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Functional electrical stimulation helps replenish progenitor cells in the injured spinal cord of adult rats☆

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ABSTRACT

Functional electrical stimulation (FES) can restore control and offset atrophy to muscles after neurological injury. However, FES has not been considered as a method for enhancing CNS regeneration. This paper demonstrates that FES dramatically enhanced progenitor cell birth in the spinal cord of rats with a chronic spinal cord injury (SCI). A complete SCI at thoracic level 8/9 was performed on 12 rats. Three weeks later, a FES device to stimulate hindlimb movement was implanted into these rats. Twelve identically-injured rats received inactive FES implants. An additional control group of uninjured rats were also examined. Ten days after FES implantation, dividing cells were marked with bromodeoxyuridine (BrdU). The "cell birth" subgroup (half the animals in each group) was sacrificed immediately after completion of BrdU administration, and the "cell survival" subgroup was sacrificed 7 days later. In the injured "cell birth" subgroup, FES induced an 82–86% increase in cell birth in the lumbar spinal cord. In the injured "cell survival" subgroup, the increased lumbar newborn cell counts persisted. FES doubled the proportion of the newly-born cells which expressed nestin and other markers suggestive of tripotential progenitors. In uninjured rats, FES had no effect on cell birth/survival. This report suggests that controlled electrical activation of the CNS may enhance spontaneous regeneration after neurological injuries.

Introduction

Electrical stimulation is being used as a therapeutic tool to promote recovery of function following nervous system injury and disease. Examples of clinical application of electrical stimulation include cochlear implants for restoring hearing, stimulation of lower motor neurons to restore breathing and hand grasp, and deep brain stimulation to treat the symptoms of Parkinson's disease (McDonald and Sadowsky, 2002b). Electrical stimulation has also been used to offset the disuse atrophy associated with paralysis (de Abreu et al., 2009).

Functional electrical stimulation (FES) assisted ergometry is currently being applied in the clinical setting for persons with spinal cord injury (SCI). There are numerous publications demonstrating practical benefits from FES in persons with SCI including: increased muscle mass (Hjeltnes et al., 1997; Scremin et al., 1999), improvements in bone density (Frotzler et al., 2009), enhanced cardiovascular function (Faghri et al., 1992), improved bowel function (Johnston et al., 2005), decreased spasticity (Daly et al., 1996) and reductions in bladder infection rate (Kutzenberger et al., 2005). In addition, electrical stimulation could someday be used in combination with other therapies to enhance functional recovery from SCI. One example is the use of electrical stimulation to facilitate partial-body-weight-supported walking in experimental animals following a spinal cord injury (Lavrov et al., 2008).

A growing body of evidence suggests that electrical stimulation can promote peripheral and central nervous system repair following injury. In a model of rat femoral nerve transection and repair, electrical stimulation promoted BDNF release from motor neurons and enhanced preferential motor reinnervation across the distal nerve stump. This stimulation paradigm also promoted functional recovery following femoral nerve repair (Ahlborn et al., 2007). In a similar model, electrical stimulation restored the specificity of sensory axon regeneration into the cutaneous branch of the femoral nerve. In addition, electrical stimulation promoted the expression of growth associated protein-43 and enhanced the number of regenerating sensory axons in the femoral nerve across the distal stump (Geremia et al., 2007). In a model of dorsal column transection (level T8), electrical stimulation promoted regeneration of CNS axons from dorsal root ganglia in a mechanism that likely involves cAMP signaling (Udina et al., 2008). Finally, electrical stimulation applied to the cortical pyramids in rats enhanced synapse formation in the spinal

Abbreviations: SCI, spinal cord injury; FES, Functional electrical stimulation.

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cord during development and following cortical–spinal tract injury (Brus-Ramer et al., 2007; Takuma et al., 2002).

This paper assesses whether FES-induced patterned activity in the chronically injured spinal cord can enhance cellular indices of CNS regeneration. Neural activity plays a critical role in nervous system development and plasticity as shown by activity-dependent gene expression (Sgambato et al., 1997), modification of synaptic strength (Daoudal and Debanne, 2003), synapse elimination (Zhou and Poo, 2004), cell survival (Jagasia et al., 2009), myelination (Coman et al., 2005), and perhaps new cell birth (McDonald et al., 2002a; Tongiorgi 2008).

Because of the dramatic reduction in neural activity below the injury level (Edgerton et al., 2001), our hypothesis is that increasing neural activity with FES may aid regeneration (Grill et al., 2001; McDonald et al., 2002a). To test this hypothesis, we provided patterned neural activity to the caudal spinal cord of chronically-injured rats. Our injury model was a complete two segment resection of the spinal cord at T8/9, which eliminated both ascending and descending activity across the lesion. Three weeks after injury, we implanted a 2-channel FES system with leads attached to the peroneal nerves. This induced stepping-like movements of the hindlimbs and patterned neural activation in the lumbar spinal cord in the area commonly associated with the central pattern generator for locomotion (Lavrov et al., 2008). We then monitored the birth and survival of neural progenitor cells, which are present in the adult spinal cord, and can generate neural cells (Horky et al., 2006; Horner et al., 2000; Ishii et al., 2001; McTigue et al., 1998; McTigue et al., 2001; Tripathi and McTigue, 2007).

Materials and methods

Subjects, animal care and surgery

Thirty six adult female Long Evans rats (275 ± 25 g; Simonsen, Gilroy, CA) were separated into six groups. These animals were housed (12:12 h light:dark cycle) and treated in accordance with the Laboratory Animal Welfare Act, and with Guidelines / Policies for Rodent Survival Surgery (Animal Studies Committee of Washington University in St. Louis). All animals received FES implants. "Short term" refers to analysis 15 days after FES device implantation, 2 h after the last BrdU injection; "long term" refers to analysis 22 days after FES device implantation, 7 days after the last BrdU injection. The six groups were:

Cell birth:
(1) SCI, NO FES activation, short-term survival ($n = 6$);
(2) SCI, FES activation, short-term survival ($n = 6$).
Cell survival:
(3) SCI, NO FES activation, long-term survival ($n = 6$);
(4) SCI, FES activation, long-term survival ($n = 6$).
Uninjured controls:
(5) NO SCI, NO FES activation, short-term survival ($n = 6$);
(6) NO SCI, FES activation, short-term survival ($n = 6$).

All SCI animals were fitted with 10.5 cm plastic collars to prevent autophagia (Ejay, Glendora, CA) and had their bladders expressed 3 times a day or until recovery of reflex urination. All rats were handled for 5 min per day and housed individually with absorbent bedding (ALPHA-Dri™, Shepherd, Kalamazoo, MI).

Spinal cord injury

Rats subjected to SCI (groups 1–4) were anesthetized (75 mg/kg Ketaset®, 0.5 mg/kg Domitor®, i.p.) and laminectomy was performed at T8-T10. A 1-mm section of the spinal cord at T9 was removed through a dural slit, using a BARON® suction tube (Roboz, Rockville, Maryland). The dural opening was covered with fascia, and the muscle and overlying skin were closed with layered sutures. Anesthesia was reversed with Antisedan® 1 mg/kg. This "suction ablation" injury model helps maintain the integrity of the dura and major blood vessels, and therefore produces less bleeding and secondary ischemia than traditional blade transection injury.

Stimulator and electrode implantation

All animals were anesthetized as described above. Twenty-one days after SCI in groups 1–4, a 2-channel battery-powered electrical stimulator (Dr. J.C. Jarvis, University of Liverpool) (Salmons and Jarvis, 1991) was implanted into each animal (Fig. 1A). In groups 5–6, an FES device was implanted into each of the uninjured rats. To insert the FES device, an incision was made in the skin of the lower back at the midline from L1 to L5. A subcutaneous pocket was created around the incision and the FES device was inserted into it. A 0.5 -cm incision was made on the lateral aspect of both legs overlying the common peroneal nerve. The stainless steel wire stimulating electrodes were tunneled bilaterally underneath the skin from the stimulator site to the incision in the leg. The wire electrodes were sutured into the tibialis anterior muscle, adjacent to the common peroneal nerve. The return electrode was sutured near the midline at the L4 paraspinal muscles. Intraoperative test stimulation was performed to ensure that the peroneal nerve was being activated during stimulation, as indicated by alternating flexion of the right and left hindlimbs.

Electrical stimulation paradigm

Three days after FES implantation the devices were activated in groups 2, 4, and 6. The FES unit was activated three times daily, for 1 h at a time, between the hours of 8:00 AM and 5:00 PM (Fig. 1B). The FES pattern consisted of 1 s stimulation of one common peroneal nerve followed by 1 s of rest; then the other common peroneal nerve was stimulated for 1 s followed by 1 s of rest and the cycle was repeated. Stimulus pulses were monophasic, 3 V, 200 μs long, and were delivered at 20 Hz. This configuration of stimulation preferentially activates large myelinated fibers within the common peroneal nerve (Gorman and Mortimer, 1983; Grill and Mortimer, 1996), and produced alternating flexion of the hindlimbs that crudely approximated bilateral stepping.

Bromodeoxyuridine injection paradigm

Beginning 10 days after FES implantation, rats received daily injections of BrdU (50 mg/kg i.p.) for 5 consecutive days. BrdU labels new cells by incorporating into replicating DNA (Dolbeare 1996; Horner et al., 2000). To determine the amount of cell birth, groups 1, 2, 5, and 6 were sacrificed at the completion of BrdU injections. To determine the ability of newborn cells to survive *in vivo*, groups 3 and 4 were sacrificed 7 days after the completion of BrdU injections.

Tissue processing and immunohistochemistry

Rats were perfused intracardially with 0.1 M PBS for 5 min followed by 4% paraformaldehyde (Sigma) for 15 min. We selected every 6th section (40 μm frozen) from spinal cord levels C2, T1, T7, T11, L1, and L5 for anti-BrdU immunohistochemistry (Horner et al., 2000). The sections were incubated in 2 N HCl for 60 min at 37 °C, transferred to 0.1 M borate buffer (pH 8.5) for 20 min, and rinsed with PBS. Non-specific labeling was blocked with 0.1% BSA in 0.1% Triton X-100/PBS for 60 min. A mouse monoclonal anti-BrdU antibody (1:600; Roche, Mannheim, Germany) was incubated with the tissue overnight at 4 °C. Then the tissue was treated with a CY3-conjugated secondary antibody (1:2000; Jackson, West Grove, PA) in 2% normal goat serum (NGS) for 60 min.

To co-label the BrdU+ cells, we fixed the sections with 4% paraformaldhyde for 30 min after applying the CY3-conjugated