as well as functional deterioration of these tissues in the lower limbs. There are no accepted treatments for lower limb muscle and bone deterioration after spinal cord injury (SCI). This muscle and bone atrophy contributes to several secondary complications such as obesity, metabolic syndrome and type II diabetes, at a much higher incidence and earlier age than seen in the general population.

Human research to identify effective treatments for musculoskeletal deterioration require multi-year studies and cannot examine the underlying mechanisms contributing to the SCI-induced pathology. Therefore, we developed an adult rat model of severe SCI, the standing frames and electrical stimulation paradigms needed to investigate this question. Thus, we tested the effect(s) of 5 hours weekly of electrical stimulation elicited hindlimb weight-bearing extension training on bone deterioration and muscle atrophy after SCI.

This poster focuses on the portion of our study that investigated the changes in muscle mass and myosin heavy chain protein phenotype in the experimental and control animals.

Hypothesis:

Five hours weekly of electrical stimulation elicited weight-bearing hindlimb extension will reduce the musculoskeletal atrophy and muscle phenotype conversion from a ‘slow oxidative’ to a ‘fast glycolytic’ that is normally observed after SCI.

Methods

Adult female Sprague-Dawley rats (n=250 g) were used in this study. All procedures complied with the Canadian Council on Animal Care and University of Manitoba ethics guidelines. Animals were randomly selected for the intact control (IC) or spinalization groups. Animals were spinalized at vertebral T8 and completeness of lesion verified under microscopic visualization. Shufeldt resection was used to separate spinal animal into either SCI-control (SCI-C) or animals that received electrical stimulation elicited hindlimb weight-bearing training (SCI-ES). After the 5-week training period, rats were anaesthetized for terminal harvest of ankle muscle tissue.

Ankle extensor [plantaris (PL) and soleus (SO)] and flexor [biceps anterior (BA), tibialis anterior (TA)] muscle wet weights were recorded and tissue was flash frozen in liquid nitrogen and stored at -80°C until immunohistochemical processing. Muscle tissue containing the mid-belly. Serial crystallographic sections (10 µm) were processed such that the first section was used to visualize fibres containing type I, IIa and IIB myosin heavy chain (MHC) proteins and a second slide for Ia, Ib and I MHC proteins, as follows:

- **Type I**
  - **Abnormal**/**non-myosin heavy chain (MHC) specific antibodies (MHC antibodies):**
  - Substance P (Sigma, H-234, 1:100)
  - Myostatin (BD, 55-8895, 1:100)
  - **MHC antibodies:**
  - **Slow** (SC; sc-12423, 1:300)
  - **Fast** (FC; sc-12424, 1:300)
  - **Type I** (0382001; 1:50)
  - **Type II** (0382002; 1:50)
  - **Type III** (0382003; 1:50)
  - **Type I** (0382004; 1:50)

Muscle sections were imaged at 10X zoom, and consistent representative sections were selected for analysis using ImageJ software with the goal of analyzing 300 muscle fibres per section per animal. As such, a homogenously representative section was selected for TA (500 µm x 500 µm), SO (500 µm x 500 µm) and PL (5000 µm x 500 µm). These images were taken of each section using each separate filter rather than a single image with all three filters to enable analysis of co-labelled cells. The cropped images were stacked and the distribution of MHC types was marked using the cell counter tool in ImageJ.

Figure 1: Experimental design and demonstration that electrical stimulation (ES) elicits increased hindlimb muscle weight bearing and improved passive stand training after SCI.

A. Experimental timeline and protocol

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>0</td>
<td>SCI</td>
</tr>
<tr>
<td>1-5</td>
<td>ES</td>
</tr>
<tr>
<td>6</td>
<td>ES</td>
</tr>
</tbody>
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B. Treadmill performance

C. Animals assigned to one of four groups: SCI Control (SCI-C), SCI Training group using electrical stimulation to elicit hindlimb weight-bearing extension (SCI-ES) or intact controls (IC).

Figure 3: Electrical stimulation-elicted hindlimb weight-bearing prevents or reduces disease-related muscle atrophy in hindlimb extensors after SCI.

- **A.** Ankle flexor muscle (TA) does not demonstrate significant disease atrophy after 6 weeks of spinalization, and is not altered by weight-bearing training (SCI-ES). (ANOVA: Tukey’s multiple comparisons: adjusted p-value).

- **C.** Type I MHC fibres are increased in SCI-ES versus IC. (ANOVA: Tukey’s multiple comparisons: adjusted p-value).

- **E.** All fibres were counted and compared to SCI-ES and IC.

Conclusions

- **•** Our model demonstrates that our method of ES and related loading is sufficient to induce training effects and reduce the atrophy normally seen in paralyzed hindlimb muscle after SCI.
- **•** Consistent with previous literature, ankle flexors (TA) do not display the large reductions in muscle mass observed in ankle extensions (SO, PL) after SCI.
- **•** The distribution of MHC proteins is altered by SCI, such that there is a general increase in the proportion of type Ia, IIB and III MHC fibres in both flexor and extensor muscles of the HL.

Based on our assessment of n=2 animals per group, **•** 5 hours weekly of ES-elicited weight bearing hindlimb did not protect against reduction in type I MHC fibres type nor against increase in proportion of type II MHC fibres.

Although this finding is consistent with other research demonstrating that electrical stimulation training does not significantly alter the ‘fatigability’ of paralyzed muscle, these findings are too preliminary to be conclusive

Limitations & Next steps:
- **•** Ongoing data analysis of the remainder of the tissue (n=6 animals per group) will determine if these preliminary findings regarding relative distribution of MHC fibre types after SCI, with and without training, are reliable.
- **•** Future studies will examine the neural and systemic factors that may contribute to muscle atrophy and conversion from ‘slow’ to ‘fast’ MHC-phenotype in paralyzed muscle.
- **•** There are many candidate neuro-peptides (e.g. leptin) that may contribute to muscle adaptations with training after SCI, and this model will be useful for investigating these neural mechanisms.

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