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

S. Wiebe, M. Aldhshan, K.C. Cowley
Departments of Rehabilitation and Physiology & Pathophysiology , University of Manitoba, Winnipeg, MB, Canada

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 (> 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. Shuffled-deck randomization was used to separate spinal animals 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 (SOL)] and flexor [tibialis anterior (TA)] muscle wet weights were recorded and then tissue was flash frozen in liquid nitrogen and stored at –80°C until immunohistochemical processing. Muscle tissue containing the mid-belly. Serial cryostat sections (12 µm) were processed such that the first slide was used to visualize fibres containing type I, IIa and IIb myosin heavy chain (MHC) proteins and a second slide for Ia, IIa and Ix MHC proteins, as follows:

MHC Isoform	Primary AB / Dilution	Secondary AB / Dilution	Ig Group	Label	Filter/cube/colour
Ia slow oxidative	mouse BA-F8 1:50	goat anti-mouse 1:500	IgG2b	Alexafluor 350	5 UV / 'DAPI filter set'/ blue
IIa fast oxidative	mouse SC-71 1:600	goat anti-mouse 1:500	IgG1	Alexafluor 488	1 / 'alexa filter set'/ green
IIb fast glycolytic	mouse BF-F3 1:100	goat anti-mouse 1:500	IgM	Alexafluor 555	2/ 'CY3 filter set'/ red
IIx fast glycolytic	Mouse 6H1 1:50	goat anti-mouse 1:500	IgM	Alexafluor 555	2 / 'CY3 filter set'/ red

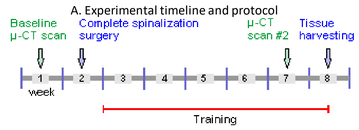
Muscle sections were imaged at 10X zoom, and consistent representative sections were selected for analysis using ImageJ software with the goal of analyzing 100 muscle fibres per section per animal. As such, a homogeneous representative section was selected for TA (500 µm X 500 µm), SOL (500 µm X 500 µm) and PL (1000 µm X 500 µm). Three 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.

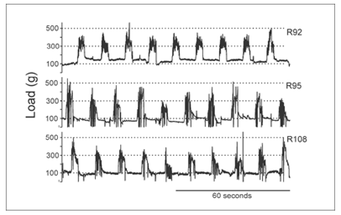
Methods

Figure 1. Experimental design and demonstration that electrical stimulation of tail afferents increases hindlimb weight bearing well beyond passive stand training alone in rats paralyzed by SCI.

A. Experimental timeline and protocol



B. With ES – load increases from ~33% to > 100% BW



C. Animals assigned to one of 3 groups: SCI Control (SCI-C). An SCI training group using electrical stimulation to elicit hindlimb weight bearing extension (SCI-ES) or intact controls (IC)

SCI-ES

SCI/C

IC

D. Area Selection Protocol

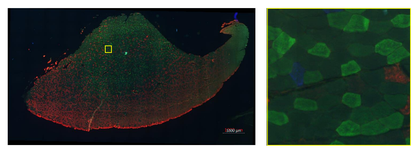
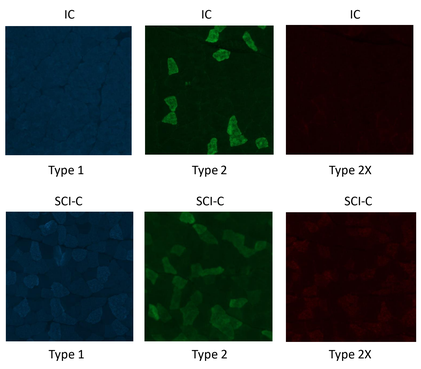


Figure 2. Raw images of stained muscle tissue: Type 1 (Blue), Type 2A (Green), Type 2X (Red)

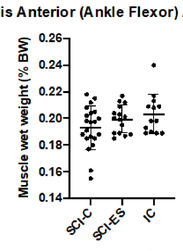
Soleus



Results

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

A. Ankle flexor muscle (TA) does not demonstrate significant disuse atrophy after 5 weeks of spinalization, and is not altered by HL weight-bearing training (p> 0.05; ANOVA; Tukey's multiple comparisons adjusted p-value)



B. PL wet-weights were similar between SCI-ES and IC whereas significant differences were observed between SCI-C and IC (p < 0.0001 Kruskal-Wallis Dunn's multiple comparison adjusted p value). SOL wet-weights in SCI-ES were reduced compared to IC (p< 0.0001; ANOVA; Tukey's multiple comparisons adjusted p-value) but were further reduced in SCI-C vs. IC (p< 0.0001; ANOVA; Tukey's multiple comparisons adjusted p-value).

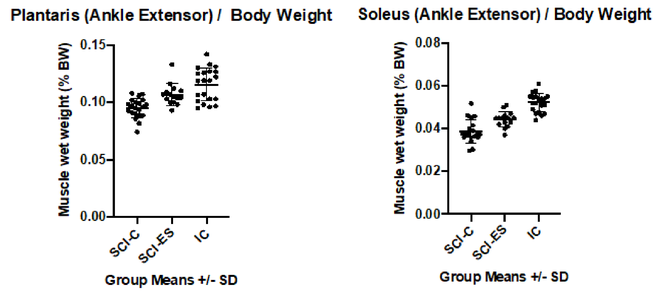
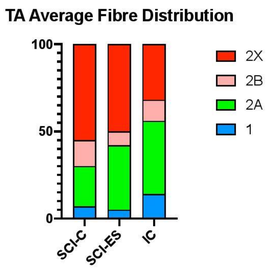
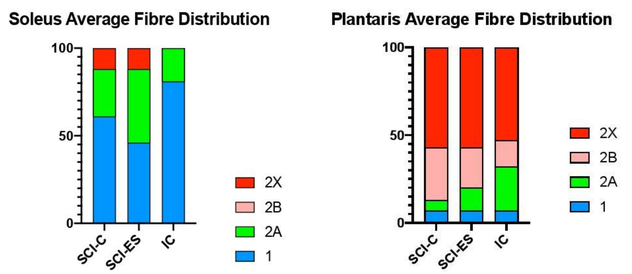


Figure 4: Preliminary results demonstrate conversion from 'slow' to 'fast' phenotype after SCI. It is unclear if electrical stimulation- elicited hindlimb weight-bearing can reduce conversion to 'fast' MHC protein phenotype after SCI.

4A. In TA, SCI caused a relative decrease in the proportion of Type 1 MHC muscle fibers and an increase in Type 2 MHC positive fibres. Electrical stimulation-elicited hindlimb weight-bearing did not protect against this conversion



4A. In SOL, SCI caused a relative decrease in the proportion of Type 1 MHC muscle fibers and the appearance of Type IIx MHC positive fibres. In PL the proportion of IIA fibres decreased and IIB increased with little change in the proportion of type I and 2X MHC. Electrical stimulation-elicited hindlimb weight-bearing did not protect against these conversions



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 extensors (SOL, PL) after SCI

- The distribution of MHC proteins is altered by SCI, such that there is a general increase in the proportion of type IIa, IIb and IIx 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-activated weight bearing hindlimb did not protect against reduction in type I MHC fibre 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 'fatiguability' 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.

Acknowledgements

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