

The Neuroregenerative Effects of Intraspinal Microstimulation (ISMS) Following Spinal Cord Injury (SCI)

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Andy Lee^{1*‡}, Mason Schindle^{1‡}, Neil Tyreman¹, and Vivian K. Mushahwar¹

¹ Department of Medicine and Sensory Motor Adaptive Rehabilitation Technology (SMART) Network, University of Alberta

* Corresponding author: adl2@ualberta.ca

‡ These authors contributed equally to this work.

ABSTRACT

Background Intraspinal microstimulation (ISMS) is a novel electrical stimulation technique that has demonstrated mobility restoration in animals with spinal cord injury (SCI). This project investigated: 1) the capacity of ISMS to restore functional walking in rats with SCI through 4 weeks of stimulation, and 2) the degree of walking deficit caused by ISMS surgery.

Methods Thirteen Sprague Dawley rats were divided into three groups: 1) rats with hemi-section SCI (hSCI) and no implants (control group), 2) rats with hSCI and passive ISMS implants (ISMS sham group), and 3) rats with hSCI and implants with active electrical stimulation (ISMS group). All groups were trained to walk on a horizontal ladder and their performance was quantified pre- and post-surgery.

Results We hypothesized that the rats with active ISMS implants would demonstrate the greatest improvement in functional walking compared to both control groups, and that the ISMS sham group would underperform the most. The preoperative functional walking scores of control, sham and ISMS rats were 5.7 ± 0.2 , 5.5 ± 0.3 and 5.7 ± 0.1 , respectively (7-point scale; mean \pm standard error). The post-surgery scores were 3.2 ± 0.9 , 2.6 ± 0.6 and 3.3 ± 0.8 for control, sham, and ISMS rats, respectively.

Conclusions As the difference between the post-surgery functional walking scores of ISMS and control rats was not statistically significant, this may indicate that four weeks of ISMS stimulation is not enough to cause rehabilitative effects. Additionally, the ISMS sham group demonstrated impaired functional walking compared to the hSCI control group as predicted. Future studies will employ a larger sample size to fully elucidate this trend and utilize thinner microwires to mitigate cellular damage.

KEY WORDS: Rehabilitation Technology, Intraspinal Microstimulation, Mobility Recovery, Rehabilitation Neuroscience, Paraplegia, Spinal Cord Injury

1 | INTRODUCTION

Spinal cord injury (SCI) is a debilitating neurological trauma that commonly results in paralysis and secondary complications including spasticity, bladder dysfunction, respiratory complications, and muscular atrophy (Bamford et al., 2017). Upwards of 500,000 individuals worldwide experience an SCI each year, presenting a plethora of complications to the quality of life of those affected and placing a substantial financial burden on healthcare systems

(New & Marshall, 2013). To put this into perspective, in Canada, depending on whether the patient is living with paraplegia (paralysis of the legs) or tetraplegia (paralysis of the legs and arms), the lifetime cost of care of a 25-year-old can range from \$1.5 million to \$3.0 million (Krueger et al., 2013). This is an especially challenging circumstance as anyone can suddenly end up with this condition; the most common causes of spinal cord injury are automobile crashes and traumatic falls (Chen et al., 2013).

Much of the sensory information perceived by the body, whether it is touch, pain, heat, or pressure, goes through sensory neurons to the spinal cord and then to the brain. Similarly, all the motor neurons that control our limbs, digits, and core traverse through the spinal cord. In effect, an injury at any level of the spinal cord can sever the transmission of this information that is so critical to daily living. The most difficult aspect of SCI is that damaged neurons often do not regenerate; this is because neurons are terminally differentiated cells, which are incapable of growing new cellular populations (Frade & Ovejero-Benito, 2015).

At a cellular level, Jara and colleagues (2020) have demonstrated that the mammalian nervous system has an innate capacity to functionally reorganize its neuron in response to pathological injuries. The specific cellular mechanism behind this is still being studied, but it has been theorized that a lesion in the central nervous system transiently increases the level of secondary messenger cyclic adenosine monophosphate (cAMP). Increasing cAMP can further catalyze the upregulation of protein kinase A (PKA), cAMP response element-binding protein (CREB), and arginase 1 (ARG1), which are implicated to support the intrinsic regeneration of neurons (Cai et al., 2001; Jara et al., 2020). Importantly, these mechanisms only facilitate a limited recovery following neural injury. However, a recent breakthrough in neuroscience discovered that central nervous system circuitry can be strengthened with continuous activation of the neural connections (Jara et al., 2020). In effect, introducing electrical stimulation to activate the desired circuitry may strengthen neurological recovery, or in some cases, cause axonal growth. Further studies have also demonstrated that electrical stimulation not only activates the aforementioned growth-promoting molecular pathways but may also speed up the intrinsic recovery process following neural injury by modulating levels of transcription factors (Zareen et al., 2018).

Intraspinal microstimulation (ISMS) provides external electrical stimulation to the spinal cord by implanting hair-thin platinum-iridium wires directly below the injury site (Moritz, 2018). By doing this, special patterns of electrical stimulation can provoke a natural gait cycle (i.e., standing, stepping, walking) to help rats with paraplegia walk again. For a constant natural gait cycle to occur, this precise electrical stimulation must be present at all times to facilitate the contraction of precise motor pools. Further beneficial effects

of ISMS are well documented in research conducted on rats, cats, and pigs (Holinski et al., 2016; Kasten et al., 2013). ISMS is advantageous because it offers the greatest selectivity of neurons and precise control of targeted motor pools vital for functional walking (Giszter, 2015). Furthermore, ISMS allows for specific stimulation and strengthening of the central nervous system circuitry that has been injured. Other methods of electrical stimulation often broadly target the general area of motor neuron pools, which often results in less effective motor movement and circuitry activation. However, many unanswered questions surrounding the translation of ISMS from animal subjects to human patients remain. Specifically, the impact of temporary, non-continuous ISMS on mobility restoration remains unknown. Additionally, the extent of cellular damage caused by microelectrode implantation in the spinal cord and the impact this may have on lasting mobility following SCI remains unknown. These are important questions to investigate as human patients with SCI may decide to cease ISMS following a temporary period of active stimulation or utilize ISMS in an intermittent fashion.

In effect, this project investigated: 1) the capacity of ISMS to restore functional walking in rats with SCI through four weeks of stimulation, and 2) the degree of walking deficit caused by ISMS surgery. The first objective sheds light on what might happen to patients with ISMS who stop receiving stimulation after four weeks, and the second objective investigates how mobility restoration is impacted by the ISMS surgery alone (no stimulation).

We hypothesized that, compared to spinal cord injured rats without ISMS, SCI rats that receive active ISMS for four weeks would demonstrate improved functional walking scores as a result of enhanced neural reorganization due to electrical stimulation. Additionally, compared to SCI rats without ISMS surgery, we believed that SCI rats that receive ISMS surgery alone (no active stimulation) would have decreased levels of mobility, as some tissue damage may occur as a result of the implantation surgery.

2 | METHODS

Pre-Operational

Thirteen Sprague Dawley rats were randomly divided into three groups: 1) rats with hemi-section SCI (hSCI) and no implants (control group), 2) rats with hSCI and passive ISMS implants (ISMS sham group), and 3) rats with hSCI and active

ISMS implants (ISMS group). Originally, five rats were included in each group. However, one rat from the sham group and one rat from the ISMS group had to be euthanized due to toe-biting following the hSCI surgery. As a result, there were five rats in the control group, and four rats each in the sham and ISMS groups. The group assignment process was randomized by generating a number from 1-13. If a number was already assigned, it was rolled again. Rats #1-5 became group 1, rats #6-9 became group 2, and rats #10-13 became group 3. All rats were housed in a standardized living environment to ensure there was no confounding variable present. To ensure the age of the rat did not have an impact on their walking performance, the deliverer made sure they were all the same age.

The experimenter was blinded to the groups when taking recordings or measurements, and during data analysis. All groups were first trained to walk on a horizontal ladder with randomly placed rungs prior to their surgery, and gait cycle performance was quantified.

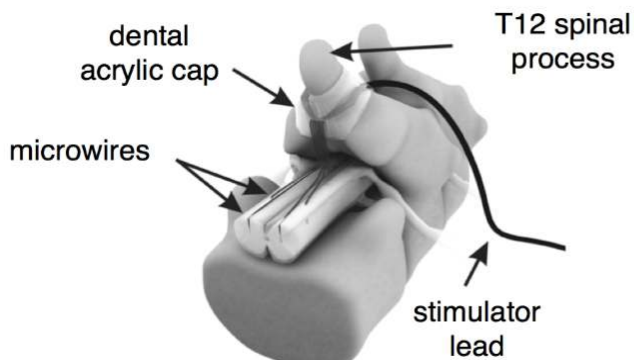


Figure 1. A model of the ISMS device. For this experiment, two microelectrodes were implanted at the T13 and L1 segments of the spinal cord, below the level of the hSCI lesion. The acrylic cap at the T12 spinal process secures the microwires in place to prevent manual disturbances to the microelectrodes. Microelectrodes are 30 μ m in diameter and connect to the electrical stimulator. Image is reproduced by authors from Bamford et al. (2010).

Surgery

Following hSCI surgery at the T8 segment of the spinal cord (performed by an experienced lab technician), and a two-week recovery period, the sham and ISMS group rats received an ISMS implant composed of an array of two 30 μ m wires

implanted in the ventral gray matter of the T13 and L1 segments of the spinal cord known to house the hind limb motor neuron pools of rats (Holinski et al., 2016). This implant was done unilaterally on the right side, below the level of the hSCI lesion. After the surgery, a threshold stimulation was introduced to make sure the wire was inserted correctly in the appropriate motor pools.

One-week post-implant, low-level, tonic sub-motor threshold stimulation through the ISMS implant was initiated in the rats in ISMS group rats. The stimulation parameter followed the standard technique described by Mushahwar and colleagues (Bamford et al., 2017). Stimulus amplitude was set at 25 μ A to prevent tissue damage. This amplitude was delivered in a 1 second train at a rate of 25 pulses per second through the microwires. Stimulation was delivered for 1 hour/day for four weeks, coupled with walking on the horizontal ladder. This was done to incorporate the common practice done in the previous ISMS procedures (Bamford & Mushahwar, 2011). This is often the optimal amount of stimulation for rats as it allows neurons to further strengthen their connections by continuously being activated whilst preventing muscle fatigue or atrophy.

Operationalization of Walking Performance

The walking performance of all rats on the horizontal ladder was video recorded twice per week and analyzed by a blinded experimenter. The horizontal ladder had two layouts: regular and irregular. The regular layout had a constant spacing of 2 cm between the rungs, and the irregular layout had varied spacing of 1-3 cm between the rungs. This variety of spacing demands the rats employ different types of weight-bearing movements to walk functionally across the ladder without slipping. Their movements were captured through VHS tape for further analysis. At first, all rats were trained on a regular layout to get accustomed to the ladder, and then switched to an irregular layout. All rats traversed the irregular ladder layout five times to ensure they understood how to perform on this layout. This same paradigm was applied following surgery. The functional walking scoring system was based on previous research by Whishaw and colleagues where scoring ranged from 0 to 6, with (0) representing a total miss of a ladder rung, (1) representing a deep slip of a ladder rung, (2) representing a slight slip of a ladder rung, (3) representing replacement, where a leg is placed then lifted quickly, (4) representing correction, where a leg is aiming for one rung but is placed on another rung instead, (5) representing partial



Figure 2. Rats engaging in the horizontal ladder task. The left photo demonstrates a rat with a score of (6) in which the rat has perfect weight-bearing in all 4 feet on the rungs. The right photo demonstrates a rat with a score of (0) where both hindlegs completely missed the ladder rungs.

placement and (6) representing correct placement where all limbs with weight are supported (Metz & Whishaw, 2009). All of the scoring data was recorded on Microsoft Excel where means and standard errors were calculated. This data was then imported into SPSS software to elucidate any statistically significant differences that existed between treatment groups and before and after the surgery. To ensure the maximum congruence among tests, we employed Tukey HSD, Bonferroni, and Sidak post-hoc tests.

Post-Operational

At the end of the experiment, all rats were euthanized, and the spinal cord was extracted and perfused for histochemistry purposes. Hematoxylin and eosin (H & E) stain was used to evaluate the extent of injury apparent in the extracted spinal cord. All of the following methods were approved by the University of Alberta research ethics committee.

3 | RESULTS

The pre-SCI functional walking score of control, sham and ISMS rats were 5.7 ± 0.2 , 5.5 ± 0.3 and 5.7 ± 0.1 , respectively.

After the hSCI was performed, the functional walking scores of the rats in all groups significantly decreased, as expected. Following hSCI and tonic stimulation of the spinal cord (in the ISMS group), the postoperative functional walking scores of control, sham and ISMS rats were 3.2 ± 0.9 , 2.6 ± 0.6 and 3.3 ± 0.8 , respectively (Figure 4).

4 | DISCUSSION

In this project, we attempted to investigate 1) ISMS's capacity to restore functional walking in rats with SCI following four weeks of active ISMS, and 2) the degree of walking deficit caused by ISMS surgery alone. Our results have shed light on the effects of hSCI, ISMS surgery, and active ISMS stimulation.

Firstly, statistical analysis (one-way ANOVA) demonstrated significantly reduced functional walking scores across all rat groups following the hSCI surgery relative to their pre-injury scores ($p < 0.05$). This highlights that the hSCI itself significantly disturbed all the rats' ability to walk.

Secondly, while the active ISMS group demonstrated slightly better functional walking following surgery and stimulation compared to the hSCI and sham controls groups, this outcome was not statistically significant (one-way ANOVA; $p > 0.05$). This was confirmed after doing three different sets of post-hoc tests. At this time, it is difficult to conclude that four weeks of active ISMS had a strong enough impact to facilitate an improvement in the gait cycle of the rats following SCI. This finding was contrary to our original hypothesis and may indicate that four weeks of ISMS stimulation is not enough to promote rehabilitative neuroregeneration at the cellular level. In previous studies, researchers have used twelve weeks of stimulation to parallel a common intervention time frame used in rehabilitative research (Kasten et al., 2013). One of the reasons why four weeks was chosen for this experiment is because in previous research it has been shown that neurons adapt to an external factor such as electrical stimulation over 30 days (Bamford & Mushahwar, 2011). This outcome may

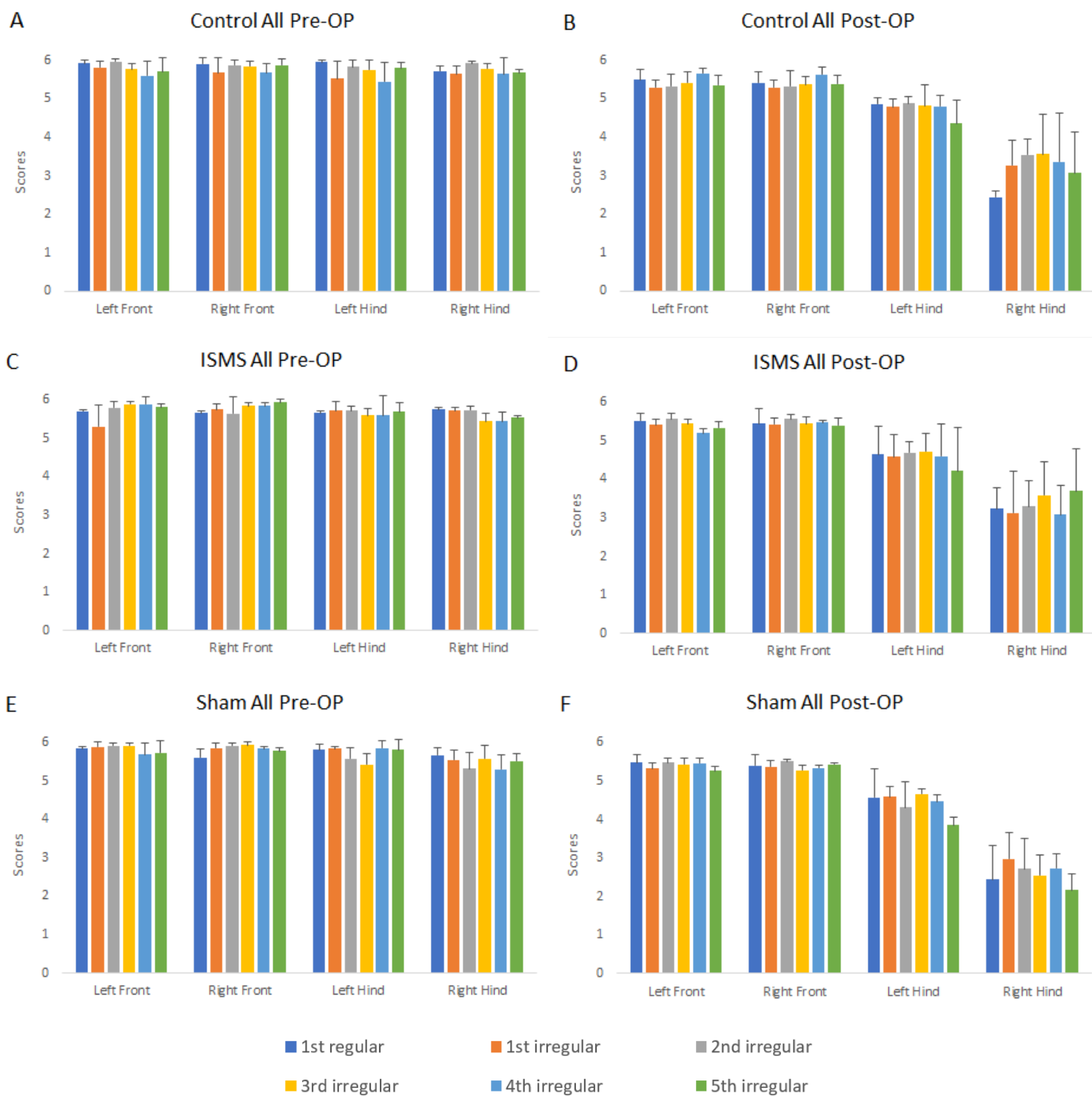


Figure 4. Pre- and post-operative functional walking scores for all groups. Ladder walking scores pre- and post-operatively of the rats in the control group (n=5), ISMS group (n=4), and sham group (n=4). 1st regular data represents a trial where rats walked on a 2 cm equally spaced ladder. Irregular trials represent walks done on a ladder with randomly placed rungs with varying separation between 1-3 cm. All scored trials were collected 6 days apart. All scores are reported as mean \pm standard error.

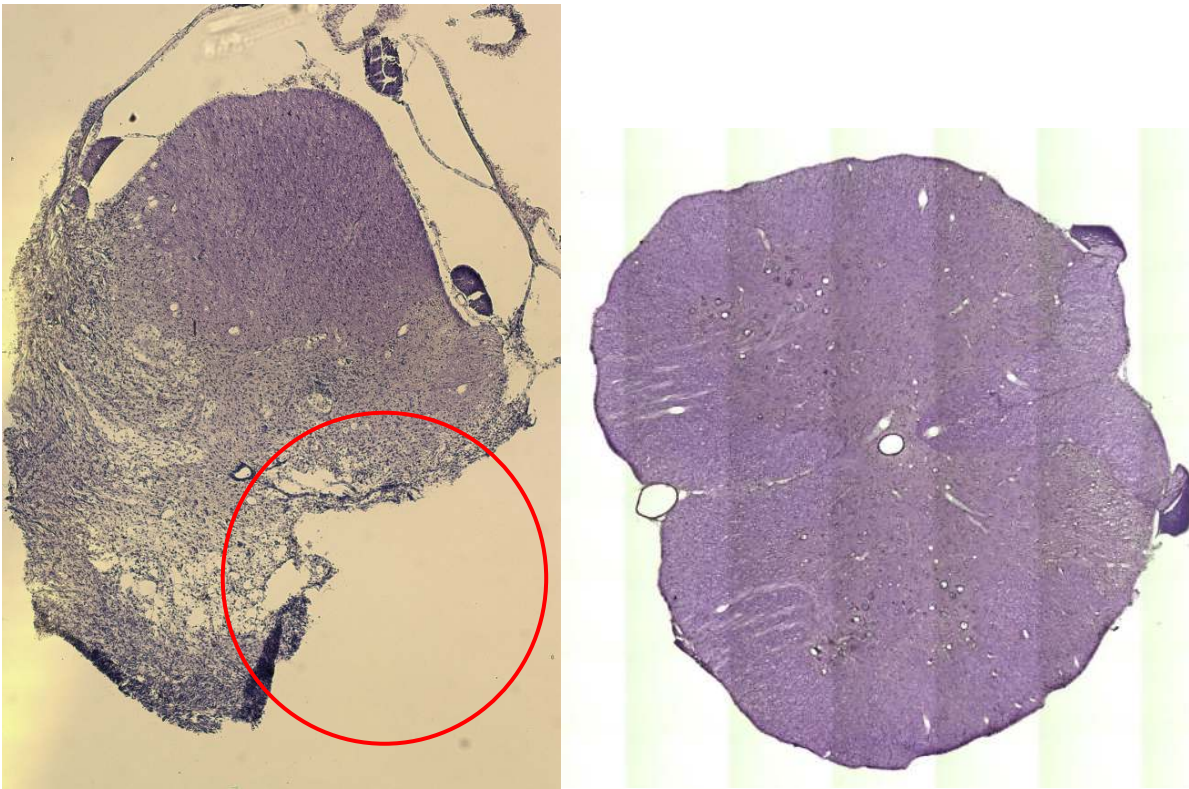


Figure 5. H & E staining was used to demonstrate the disruption of the spinal cord at the site of hemi-section injury (left) relative to an intact cord (right). The red circle shows that the hemi-section has removed a section of spinal cord, indicating that spinal cord injury was the cause of the mobility deficit.

also be a result of our sample size across all three groups. Nevertheless, the four-week stimulation time frame and small sample size are admittedly limitations of the present study and an area we hope to address with future research.

In addition to the aforementioned finding, statistical analyses comparing all groups post-injury and ISMS (one-way ANOVA followed by Tukey HSD post-hoc analysis) demonstrated that the functional walking scores of the rats in the sham group were significantly lower than those in the control and ISMS groups ($p < 0.05$). This finding confirmed our original hypothesis that spinal cord injured rats that receive ISMS surgery alone (no active stimulation) will have decreased levels of mobility as some tissue damage may occur as a result of implantation surgery. In previous studies, it has been shown that the thinness of wires is positively associated with fewer physical side effects following ISMS surgery (Kasten et al., 2013). Even though 30 μ m thick microwires are typical for larger animals (e.g., cats, pigs), we suggest that future research in rats employ thinner, 25 μ m wires to mitigate tissue injury.

In future ISMS studies, an area to explore with this experiment is 3D spinal cord imaging to measure injury volume. Injury volume can function as an objective way to quantify the degree of injury incurred in the spinal cord and would allow us to explore whether performance may be attributable to the size of the injury as opposed to the treatment. Similarly, injury volume could allow us to explore whether ISMS (with and without stimulation) modulate the size of the lesion. In addition to this, immunohistochemistry could also be utilized to identify levels of inflammation in response to the ISMS implant, as well as any neuroregeneration promoted by ISMS through the visualization of synaptic density in the spinal cord.

ETHICS

All the procedures have been approved by the ethics committee at the University of Alberta, AUP 302. All the scientists involved in the study have been trained to handle animals and procedures were proceeded under supervision.

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