Cervical intraspinal microstimulation improves forelimb motor recovery after spinal contusion injury

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Abstract

Incomplete injuries to the spinal cord are the most commonly observed in human patients. These injuries spare neural tissue bypassing the lesion that could be influenced by neural devices to promote recovery of function. Intraspinal Microstimulation (ISMS) is a promising method of activating the spinal cord distal to an injury site, either to directly produce movements or to improve subsequent volitional control of the paretic extremities. Here we tested whether long-duration ISMS can improve recovery of forelimb reaching movements following a cervical spinal cord contusion injury at level C4-C5 by examining whether motor improvements would last beyond the period of stimulation. When tested without the stimulation active, animals that received daily ISMS performed better than unstimulated animals with identical injuries and implants. Animals in the stimulated group improved rapidly within the first several weeks of daily stimulation, reaching a plateau in function after 4 weeks. Long-duration stimulation (7 hours/day) delivered to ISMS electrodes did not adversely affect the threshold needed to evoke a forelimb movement over the 12 weeks of the study. While stimulus threshold currents gradually increased over time (~8μA/week), there were no consistent differences in thresholds between chronically stimulated and unstimulated electrodes. These results indicate that neuroprosthetic stimulation may have benefits that extend beyond the period of stimulation, and suggest future work developing neural devices to promote regeneration of damaged neural tissue.

Keywords: *spinal cord injury, ISMS, regenerative stimulation, therapeutic stimulation*

Introduction

Largely due to improved safety equipment and acute care, incomplete injuries to the cervical spinal cord are now the most common spinal cord injury in human patients[1]. Cervical spinal segments C4-C5 are the most often affected, resulting in incomplete tetraplegia in the majority of cases [1, 2].

Intraspinal micro-stimulation (ISMS) of the cervical and lumbar spinal cord may assist in reanimating paralyzed limbs, and perhaps even promote long-term recovery of function. ISMS delivered to the lumbar spinal cord is capable of evoking functional muscle synergies of the hindlimbs in cats [3], rats [4, 5] and frogs [6]. Cervical ISMS evokes highly functional synergies such as grasp from single stimulating locations in spinally-intact monkeys [7]. Here we apply long-duration spinal stimulation with the goal of promoting recovery that lasts beyond the application of ISMS.

Tonic epidural stimulation applied to the dorsal surface of the spinal cord after injury appears to activate rhythmic pattern generators and facilitate volitional control of movements. Epidural stimulation centered on the L2 spinal segment, and often combined with the serotonin agonist quipazine, is capable of evoking hindlimb stepping movements in completely transected rodents when placed on a moving treadmill [8, 9]. A recent clinical case study revealed that tonic epidural stimulation facilitated volitional control of leg movements in a subject with largely motor complete (ASIA B) T1 subluxation injury [10].

While the preceding results are quite exciting, it should be noted that in all cases the stimulation must be active for the motor benefits to be observed. Therefore, the goal of the present study was to examine whether long-duration intraspinal stimulation applied to the motor pools below a cervical contusion injury could have positive effects on motor function even after the stimulation was discontinued. ISMS delivered to the lower cervical segments at locations evoking forelimb movements resulted in a rapid and sustained increase in forelimb reaching ability when tested with the stimulator inactive.

Materials and Methods

Experiments to quantify the effect of long-duration intraspinal stimulation on recovery of forelimb reaching were performed in twenty-four adult female Long-Evans rats. All procedures were approved by the University of Washington Institutional Animal Care and Use Committee (IACUC).

Forelimb reaching task

Rats were trained to perform a precision forelimb reaching task to greater than 70% success prior to injury [11, 12]. Animals reached across a 1 cm to gap to retrieve 45 mg chocolate-flavoured food pellets (BioServ) at a total distance of 2 cm from the inside of an acrylic arena.

To more precisely quantify graded recovery of function, the following scoring metric was used to capture attempted but unsuccessful pellet retrievals. Each animal was allowed 20 individual attempts to retrieve a food pellet. A successful reach was scored a full point, grasping the pellet but dropping before returning to the arena was scored 0.5 points, and touching but failing to grasp a pellet was scored 0.25 points. Total scores (divided a maximum of 20) were then calculated for each animal (Modified from [13]).

Cervical Injury and Microwire Implant

All animals received a moderate, lateralized contusion injury to spinal segments C4-C5 using a modified Ohio State injury device [14, 15]. Animals were deeply anesthetized via intraperitoneal injection of ketamine (80 mg/kg) and xylazine (12 mg/kg). After performing a C4/C5 dorsal hemi-laminectomy, the electromagnetic injury device compressed the spinal cord by 0.8 mm for 20 ms. Muscles were sutured in layers and the skin closed. Buprenorphine (0.05 mg/kg) was given twice daily for three days for analgesia.

Three weeks after injury, all animals were implanted with intraspinal stimulating electrodes in spinal segments C6-C7 ipsilateral to the injury. Stimulating electrode arrays were based on those developed by the Mushahwar group for rodents [4]. Briefly, an array of five 30 μm polyimide-coated platinum-iridium wires (California Fine Wire) were threaded through a 19 gauge epidural catheter (Arrow International) to a head-mounted connector (PlasticsOne). Stimulating wires were arranged to target different medio-lateral and rostro-caudal position within the spinal cord below the injury.

Animals were anesthetized by inhalation of 1-3% isoflurane in 100% oxygen. An incision was made from C4-T2 and muscle layers were separated and retracted and hemilaminectomies were performed at C6 and C7. The catheter containing the stimulating wires was secured to the T2 dorsal spinous process with non-resorbable silk sutures.

A longitudinal slit was made in the dura and the wires inserted into the spinal cord targeting the forelimb motor pools in the ventral horn of the grey matter (depth of 1.2-1.8 mm below dorsal surface of spinal cord). The dura was sewn over the top of the wire bundle using 7-0 silk suture and cyanoacrylate glue was used to seal the surface of the dura. A reference wire was sutured to the muscles above the spinal cord. The catheter was routed under the skin and the connector was fixed to the skull using stainless steel screws and dental acrylic. Muscle layers and skin were closed, and buprenorphine (0.05 mg/kg) was given twice daily for three days for analgesia.

Daily stimulation and reaching task.

Animals were paired based on movement deficit one week later (4 weeks after injury) and assigned to the stimulated or unstimulated condition ($n = 12$ in each group). Animals in the stimulated group then received long-duration ISMS for an average of 7 hours/day, 5 days/week for 12 weeks.

Daily stimulation was delivered during the animal's active (dark) cycle. Bi-phasic pulses (300 μs width each phase) were delivered at a rate of 4 ± 1.5 Hz (Gaussian distribution). The stimulator was active for 15 minutes followed by 5 minutes of no stimulation throughout the day. Stimulation was delivered via a single electrode (returning to the reference) at threshold currents to just evoke a visible forelimb movement. Spinal stimulation thresholds were measured weekly on all implanted electrodes, and the electrode with the lowest threshold to evoke forelimb movements was selected for stimulation during the following week.

All animals were tested daily at the forelimb reaching task by a blinded scorer. Stimulation was not applied during the reaching task. Reaching scores were averaged across days for a weekly total for each rat, and then normalized to a percentage of each animal's pre-injury skill level. Reaching data were normally distributed, so T-tests were used to compare reaching scores between groups at weekly time points.

Results

Long-Duration ISMS leads to rapid recovery of reaching after cervical contusion injury.

Animals receiving long-duration intraspinal microstimulation rapidly improved forelimb reaching function when tested with the stimulator inactive. Improvements began within one to two weeks of stimulation. In a particularly interesting example, an animal in the stimulated group recovered to 75% of pre-injury reaching ability within just 4 weeks (Fig. 1). Notably, stimulation in this animal was discontinued after week 5 due to implant failure, providing the opportunity to observe lasting recovery with no further stimulation. Although reaching scores reduced immediately upon halting daily stimulation, success rates stabilized well above pre-stimulation levels and those of untreated animals (Fig. 1). This animal and her unstimulated pair were not included in group statistics below.

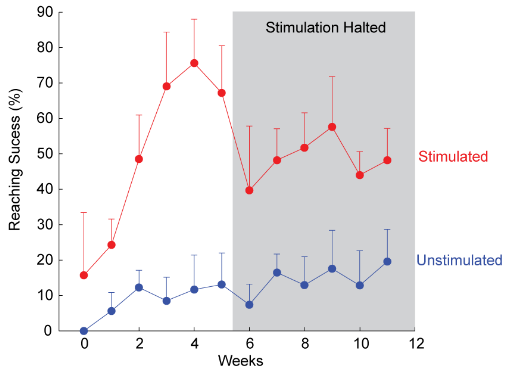

Fig 1: Example of rapid recovery of forelimb reaching ability for one animal receiving long-duration ISMS, compared to an unstimulated animal. After 5 weeks, stimulation was halted due to implant failure, allowing examination of sustained recovery with no further stimulation. Mean + SD for each animal.

The remaining animals continued to receive stimulation for a total of 12 weeks (n = 11 animals in each group). Figure 2 shows that the stimulated group performed forelimb reaching significantly better than the unstimulated group for the first three weeks of stimulation, reaching an asymptote around the 4th week of stimulation that was sustained through the 12th week. Although the unstimulated group spontaneously recovered somewhat during weeks 4-8, this improvement was not sustained, resulting in significantly better reaching for the stimulated groups on weeks 10 and 12. Notably, one animal in the stimulated group demonstrated perfect reaching (score of 100%) during week 11, increasing the variance of the data on that week and likely contributing to the lack of significance during that week.

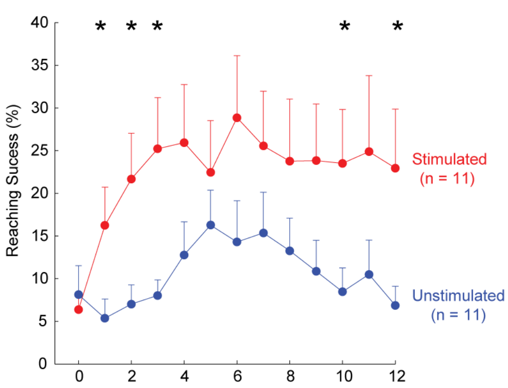

Fig 2: Weekly forelimb reaching success for stimulated animals that received long-duration ISMS and unstimulated animal in the control group. (Mean ± SE; n = 11 each group; * = $p < 0.05$ between groups).

Long-Duration ISMS did not adversely affect the threshold to evoke forelimb movement.

The lowest spinal stimulus current to evoke a forelimb movement was measured each week for animals in the stimulated group. These data permit comparison of electrodes that delivered long-duration stimulation (n = 15) and those that did not (n = 12) across the 11 animals in this group.

Although the threshold current needed to evoke a movement gradually increased over the 12 week study, there was no difference between wires that delivered long-duration stimulation and wires that were not stimulated (Fig. 3). Single pulse stimulus thresholds increased by an average of 7.9 ± 4.6 μA/week for the stimulated wires, compared to 7.2 ± 8.3 μA for the unstimulated wires (mean ± SD; T-test $p = 0.78$). Further, there was no significant correlation between the number of weeks a given wire was stimulated (mean 7.8, range: 1-12 weeks) and its change in threshold over the study (linear regression $p = 0.18$).

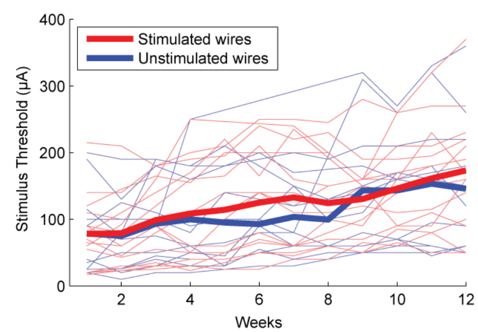

Fig 3: Weekly single-pulse stimulus threshold to evoke visible forelimb movement for individual wires (thin lines) and group averages (thick lines). Figure compares stimulated wires (blue) that delivered long-duration ISMS with unstimulated wires (red) that were not stimulated except during threshold testing.

Discussion

The results demonstrate that long-duration intra-spinal stimulation below the level of an injury improves motor function even beyond the period of stimulation. This effect occurs within the first several weeks of stimulation, reaching a plateau after approximately four weeks if daily stimulation is continued. When stimulation was discontinued after five weeks in one animal, forelimb function regressed somewhat, but then stabilized, suggesting that gains may persist many weeks beyond the completion of stimulation.

It is possible that improved function observed in the group of animals exposed to long-duration electrical stimulation acts via similar mechanisms as stimulation of the brainstem or cortex. Neuron sprouting and locomotor recovery was observed after electrical stimulation was applied to the motor cortex or pyramidal tract following selective lesions to the cortico-spinal tract [16, 17]. Spinal stimulation may also act to re-regulating neural circuits deprived of natural descending drive after spinal cord injury.

Conclusions

Intraspinal microstimulation delivered below a spinal cord injury improves forelimb motor function even after the stimulation is discontinued. This provides exciting evidence for the therapeutic or perhaps even regenerative capacity of neuroprosthetic devices. Studies are underway to determine the long-term benefits of neural stimulation, and the potential for stimulation to positively affect stem cell transplants and combine with other pharmacology to improve recovery from spinal cord injury. Intraspinal stimulation could improve hand and arm function for individuals with high level spinal cord injuries.

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