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Chronic Electrical Nerve Stimulation as a Therapeutic Intervention for Peripheral Nerve Repair

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When a peripheral nerve is injured after either trauma or a neurodegenerative disease, motor function and sensory perception are impaired. Repair strategies aim both at reconstructing the damaged nerve and in promoting regeneration to enhance target reinnervation and functional recovery. Advanced surgical procedures can enable efficient repair, but restoration of function remains challenging. Among various factors influencing nerve regeneration, electrical stimulation is often cited as a potential therapeutic approach to nerve repair, engaging regenerative transcriptional programs. In this report, we review both reported effects on axonal growth and functional outcomes of electrical stimulation on peripheral nerve repair and the techniques for chronic nerve stimulation, highlighting the challenges and opportunities of such repair strategies.

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INTRODUCTION

Peripheral nerve damage is an important clinical problem, with an occurrence of up to 3% of all trauma patients (1–3). Peripheral nerve injuries often result in loss in motor function, sensory function or both and are associated with a high socioeconomic impact (4–6). After nerve injury, the damaged axons of sensory and motor neurons can regenerate and, under optimal circumstances, can reinnervate peripheral target organs such as the skin and muscles (7). However, functional outcome after repair (despite modern microsurgery techniques) is often poor and incomplete. Extensive soft tissue injury, a large gap between nerves stumps, long distances to reach receptive targets and misdirected axons contribute to the challenging repair environment (8–11). There is an urgent need to find new treatments for promoting regeneration after peripheral nerve injury, especially since chronic denervation results in muscle atrophy and progressive decline in Schwann cell support, making the distal nerve progressively less growth-permissible (12).

After trauma or neurodegenerative diseases, electrical stimulation of neural tissue can be used to either alleviate symptoms or restore function (13). For example, deep brain stimulation relieves the effects of Parkinson's disease; cochlear implants convert sound waves into stream of electrical impulses that are transmitted to the auditory nerve to partially restore hearing; direct electrical stimulation enhances wound healing; and transcutaneous electrical nerve stimulation is a nonpharmacological therapy for pain relief.

Electrical stimulation has been also envisioned as a potential complementary therapy to microsurgical repair after peripheral nerve injury to improve functional outcomes by promoting a greater and faster axonal regeneration. A number of preclinical studies across a number of nerve injury models have shown that direct stimulation of injured peripheral nerves can enhance peripheral sensory (14,15) and motor (16–18) axon regeneration, increase target reinnervation (17,19) and improve electrophysiological outcomes (20,21) and functional recovery (14,15). To date, however, electrical stimulation is rarely used in a clinical context of nerve repair. One study has reported accelerated reinnervation of the thenar muscle following nerve electrical stimulation after carpal tunnel release surgery (22).

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Reviewing the effect of electrical stimulation on nerve repair is challenging because results from preclinical studies are often inconsistent, and their impacts on peripheral nerve repair vary substantially. This can be attributed to the vast number of experimental protocols, for which parameters include: type of injury (crush versus transection), nature of microsurgical repair, type of stimulation used (direct current or pulsed stimulation), location of the stimulation (transcutaneous, proximal or distal nerve stump), onset of stimulation (immediately after injury or delayed), its duration (acute versus chronic), stimulation waveforms (frequency, amplitude, pulse duration, biphasic) and ways to assert physiological and functional outcomes. Given this large methodological variance and consequent inconclusive data, the justification for such interventions at the clinic remains uncertain. Furthermore, it is still not clear whether any failure to detect an improvement is due to a lack of biological impact of electrical stimulation or a technical failure, or both.

Here we review the effects of electrical stimulation on peripheral nerve repair, distinguishing regenerative effects and functional outcomes. On the basis of preliminary work that we have performed to enable chronic stimulation of the sciatic nerve after a crush injury, we discuss the challenges and opportunities for developing tools and methods for chronic stimulation of injured peripheral nerves.

PERIPHERAL NERVE ELECTRICAL STIMULATION

Electrodes

Electrical stimulation may be applied with surface skin electrodes or by means of invasive electrodes placed in the direct vicinity of an injured nerve. Few studies report the use of transcutaneous electrical stimulation for nerve repair, with inconsistent outcomes for both regeneration and function (23,24). Surface electrodes are often >1 cm^2 cloth-like pads coated with a coupling gel above the site of nerve injury and have relatively low impedance, in the kΩ range.

Invasive nerve electrodes are either designed as wires/needles (mainly used for acute stimulation) or cuff electrodes. A metallic wire (cathode) is typically wrapped around the proximal nerve, and a second wire electrode (anode) is inserted within a distal muscle or wrapped along the nerve distally. Such an electrode configuration is commonly implemented for acute stimulation following a nerve crush injury, nerve transection with end-to-end suturing of the proximal and distal nerve stumps (16–19,25–27) or nerve repair facilitated by a biocompatible conduit sutured between both nerve stumps (21,28). Their impedance is typically in the 10-s MΩ range because of their small effective surface area.

Cuff electrodes in contrast are nerve diameter-sized tubes hosting metallic contacts with a monopolar, bipolar or tripolar configuration. This design provides selective activation of the nerve fibers. The impedance of the contacts is in the kΩ range. Chronic (long-term, >2 wks) stimulation of the injured nerve is possible, provided the cuff is secured to the tissue and induces negligible constriction of the nerve during movement. Subcutaneous wiring to a back- or headplug enables minimally disruptive experiments. Ultimately long-term electrodes could interface a wireless, implanted microstimulator.

Parameters of Electrical Stimulation

In most cases, a pulsed stimulation is implemented. Four criteria defining electrical stimulation protocols need to be preselected: (a) pulse waveform (frequency, width, amplitude and shape), (b) duration of stimulation, (c) onset time of nerve stimulation after injury and (d) overall duration of the stimulation treatment. The most frequently used parameters are listed below:

- Threshold stimulation current: 0.5–5 mA range, but depends on electrode type, configuration and distance from the injured nerve;
- Cathodic pulse width: 50–400 μs;
- Pulse frequency: 1–20 Hz;
- Session duration: 15 min to 1 h;
- Acute stimulation: immediately after nerve injury or after surgical repair of the injured nerve;
- Long-term stimulation: 5 d/wk or every other day for up to 8 wks.

Different types of stimulation (direct current [DC] and alterative current [AC]) and various stimulation parameters used and their impact on peripheral nerve regeneration are summarized in Table 1.

An important issue lies in the identification of those fibers that need to be activated by electrical stimulation. The nerve contains motor and sensory fibers, which have different stimulation thresholds: motor axons are activated with lower stimulation amplitude than unmyelinated sensory axons. Recruitment of small myelinated or unmyelinated fibers, which signal pain, itch and thermal sensations, will produce nocifensive responses and will generally not be tolerated. As motor axons begin to reform functional neuromuscular junctions, movement will be elicited, and this may not be tolerated in a freely moving animal. The duration of the stimulation session may be important for regeneration. For example, both 1-h-long and 2-wk-long electrical stimulation have been reported to lead to a similar increase in the number of regenerated motor neurons (17), whereas a 3-wk stimulation was found to reduce the number of back-labeled sensory neurons compared with a 1-h, acute electrical stimulation session (26).

ELECTRICAL STIMULATION MODULATES THE MOLECULAR AND CELLULAR ACTIVITY INVOLVED IN NERVE REGENERATION

Peripheral nerve injury results in plastic changes in injured neurons. The sudden exposure to an extracellular medium triggers an immediate influx of sodium and calcium ions into the injured axons, causing a high-frequency burst of action potentials in the severed axons. A phenotypic switch of the injured neurons from a maintenance to a regenerative state then ensues, characterized by up- and downregulation of hundreds of genes. These include increased expression of

Table 1. Reported effects on nerve injury recovery after electrical stimulation.

Authors	Journal (year)	Type of current used, site of stimulation and parameters	Type of injury	Outcomes
Hoffman H	*Aust. J. Exp. Biol. Med. Sci.* (1952)	AC, 6.3 V, 50–100 cycles, 1.5 mA, 0–5,000 Ω variable resistance	Spinal cord injury	Enhanced reinnervation and sprouting
Maehlen J, Njå A	*J. Physiol.* (1982)	Preganglionic stimulation for 1 h immediately after the partial denervation with 100 pulses at 20 Hz every 25 s	Thoraco-cervical sympathetic trunk transection	Increased rate of sprouting
Nix WA, Hopf HC	*Brain Res.* (1983)	AC, 0.2 ms duration, frequency of 4 pulses per second (pps), applied (24 h daily) for 4 wks, and stimulation started 1 d postoperatively	Sciatic nerve transection	Improved electrophysiological recovery
Pockett S, Gavin R	*Neurosci. Lett.* (1985)	AC, proximal stump, 0.1-ms pulses, supramaximal voltage	Sciatic nerve crush	Improvement in toe spread function
McDevitt, *et al.*	*Brain Res.* (1987)	DC, 10 μA/cm^2, Distal cathode, hind paw, field strength ~100 mV/cm, 100 kΩ resistance, daily for 20 d	Sciatic nerve transection and repair	Enhanced motor axon regeneration
Román GC, *et al.*	*Exp. Neurol.* (1987)	DC, distal cathode implantation with a 10-μA for 3 wks	Sciatic nerve transection and repair	Increased number of myelinated axons
Zanakis MF, *et al.*	*Acupunct. Electrother. Res.* (1990)	DC, 1.4 μA (about 8 mV/cm field strength) to a nerve cuff	Sciatic nerve crush	Enhanced number of regenerating axons in the distal stump
Kerns JM, *et al.*	*Exp. Neurol.* (1992)	DC, 10 μA/cm^2, Distal cathode	Sciatic nerve crush	No change in sciatic function index (SFI)
Pomeranz B, *et al.*	*Brain Res.* (1993)	DC of 10 μA, 200–270 kΩ resistor, stimulation for a month	Sciatic nerve crush	Improved electrophysiological outcomes
Al-Majed AA, *et al.*	*J. Neurosci.* (2000)	AC, proximal stump, 0.1 ms, 3 V, 20 Hz, 1 h immediately after repair and up to 2 wks	Femoral nerve transection and repair	Accelerated motor axons regeneration across repair site after 1 h of stimulation but no further benefits with chronic stimulation
Mendonça CA, *et al.*	*J. Neurosci. Methods* (2003)	DC, proximal stump, low-intensity continuous current circuit (1 μA), 1.5 V battery and a 1.3-MΩ resistor for 3 wks	Sciatic nerve crush	Improved SFI
Ahlborn P, *et al.*	*Exp. Neurol.* (2007)	AC, proximal stump, square 0.1-ms pulses, 20 Hz, 3–4 V, acute	Femoral nerve transection	Improved motor axon regeneration and behavioral recovery
Geremia NM, *et al.*	*Exp. Neurol.* (2007)	AC, proximal stump, 0.1 ms, 3 V, 20 Hz, 1 h immediately after repair and up to 3 wks	Femoral nerve transection and repair	Improved sensory axon regeneration with acute stimulation but reduced benefits with chronic stimulation
Singh B, *et al.*	*J. Neurosurg.* (2012)	AC, proximal stump, 0.1 ms, 3 V, 20 Hz, 1 h immediately after repair	Sciatic nerve transection and repair	Improved axon regeneration and target reinnervation
Huang J, *et al.*	*Eur. J. Neurosci.* (2013)	AC (3 V, 20 Hz, 20 min) applied proximally to the transected nerve while repairing (2, 4, 12 and 24 wks)	Sciatic nerve transection and delayed repair	Increased motoneurons and sensory neurons regeneration, improvement in CMAP and NCV up to 24 wks of delay
Zhang X, *et al.*	*Mol. Med. Rep.* (2013)	AC (3 V, 20 Hz, 1 h) applied proximally to the nerve	Sciatic nerve crush	Improved remyelination, axon diameter and electrophysiological measures
Calvey C, *et al.*	*J. Hand Surg. Am.* (2014)	A direct current of 24 V/m (24 mV, DC 1.5 mA), applied across the electrodes for 10 min and 60 min	Sciatic nerve transection and repair	Enhanced behavioral and histological recovery
Xu C, *et al.*	*PLoS One* (2014)	AC, proximal stump, 0.1 ms, 3 V, 20 Hz, delayed nerve repair after 1 d, 1 wk, 1 month and 2 months	Sciatic nerve transection and repair at different time points	Improved electrophysiology parameters but the impact reduced with the delay
Thompson NJ, *et al.*	*Dev. Neurobiol.* (2014)	AC, short (0.1 ms) pulses at 20 Hz, 1 h immediately before nerve transection	Sciatic nerve transection and repair	Enhanced axon regeneration

brain-derived neurotrophic factor (*BDNF*) and its receptor *tropomyosin kinase receptor B* (*trkB*), *growth associated protein 43* (*GAP-43*) and the cytoskeletal proteins *Tα-1 tubulin* and *actin*, and a downregulation of enzymes such as choline acetyltransferase (ChAT) and acetylcholinesterase (AchE). To facilitate retrograde protein transport along the axons, neurofilament genes (*NF-L/M/H* mRNA) are concomitantly downregulated (7).

Acute electrical stimulation (1 h, 20 Hz) applied immediately after injury proximal to the injured nerve appears to lead to a more robust and earlier phenotypic switch (16,17) with, for example, enhanced mRNA and protein expression of *BDNF* and *GAP-43* in adult sensory neurons (21,26,29,30). In a recent study, androgen receptor signaling was elucidated as a possible cascade activated by electrical stimulation (31). Electrical stimulation also was found to induce an upregulation of *NT-3/4* in sensory neurons, which may promote axonal regeneration (32).

The specific mechanisms by which electrical stimulation may induce improved nerve regeneration are not yet clear. However, the available data suggest that the enhanced production of growth factors, especially BDNF, may have a significant role. Blockade of the electrostimulation-induced regenerative response in both motor and sensory neurons by tetrodotoxin (TTX) application at the site of injury implies involvement of neuronal cell body mechanisms triggered by electrical activity (16,26). However, a contribution of non-neuronal cell types (Schwann cells at the site of injury and in the distal stump and periganglionic satellite cells in the dorsal root ganglia) cannot be excluded. Enhanced glial fibrillary acidic protein (GFAP) expression in Schwann cells was, for example, observed after local electrical stimulation of injured nerves (33). Furthermore, Schwann cells respond to electrical stimulation by producing increased nerve growth factor (NGF) due to rises in calcium levels (34), and this could increase growth of TrkA-expressing nociceptors. Overall, although there are several phenomenological studies indicating some benefit of acute electrical stimulation on peripheral nerve regeneration, the specific mechanisms responsible still remain elusive.

BENEFITS OF PROLONGED ELECTRICAL STIMULATION

Few studies have examined the effects of chronic electrical stimulation of injured nerves over time (35–37). Changes in histological metrics (myelin thickness, axon density, blood vessel area and density, axon and fiber density, g-ratio, reinnervation specificity, mRNA and proteins expression), electrophysiological responses (compound muscle action potential, nerve conduction velocity, M/H wave ratio, latency and amplitude of electrical response) and functional outcomes (functional stance recovery, sciatic function index, thermal and mechanical algesimetry, toe-pinch test) have been reported for stimulation up to 24 wks after injury. However, results are inconsistent. Single time-point analyses have revealed either significant improvements at 2, 3 and 6 wks after repair (19,38,39) or no improvement at 4 wks (40). Over longer periods, between 6 and 12 wks after injury, no significant differences were observed between stimulated versus nonstimulated nerves. This result may reflect an action of electrical stimulation only in an earlier activation of intrinsic growth programs, which is not sustained so that the normal pattern of growth eventually "catches up" (17,39,41). Chronic stimulation delivered for 8 h a day was actually found to be counterproductive over longer periods, exacerbating nerve atrophy in a rabbit model (42). Daily stimulation of 1 h, 5 d a week, failed to promote motor reinnervation in comparison to single acute stimulation but reduced the H/M wave ratio, indicating decreased hyperreflexia 60 d after injury (27,43). Hyperreflexia is a reflection of neuronal reorganization and plasticity in the spinal cord after peripheral nerve injury (7). Whereas 1 h of stimulation applied every other day on eight occasions was found to produce significant recovery of the sciatic function index at 12 wks as well as a higher reinnervation of both sensory and motoneurons, reducing the electrical stimulation to 15 min every other day starting at 1 wk, post-injury repair did not produce any improvement of motor responses at 6 wks. However, a significant increase in blood vessel density in the nerve was observed (39).

Table 1 summarizes different experimental protocols used to test if electrical stimulation alters regeneration and lists the major findings for each study.

CHALLENGES OF CHRONIC PERIPHERAL NERVE STIMULATION

We performed a pilot study to evaluate the technical challenges of chronic nerve stimulation in the rat. A cuff electrode was applied proximal to a sciatic nerve crush injury site and the nerve was stimulated for 2–3 wks after the injury.

We tested several types of silicone cuff electrode, which had subtle variations in their geometry. All implants were prototyped by using silicone rubber, multistrand stainless steel wires (300-μm outer diameter [o.d.]) and an Omnetic connector mounted on the head of the animals. After braiding three wires equally spaced around a polystyrene cylindrical mandrel (tripolar configuration, 1.5 mm diameter, matching the sciatic nerve diameter), the structure was flooded with quick-cure silicone (KWIK-SIL, World Precision Instruments, Sarasota, FL, USA). The distal ends of the wires were then cut and the plastic mandrel dissolved in acetone. Small additional amounts of silicone were added to cover any exposed portion of the steel wires. The cuff was then cut to the desired length. The cuff endings were terminated with either flat or tapered edges. To form the soft edge, the cuff was briefly dipped in freshly mixed PDMS (polydimethylsiloxane) before removal of the mandrel. This ensured formation of a gradually tapered edge. Next, a longitudinal slit was made in the cuff with fine surgical scissors, allowing for easy opening with a pair of forceps and gentle insertion of the nerve.

Implantation of the soft cuff electrodes designed to wrap around the rat sciatic

nerve proved challenging. Anchoring the cuff implant to the soft tissue surrounding the nerve did not provide a stable way to secure the cuff in position. The stretching or twisting of the nerve within the electrode cuff during leg movement often triggered mechanical damage to the nerve and inflammation. "Hard," straight, edge cuffs induced much more damage than soft. However, extremely soft cuffs then proved difficult to anchor to the surrounding tissues with nylon sutures, without compressing the nerve.

Another challenge associated with long-term peripheral nerve stimulation was management of the wiring cable connecting the cuff electrodes to the head-plug connector. As the rat grew over the course of the experiment, tension in the cable in some cases pulled the connector wires out of the cuff. Adding length to the cables to provide slack did not solve this issue because the cables became surrounded with scar tissue and fixed in position.

Our experience indicates that the technical challenges associated with long-term peripheral nerve interfaces in the rat are considerable and should first be solved before physiological, anatomical and functional assessment of nerve regeneration and its modulation by electrical stimulation can be performed. Nevertheless, we feel that this should be possible and needs to be done.

CONCLUSIONS

Although considerable effort has been devoted by many investigators to the preclinical analysis of the effects of peripheral nerve stimulation on axonal regeneration in rodents, these effects have not yet translated into therapy. To better understand the extent to which electrical stimulation may enhance sensory and motor axon regeneration and if there is a correlation between such regeneration and improved functional outcome, further neurotechnology developmental efforts are needed to enable the necessary long-term chronic stimulation studies to be conducted. Minimizing nerve damage by using soft, nerve-like materials and ultra-compliant wiring, together with the development of new surgical techniques for inserting and stabilizing the electrodes, as well as development of sensitive behavioral outcome measures accurately reflecting successful sensory and motor recovery in freely moving animals, would enable the long-term preclinical studies needed to assess if chronic stimulation is a beneficial strategy for treating axon damage in peripheral nerves and what stimulation protocol is most effective.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Bioelectronic Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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