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Nerve lesioning with direct current

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Abstract

Spastic hypertonus (muscle over-activity due to exaggerated stretch reflexes) often develops in people with stroke, cerebral palsy, multiple sclerosis and spinal cord injury. Lesioning of nerves, e.g. with phenol or botulinum toxin is widely performed to reduce spastic hypertonus. We have explored the use of direct electrical current (DC) to lesion peripheral nerves. In a series of animal experiments, DC reduced muscle force by controlled amounts and the reduction could last several months. We conclude that in some cases controlled DC lesioning may provide an effective alternative to the less controllable molecular treatments available today.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

There are over 3 million stroke survivors in North America, about 30% of whom have severe motor impairments (Lloyd-Jones *et al* 2009). There are also about 225 000 persons with spinal cord injury (SCI) in the USA today, over 50% of whom are quadriplegic (NSCISC 2008).

One of the most debilitating outcomes of stroke and cervical SCI is the loss of hand and arm function. This has a direct effect on the level of independence in activities of daily life (ADL), often preventing the injured persons from performing basic tasks such as eating or dressing. The reduced function of the extremities in stroke and SCI survivors may be further complicated by the development of overactivity of the muscles controlling the limb. This is known as spastic hypertonus. Apart from restricting the range of motion (ROM) of voluntary movement it is also associated with painful spasms (Rizzo *et al* 2004). Spastic hypertonus develops in about 20% of stroke survivors (Sommerfeld *et al* 2004) and 65% of persons with SCI (Skold *et al* 1999).

The most common treatments for hypertonus include strengthening exercises, muscle stretching, brushing, vibration, casting, pressure splinting and transcutaneous electrical stimulation. The efficacy of these treatments is often quite limited, variable and of short duration (Goldstein 2001; Bovend'Eerd *et al* 2008). Oral medications include benzodiazepine, dantrolene and baclofen (Kita and Goodkin 2000; Goldstein 2001; O'Brien 2002). These drugs have

adverse side effects such as drowsiness, fatigue, weakness, sedation and dizziness (Kita and Goodkin 2000; Montane *et al* 2004), an increased risk of falling (Esquenazi 2006). In people with SCI, baclofen is sometimes delivered intrathecally via an implantable pump (Barker 2008). The dosage required is lower than the oral dose, reducing systemic side effects, but surgery is required and other complications such as mechanical failure of the pump and infections may occur (Kita and Goodkin 2000). Surgical interventions of a biomechanical type such as tendon lengthening, tendon transfer and osteotomy (Woo 2001) are sometimes considered when muscle changes such as contractures or paralysis occur (Esquenazi 2006).

Chemical denervation by injection of phenol or, more commonly, botulinum toxin (BtA) is widely used to reduce spastic hypertonus (Kirazli *et al* 1998). Phenol causes immediate nerve block that lasts between 6 weeks and 6 months, but the injections can be painful and muscle atrophy can occur as a result of neurolysis, demyelination and axonal damage (van Kuijk *et al* 2002). BtA acts on motor nerves by blocking the release of acetylcholine, thus inhibiting synaptic transmission. It has fewer side effects than phenol (Kirazli *et al* 1998). However it takes up to 2 weeks to act, so producing the desired level of nerve block requires skill and experience. The most important limitation of BtA however is the relatively short duration of its nerve-blocking effect, typically 4 to 6 months (Dolly 2003), necessitating repeated sets of expensive injections. In this paper we introduce a new approach to treating hypertonus by means of controlled lesioning of nerves using direct current (DC) stimulation.

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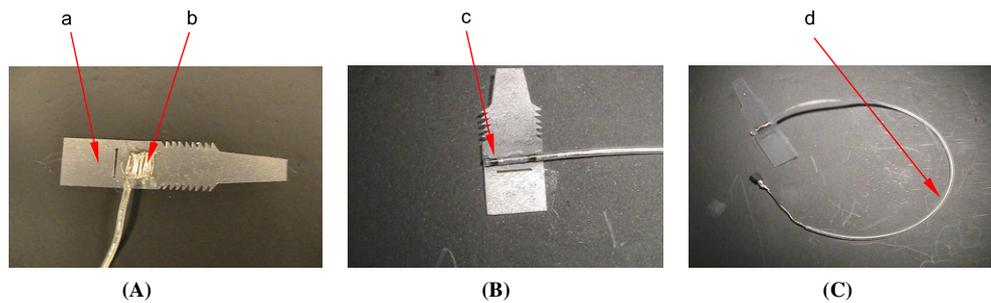


Figure 1. The nerve cuff electrodes used in the study. (A) Pt-Ir nerve cuff, which delivered DC to cause controlled ablation. Cuff is shown open, prior to placement on nerve; (a) silicone strip in the form of a tie-wrap; (b) A single terminal of the Pt-Ir wire was soldered to the stainless steel wire emerging from the silicone tubing of the lead. The Pt-Ir wire was sewn into the silicone strip, looping back and forth between two sutures, also sewn into the strip. (B) Proximal cuff which delivered pulses to the nerve to generate test muscle twitches; (c) two insulated stainless steel wires emerged from inside the silastic tubing of the lead were stripped of insulation and tightly coiled around the silastic tubing of the lead to form a bipolar terminal. (C) Control electrode implanted on the nerve in the contralateral leg; (d) silastic tubing through which the lead was inserted.

Previously DC has been used in acute physiological studies to selectively block large nerve axons (Mendell and Wall 1964; Whitwam and Kidd 1975; Bhadra and Kilgore 2004). To our knowledge, long-term nerve ablation for clinical purposes has not been previously suggested.

The mechanism by which DC ablates axons is not well understood; however it is known that short-duration, charge-balanced biphasic pulses involve reversible reactions at the electrode–tissue interface (McCreery *et al* 1990), while long-duration, monophasic pulses allow time for irreversible reactions to occur (Merrill *et al* 2005). DC could be viewed as an extremely long monophasic pulse.

Here we show that the damaging effect of applying DC to a nerve can be used to gradually lesion it, reducing in a controlled manner the motor activity transmitted by the nerve. We suggest that this could provide an effective treatment of spastic hypertonus in some cases.

2. Methods

All the procedures described below were approved by the University of Alberta Animal Care and Use Committee.

2.1. Animals and surgical procedures

2.1.1. Acute surgical procedure. Seven white New Zealand rabbits were used in a non-recovery experimental procedure. The animals were surgically anaesthetized using isoflurane to effect (Forane, Baxter Corp., Toronto, ON, Canada). The left sciatic nerve was exposed through an incision overlying the hamstrings muscle. The common peroneal nerve was separated from the tibial nerve and transected. Two nerve cuff electrodes were attached to the tibial nerve, approximately 1 cm apart. The cuffs each comprised a strip of silicone with a serrated tongue at one end, designed to be inserted into a slot close to the conductive terminals (figure 1(A)). The strip functioned like a ‘tie-wrap,’ which was used to fix electrodes to the nerve (Kowalczewski 2009). The nerve cuff leads were made of insulated multi-stranded, stainless steel wire (AS632, Cooner Wire, Chatsworth, CA) inside a silicone tube (508–003, Dow Corning Corporation, Midland, MI). The more distal

of the two cuffs, which was used to deliver DC to the nerve, had a single lead terminating in a length of the bare Pt-Ir wire (0.003" A-M Systems Inc., Carlsborg, WA) which was sewn back and forth between two sutures in the strip forming an approximate rectangle of sides 0.3 and 0.5 cm. (figure 1(A)). The more proximal cuff contained two stainless steel terminals formed by tightly coiling the bared ends of two insulated lead wires emerging from the silastic tube around the outside of the tube (figure 1(B)). Each terminal was 1 mm in length and the distance between them was 4 mm. A third cuff, similar to the proximal cuff but with only a single bared terminal 5 mm in length, was implanted on the tibial nerve of the contralateral leg, to serve as a control (figure 1(C)). Finally, an indifferent (anodal) electrode comprising an insulated lead terminating in a bare stainless steel disk 3 cm in diameter was placed under the skin over the paravertebral muscles of the lower lumbar region.

2.1.2. Chronic surgical procedure. In a single cat two monopolar nerve cuffs were implanted on the common peroneal nerve of each leg. The implant surgery was performed in a fully equipped operating room with sterile equipment and procedures. Acepromazine (0.1 mg kg⁻¹ i.m.), hydromorphone (0.05 mg kg⁻¹ i.m.) and glycopyrrolate (0.01 mg kg⁻¹ s.c.) were administered pre-operatively. The cat was then anesthetized with isoflurane and intubated using a pediatric tracheal tube. Anesthesia was maintained with 2–3% isoflurane in carbogen at 1.5 L min⁻¹. An intravenous catheter was inserted in the cephalic vein and a saline drip was administered. Body temperature was maintained using a warm-water heating pad. Respiration and heart rate were monitored throughout the surgery. The legs and back were closely shaved, cleaned with soap and swabbed with an iodine solution.

The distal cuffs in the chronic implant had 5 mm Pt-Ir terminals as in figure 1(C) and the proximal cuffs were identical, except for stainless steel terminals. The other ends of the four implanted leads terminated in an insulated connector, which was tunneled under the skin of the animal’s back to an exit point on the head. Accompanying these leads and also

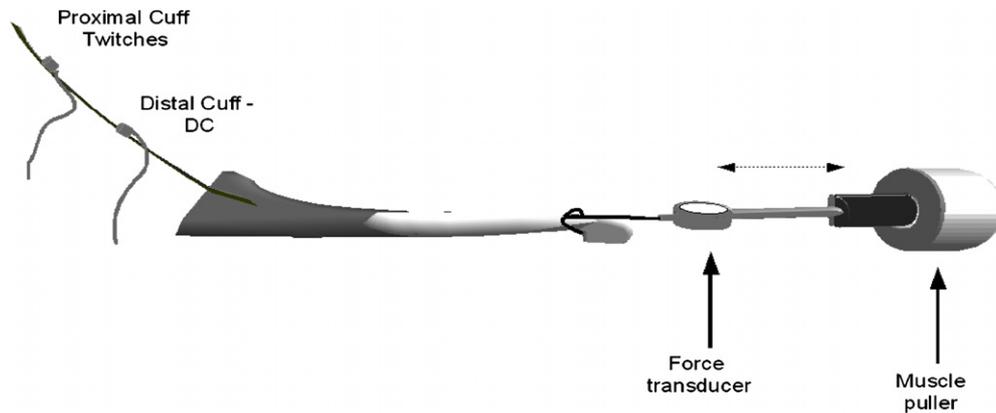


Figure 2. Acute experiment setup. The tendon of the triceps surae muscles was exposed and dissected free through a skin incision, detached with a small portion of the calcaneus and connected to a force transducer. The bony origin of the muscle at the knee was immobilized by a clamp attached to a metal frame. The nerve innervating the triceps surae was exposed in the thigh and the two nerve cuffs were attached as shown. The force transducer was attached to an electromagnetic servo muscle puller that received as input a triangular signal (0.05–0.08 Hz cycle frequency), causing the muscle to be stretched slowly back and forth through 15 mm.

terminating in the connector were two insulated stainless steel reference leads, whose bared terminals (1.5 cm in length) were implanted under the skin overlying the lower lumbar spine. All electrodes were secured in place by suturing silicone tie-downs to connective tissue. Four stainless steel screws were attached to the skull through small skin incisions. A rubber mold with slits in its base was pushed onto the screws to act as a former for the manufacture of a dental acrylic headpiece. The connector was pulled into this mold through a slit. Dental acrylic was then poured into the mold and allowed to harden. During subsequent experiments, the sockets in the connector embedded in the headpiece enabled delivery of test pulses via the proximal cuff and lesioning DC via the distal cuff in either leg.

All skin incisions were closed with 3–0 prolene suture. At extubation, the cat was given ketoprofen ($0.5\text{--}1\text{ mg kg}^{-1}\text{ sc}$) to minimize discomfort. During post-operative recovery the cat was kept warm in a heated cage and provided with blankets. Analgesia was maintained by giving hydromorphone ($0.05\text{--}0.1\text{ mg kg}^{-1}\text{ sc}$) 6 h after extubation. The antibiotic clavamox ($10\text{--}20\text{ mg kg}^{-1}$) was administered for 7 days after surgery.

2.2. Experimental procedures

2.2.1. Acute experiments. The rabbit was surgically anesthetized and placed prone, with its abdomen supported by a sling strung between the parallel bars of a stereotaxic frame, its head resting on the front of the frame. The animal's left knee was stabilized with a clamp containing two pins that were tightened onto the distal end of the femur. The tendon of the triceps surae muscle was exposed and detached from the foot along with a small part of the calcaneal bone. The tendon was secured to a force transducer via a strong suture. The force transducer was mounted on an electromagnetic, servo-controlled muscle puller that could be configured to stretch the muscle at different velocities and through different lengths (figure 2).

Pulses of electrical current ($200\text{ }\mu\text{s}$, 2 pulses s^{-1}) were applied through the proximal nerve cuff to elicit test muscle

twitches throughout the experiment. The pulse amplitudes were adjusted to be 1.5 times threshold (1.5 T) to activate all the alpha motoneurons in the nerve. In the first two experiments a monophasic, constant voltage, pulse generator was used (Grass SD9, Grass Medical Instruments, Quincy, Mass. USA). In the remaining five experiments, a custom, biphasic, charge-balanced, constant-current pulse generator was used. The lead of the distal cuff was connected to the negative (cathodic) output of a custom, feedback-controlled DC source. The indifferent lead under the skin of the animal's back was connected to the positive (anodic) terminal of the current source. DC was applied in the range 0.1 to 1 mA for durations ranging from 5 to 45 min, according to the lesioning effect. No current was delivered through the control nerve cuff in the contralateral leg.

The muscle puller was controlled with a triangular signal 0.05–0.08 Hz (Function Generator FG600, Feedback Instruments, England) slowly pulling the end of the tendon back and forth through 15 mm. This was done to ensure that twitch force at the maximum of the force-length curve of the muscle was captured in each stretch cycle.

The signals from force and displacement transducers attached to the muscle puller were viewed on an oscilloscope (Tektronix TDS3014B, Tektronix Inc., Beaverton, OR USA). The digitized signals were downloaded from the oscilloscope to a desktop computer via a local area network and Tektronix Visa software. The oscilloscope was set to sample at 250 or 500 samples s^{-1} enabling either 20 or 40 s segments of data to be stored. These segments were recorded at intervals of 1 to 5 min throughout the experiment.

At the beginning of each experiment muscle twitches were recorded during triangular muscle stretching in the absence of DC, in order to establish baseline length–tension curves. The duration of these baseline measurements varied from 5 to 15 min across all animals. After these initial measurements, DC was applied through the distal nerve cuff for various lengths of time, depending on how much the peak force of muscle twitches was reduced during DC application, and on how much it recovered thereafter. Once the twitch force had

stabilized for a few minutes, a new DC application would start. If the twitch force was reduced to zero and did not recover within an hour or more, the experiment was terminated and the animal was euthanized with concentrated intravenous pentobarbital (euthanol).

At the end of each experiment, the force transducer was calibrated with a spring balance and approximately 20 mm lengths of each tibial nerve were dissected and flash-frozen in isopentane for histochemical analysis of neurofilament H (NFH).

2.2.2. NFH staining. Longitudinal nerve sections were stained with antibodies that bind to NFH. From the left tibial nerve (which had been stimulated in the acute experiment), sections were taken from five sites: distal to the DC cuff, within this cuff, between the two cuffs, within the proximal cuff and from an area proximal to the proximal cuff. From the right, unstimulated tibial nerve, sections were taken from within the control cuff and distally and proximally to the cuff.

2.2.3. Chronic experiments. DC lesioning of the right common peroneal nerve was performed in a graded manner in the temporarily anesthetized cat, once every 7 to 14 days over a period of 8 weeks, culminating in a complete loss of nerve conduction. From weeks 1 to 20 post-lesioning, at intervals of 2 to 4 weeks, twitch thresholds and responses to tetanic nerve stimulation through the left proximal cuff (Lloyd-Jones *et al*) and the right proximal and distal cuffs (R_p and R_d) were determined during brief periods of anesthesia.

In the DC lesioning experiments the cat was anaesthetized with isoflurane. It was laid on its left side on a heated pad and its right hindlimb was held by a retort clamp applied above the ankle. The clamp allowed foot dorsiflexion, while minimizing movement of the shank. Care was taken not to tighten the clamp to a point where it would damage the tissues. A 1 cm wide band of webbing material was looped around the metatarsals 60 mm from the pivot point of the ankle joint and attached to a force transducer so that force was applied in the plantarflexion direction, at right angles to the long axis of the foot. The leg clamp and the force transducer were secured to a heavy metal frame, which in turn was clamped to a table. As in the acute rabbit experiments, biphasic current pulses ($200 \mu\text{s}$, 2 pulses s^{-1}) were applied via the R_p nerve cuff to elicit test muscle twitches. This was achieved by connecting the cathodal output of the biphasic pulse generator to a socket in the headpiece connector leading to the R_p cuff. In the first few trials the anodal output was connected to a socket leading to one of the implanted indifferent electrodes terminating under the skin over the lumbar spine. In later experiments a surface indifferent electrode was used, as explained below. The pulse amplitudes were again adjusted to 1.5 T. At the beginning of each experiment the baseline values of peak twitch force were obtained.

After these initial measurements, lesioning DC was applied through the R_d cuff by connecting the cathodal output of the DC source to the headpiece socket. The anodal output of the DC source was connected in one of the following three ways. In the first 2 weeks it was connected to one of the

stainless steel indifferent electrodes implanted in the lower back. At the end of the second week the voltage required to maintain a constant current had increased to nearly the compliance level of the DC source (45 V), which suggested corrosion of the indifferent electrode. In week 3 we therefore switched to a self-adhesive gel surface electrode (Kendall ES40076) as the indifferent. By week 5, after repeated DC applications the voltage had again reached the compliance level and we found that the Kendall electrode was discolored, indicating corrosion. From week 6 onward, we used a wettable pad surface electrode 3 cm in diameter, which enabled DC to be applied for long periods at well below the compliance voltage.

DC was applied at amplitudes in the range 0.3–0.4 mA for durations of 10 to 40 min according to the lesioning effect. The twitch force responses to 1.5 T pulse trains delivered through the proximal cuff were monitored on the oscilloscope and segments of recording were stored digitally at intervals of 1 to 5 min. After each period of application of DC, recording was continued for up to 1 h to monitor the recovery of twitch force. After the final DC application in the last DC lesioning session, the twitch responses were completely abolished (see section 3). From weeks 1 to 20 post-lesioning, the cat was briefly anesthetized every 2 to 4 weeks and muscle response thresholds to stimulation via the L_p , R_p , and R_d cuffs were determined visually. At week 20 post-lesioning, the thresholds had returned close to baseline and force measurements were carried out as above.

2.3. Data analysis

2.3.1. Acute experiments. A custom Matlab (The MathWorks, Inc., Natick, MA) program was used to detect the maximal twitch force occurring within each muscle stretching cycle, that is the twitch force at an optimal muscle length. Reductions in this parameter during and after the application of DC were taken as a measure of the extent of the lesioning effect.

The twitch forces were superimposed on a slow waxing and waning passive component of force resulting from the visco-elastic resistance of the muscle to the triangular displacement. The passive component was isolated in software with a digital low pass filter (1 Hz, fifth-order Butterworth) and subtracted from the unfiltered signal to leave just the twitch forces. Finally, a low pass filter (100 Hz, fifth-order Butterworth) was used to attenuate high-frequency noise. Figure 3 (top panel) shows unfiltered force signals for three stretch cycles with and without DC and the same signals after subtraction of the passive force component (third panel).

The filtered force signal was further analyzed by the software to select the maximal peak force in each muscle stretch cycle. The first five maximal peak force values of the baseline measurements in a given experiment were averaged and all subsequent maximal peak twitch forces sampled in that experiment were normalized to that mean value. The normalized twitch forces are plotted in figures 4–7.

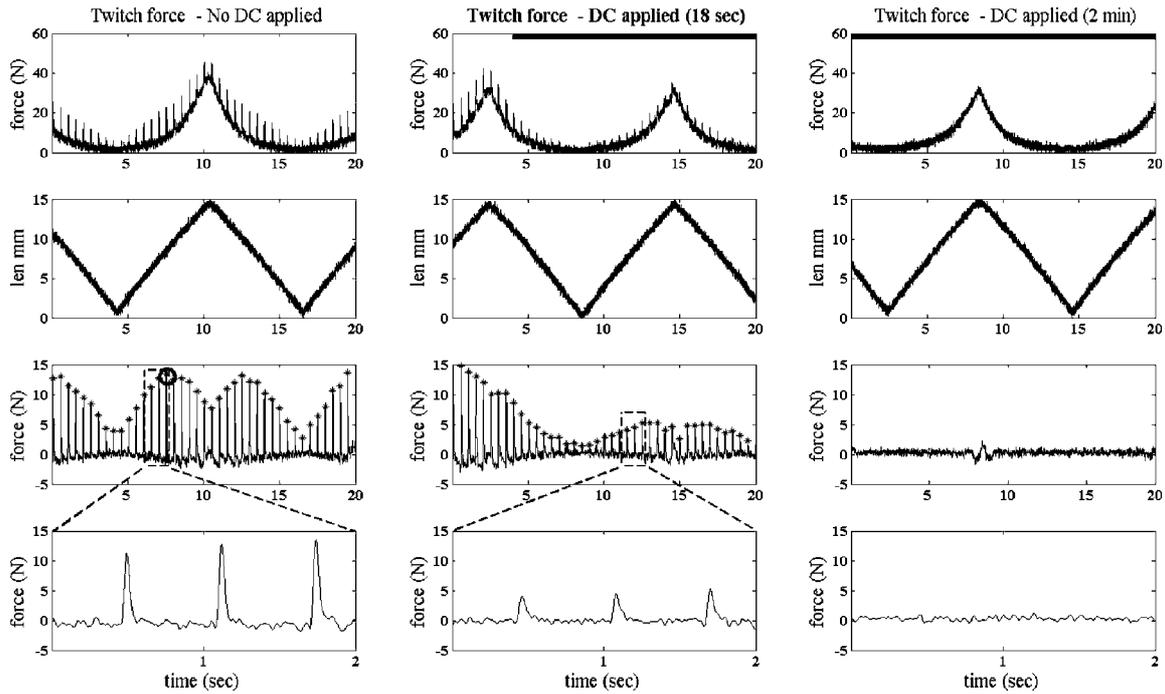


Figure 3. Muscle twitches were generated by pulsatile stimulation at 2 Hz through the proximal nerve cuff. Top row: force transducer signal during triangular changes in muscle length. Black bars represent the time DC was applied through the distal nerve cuff. Second row: imposed displacement. Third row: twitch forces after the passive force was removed by filtering. The peak force in each twitch, shown by asterisks, and the maximal peak force within each stretch cycle (circled asterisk) was automatically identified by a software program.

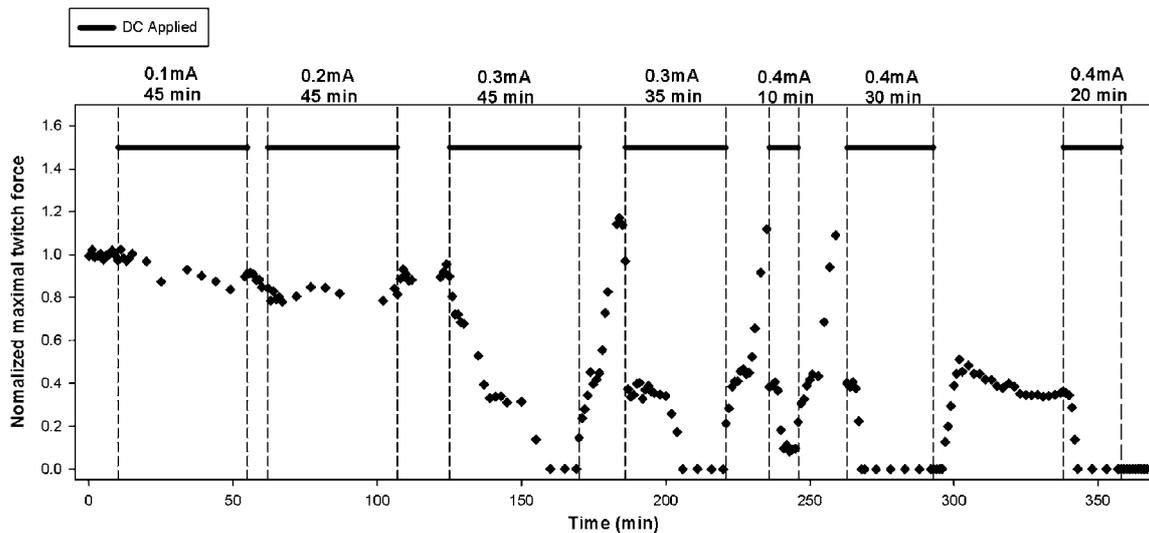


Figure 4. Changes in twitch force during episodes of DC application over a 6 h experiment. Top: amplitudes and durations of DC. Horizontal bars: duration of DC application. Data points: normalized maximal twitch forces in stretch cycles measured at intervals of 1 to 5 min throughout the experiment. Peak twitch forces were normalized to the baseline value: the mean of five peak twitch force values measured just prior to the first application of DC. The larger the DC amplitude the faster the twitch force was attenuated. In three cases peak twitch forces rebounded to higher than baseline values between DC applications. There was a cumulative effect of repeated DC applications at a given amplitude: peak twitch force attenuated faster on repeated applications. The estimated charge densities for each consecutive DC application in this figure were: 1.14, 2.29, 3.43, 2.67, 1.02, 3.05 and 2.03 C cm⁻².

2.3.2. Chronic experiments. As in the acute experiments, the force signal was digitally band-pass filtered (1 Hz, 100 Hz) to attenuate DC drift and high frequency noise. The same Matlab algorithm to detect peak twitch forces was also used. The average peak twitch force per 40 s sample was computed.

2.3.3. Charge density calculations. In previous studies examining safe stimulation of nerves, charge density per phase was a key variable. For each of the nerve cuff electrodes used to deliver DC, we estimated the surface area in contact with the nerve, taking into account the semi-circular profile of the exposed wires. Thus the DC cuff used in the acute rabbit

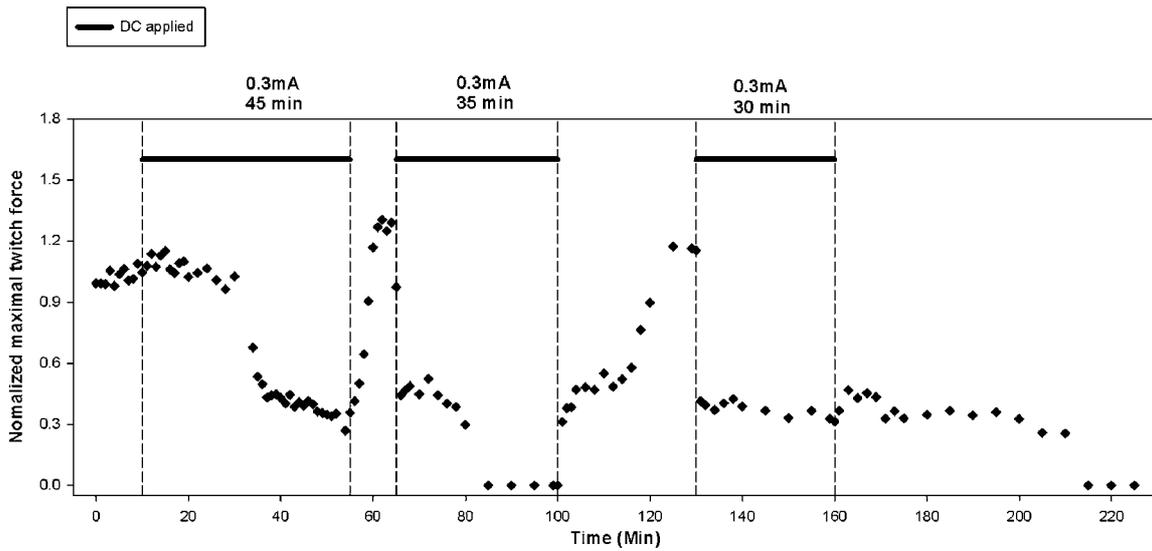


Figure 5. Changes in twitch force during episodes of DC application over a 4 h experiment, data displayed as in figure 4. Clear examples of rebound and increased efficacy and speed of attenuation with repeated DC applications at the same amplitude (0.3 mA). The estimated charge densities for each consecutive DC application in this figure were: 3.43, 2.67 and 2.29 C cm⁻².

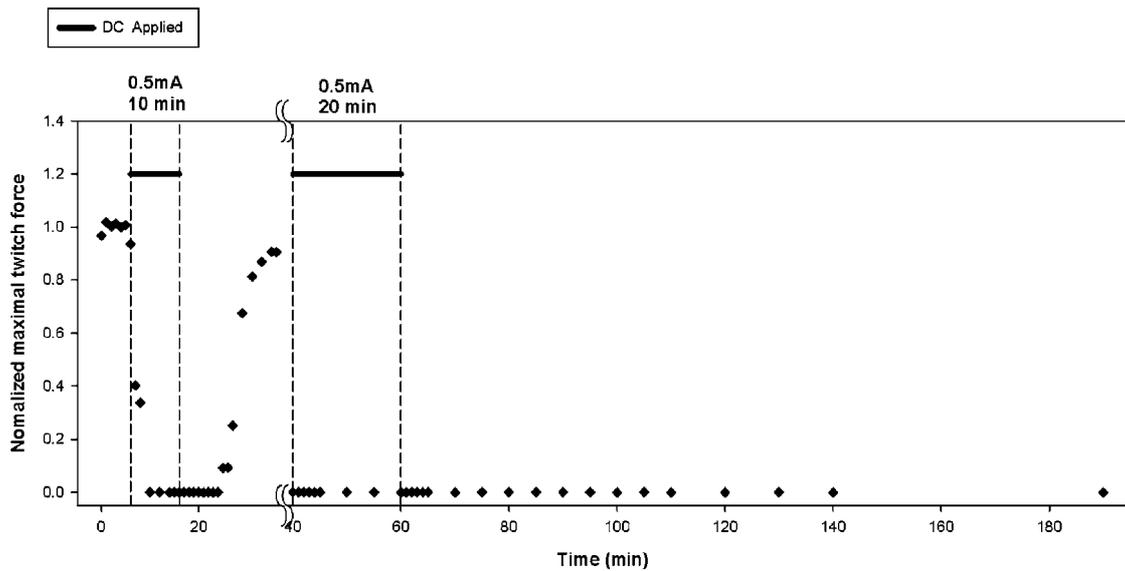


Figure 6. Changes in twitch force during episodes of DC application at an amplitude of 0.5 mA. Data displayed as in figure 4. In this experiment after a first application of DC (not shown) we discovered that the test nerve stimuli were not supramaximal. This was corrected and the experiment was restarted. This may explain the immediate and complete attenuation caused by the first of the two DC applications illustrated. At this amplitude, force attenuation was rapid, and after the second application, it was maintained for over 2 h until the end of the experiment. The wavy lines in the horizontal axes indicate a break of 10 to 15 min in data collection. The estimated charge densities for each consecutive DC application in this figure were: 1.27 and 2.54 C cm⁻².

experiments had an estimated surface area of $0.3 \cdot 0.5 \cdot \pi / 2 = 0.236 \text{ cm}^2$. The cuff used in the chronic implant in the cat had an estimated surface area of 0.093 cm^2 . In figures 4–7 and 9 we provide the values of total charge density based on the level and duration of DC and the above estimates of the surface area.

3. Results

3.1. Definitions

Baseline. Test period at the beginning of each experiment prior to delivering DC. Muscle twitch forces elicited by a

train of test stimuli applied through the proximal cuff were continuously monitored. Durations of the baseline period ranged from 5 to 15 min.

DC application. A single duration of DC applied to the nerve.

DC session. An experiment in which one or more DC applications occurred.

Recovery. The period following the cessation of DC delivery. Test stimuli continued to be applied through

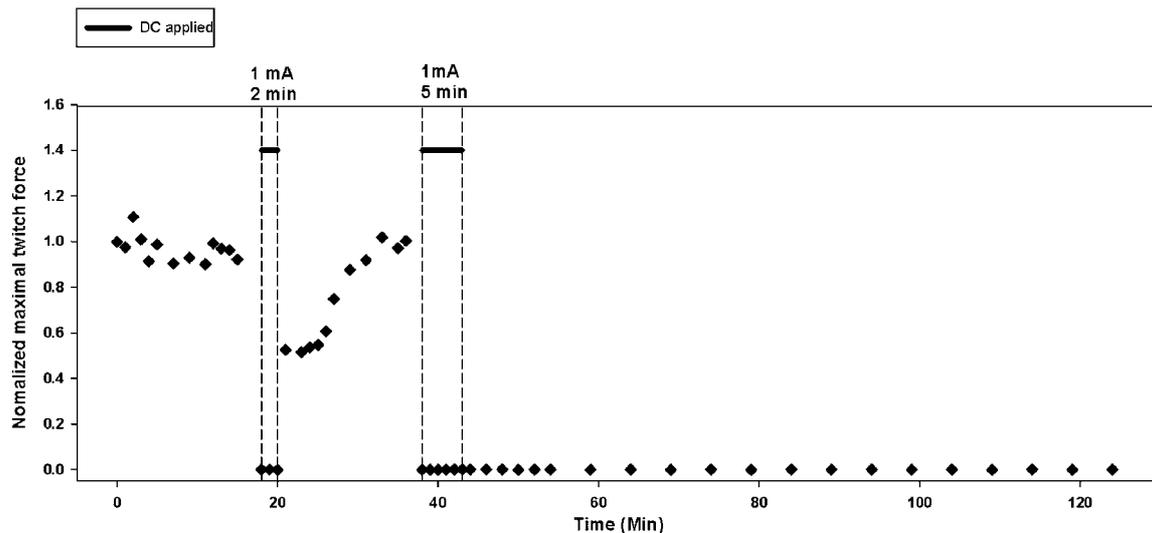


Figure 7. Changes in twitch force during episodes of DC application at an amplitude of 1.0 mA. Data displayed as in figure 4. As in figure 6, force attenuation by DC was rapid and complete, after the second application, it was maintained until the end of the experiment. The estimated charge densities for each consecutive DC application in this figure were: 5.08 and 12.7 C cm⁻².

the proximal cuff and twitch force was monitored and recorded.

Force overshoot. Peak twitch force values during recovery exceeded either the mean baseline peak twitch force value or the value recorded just prior to the onset of the preceding DC application.

3.2. Acute experiments

3.2.1. Effect of DC amplitudes in the range 0.1–0.4 mA. DC at 0.1 mA was applied in two different animals for durations of 2.5, 10 and 45 min. This had little attenuating effect on the peak force of muscle twitches. Similarly, DC at 0.2 mA had little attenuating effect. It was not until DC reached 0.3 mA and above that attenuation of peak twitch forces became obvious. This is shown in the experiment in figure 4. After each of the two applications of DC at 0.3 mA and after the first two applications at 0.4 mA, force recovered to levels above baseline. This was an unexpected but repeated finding in our experiments. This did not occur after the third and final DC application at 0.4 mA, which resulted in a complete abolition of force that endured for the remainder of the experiment in this case. Similar results are shown in a second animal in figure 5, this time for three repeated applications of DC at 0.3 mA. Again, there was a cumulative effect of consecutive DC applications, leading to a long-lasting attenuation of force, with complete abolition at the end of the experiment. Why force declined in two stages in this case is not known, but this kind of variation in repeated applications of DC at 0.3 to 0.4 mA was seen in other animals in this series.

3.2.2. Effect of DC amplitudes in the range 0.5–1 mA. DC amplitudes in this range had a large effect, often resulting in a rapid and sharp decline in force during DC application. Short applications of DC at 0.5 mA for 2.5 to 5 min (not illustrated)

resulted in rapid declines in twitch force during DC application followed by a recovery of force after DC was withdrawn, to levels equal to or exceeding baseline values (not illustrated). Longer applications of DC at 0.5 mA (e.g. 10 and 20 min in figure 6) resulted in a complete abolition of twitch force, with incomplete recovery after the first application, and long-lasting attenuation after a second application. A similar pattern was seen for DC at 1 mA applied for durations of 2 to 5 min in two animals. Figure 7 illustrates one such case. After the first application, force recovered, but after the second, longer, application, no recovery was seen for the remaining 80 min of the experiment.

3.2.3. Force overshoot. Because force recovered beyond the baseline levels in some of the DC applications, especially after having been abolished or strongly attenuated, in some experiments we verified that proximal test stimuli were supramaximal by increasing their amplitude without observing an increase in twitch forces.

3.2.4. Condition of electrodes. The same electrodes were used in all of the experiments. No corrosion was observed in either the proximal or distal (DC) nerve cuff electrodes.

3.2.5. Histochemical results for NFH staining. Staining for NFH showed localized axonal damage under the distal cuff delivering DC. No damage was visible in the area distal or proximal to this. Figure 8(A) shows undamaged NFH-stained axons in one of these regions. The neurofilaments are straight and discontinuities are attributable to out-of-plane sectioning, the ends are not curled. In contrast, in the section shown in figure 8(B), taken from under a DC cuff, most of the discontinuities have curly ends, indicating axonal damage.

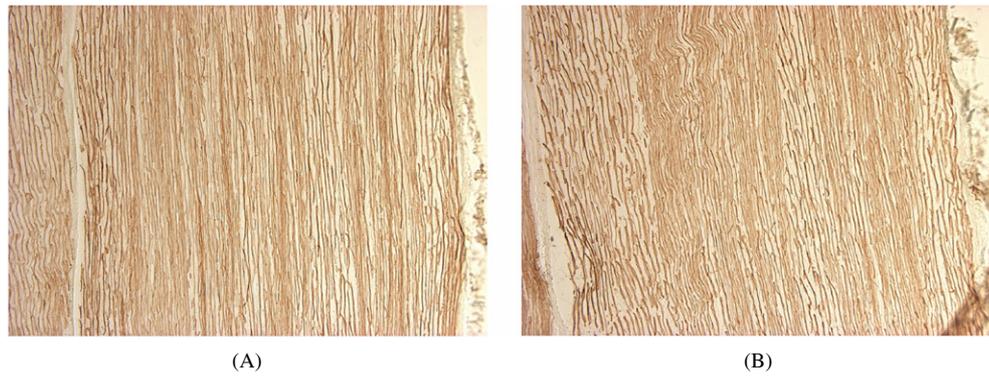


Figure 8. Photomicrographs of longitudinal sections of the nerve stained with neurofilament H. (A) Segment of nerve taken from within the proximal cuff used to deliver test stimuli. Nerve filaments are straight and continuous with little sign of damage. (B) Segment of nerve taken from within the distal cuff used to deliver DC. Nerve filaments in the left, right and middle of the picture are broken, with curly edges, indicating localized axonal damage.

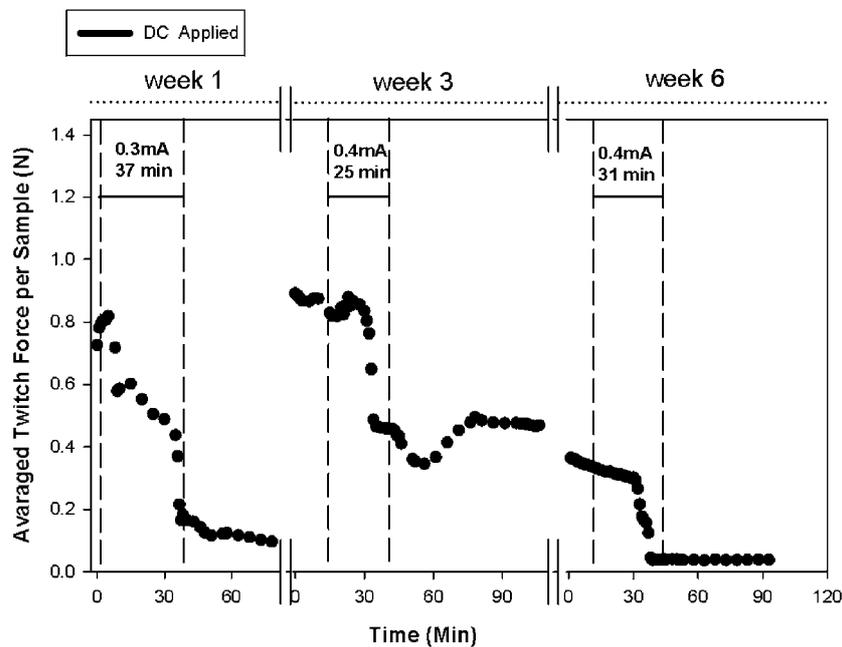


Figure 9. Three separate DC lesioning sessions in an anesthetized cat chronically implanted with test and DC cuffs on the common peroneal nerve innervating the pretibial muscles extensor digitorum longus and tibialis anterior. The force measurements were performed by immobilizing the leg just above the ankle with a retort clamp attached to a frame and attaching the transducer shown in figure 2 via a loop of canvas webbing to the foot close to the metatarsophalangeal joint. Data displayed as in figure 4. In the first session the DC amplitude was 0.3 mA. In sessions 3 and 6 it was 0.4 mA DC. Following the sixth (final) session, no ankle dorsiflexion could be elicited by pulsatile or tetanic test stimuli for 8 weeks, after which a gradual recovery was observed. The cat recovered quickly after each lesioning session, with no sign of discomfort. The estimated charge densities for each consecutive DC application in this figure were: 7.1, 6.4 and 8.0 C cm⁻².

3.3. Chronic experiments

3.3.1. DC delivery in the anaesthetized cat. As mentioned in section 2, six DC lesioning sessions took place at intervals of 7 to 14 days over a period of 8 weeks. On each occasion the cat was anesthetized for approximately 2 h and DC was delivered to the right common peroneal nerve through the R_d cuff via the headpiece while test twitches were elicited via the R_p cuff and muscle force was measured. The cat was then allowed to recover and rest until the next session. Figure 9 shows the results obtained in sessions 1, 3 and 6. The DC levels during the experiments at weeks 2, 4 and 5 fluctuated, due to the corrosion problems with the anodal electrodes described in

section 2, so the results of these experiments are not included in the figure. Twitch forces were progressively reduced by DC in each experiment in figure 9, culminating in a complete abolition of force at the end of the sixth session.

At weeks 1, 3, 5, 8, 12, 16 and 20 post-lesioning, the cat was briefly anesthetized with isoflurane and muscle response thresholds to stimulation via the left and right proximal nerve cuffs (L_p and R_p) were determined visually. As before, the cathode of the pulse generator was connected to the appropriate socket in the headpiece and the anode to a wettable surface electrode on the animal's back. At week 1, threshold to tetanic stimulation through the L_p cuff (200 μ s pulses at

40 pulses s^{-1}) was 0.12 mA, while in the right leg tetanic stimulation via the R_p cuff at amplitudes up to 1.5 mA failed to produce ankle dorsiflexion, though a slight toe dorsiflexion was visible at the highest amplitudes. Similar results were obtained at weeks 3 and 5.

To ensure that the absence of muscle responses on the right side were not due to a failure in the headpiece connector, the lead or the electrode terminal in the R_p cuff, at week 5, we also applied stimulation through pairs of stainless steel wires inserted into the left and right tibialis anterior muscles (TA) with hypodermic needles. The wires had 5 mm bared ends. In the left leg, tetanic intramuscular stimulation with 2 mA pulses elicited powerful ankle dorsiflexion, whereas in the right leg pulse amplitudes up to 23 mA failed to produce ankle dorsiflexion. The TA and Extensor Digitorum Longus (EDL) muscles were clearly atrophied at this stage. In addition, surface stimulation was applied over these muscles through a pair of wettable pad electrodes. On the left side the threshold to elicit muscle twitches was 3 mA, whereas on the right side stimulation at up to 6 mA failed to elicit muscle responses.

From weeks 8 to 20, responses in TA and EDL began to reappear. The following twitch thresholds to stimulation through the L_p , R_p and R_d cuffs were determined: week 8: 0.1 mA, 0.3 mA, 0.7 mA; week 12: 0.1 mA, 0.3 mA, 0.6 mA; week 16: 0.1 mA, 0.2 mA, 0.3 mA; week 20: 0.1 mA, 0.2 mA, 0.4 mA. Because thresholds at week 20 had returned close to pre-lesioned values, twitch forces were measured with a force transducer as described in section 2.2.3. The mean peak twitch force had returned to the values measured prior to DC lesioning in sessions 1 and 3. This indicates that the muscle nerve had successfully regenerated.

3.3.2. DC delivery in the awake cat. As described in section 2, on one occasion DC (0.4 mA) was delivered in the awake animal through the R_d cuff by connecting the cathodal output of the constant current stimulator to the headpiece and the anodal output to the implanted indifferent electrode via the socket in the headpiece. DC was delivered for 1.5 min. The animal showed no signs of discomfort during or after this procedure. As it was impossible to measure twitch forces under these circumstances, it is unknown whether nerve block occurred. However the experiment showed that DC could be delivered at an amplitude that caused nerve block during anesthesia, without causing signs of discomfort. Future experiments are planned to compare twitch forces measured during brief periods of anesthesia before and after DC application in the awake animal.

3.3.3. Condition of electrodes. An attempt to re-lesion the nerve following the 20 weeks recovery period using the same DC parameters (0.4 mA for 40 min) did not result in force attenuation. In a brief surgical procedure, it was found that the solder joint connecting the Pt-Ir terminal to the stainless steel lead was corroded. This joint was insulated and therefore had not been in contact with the nerve. The Pt-Ir terminal itself was intact, with no sign of corrosion. The proximal cuff was fully intact, no corrosion was evident.

4. Discussion

Because this method of nerve ablation was novel, the choice of electrodes, stimulation parameters and outcome measures was exploratory in nature and the results are therefore preliminary. However, some tentative conclusions may already be drawn. The experiments showed that it is possible to lesion peripheral nerves in a controlled manner by applying DC to them. This causes a controlled amount of reduction in muscle force. Consecutive DC applications showed a cumulative effect on muscle force, whereby the force declined faster and to a greater extent in each subsequent DC application. This was also observed by Whitwam and Kidd (1975).

DC amplitudes of 0.1 or 0.2 mA required long durations of application in order to attenuate muscle force, and the attenuation was temporary, which agrees with previous results (Bhadra and Kilgore 2004). DC amplitudes of 0.3 and 0.4 mA were effective in damaging the nerve in a controlled fashion but they required 20–40 min to take effect, and complete blockade was usually not achieved. DC amplitudes of 0.5 to 1 mA produced large force reductions in a relatively small amount of time, which may be desirable in some cases, but not necessarily if graded ablation is desired. The recovery in twitch force seen between the end of one DC lesioning session and the next (e.g. figure 9) can be attributed to the recovery of conduction in transiently blocked axons.

The DC charge densities in our study ranged from 1 to 13 $C\ cm^{-2}$, i.e. one to two orders of magnitude greater than the largest charge densities ($1\ mC\ cm^{-2}\ phase^{-1}$) explored in previous studies of safe and damaging pulsatile stimulation (Merrill *et al* 2005). Long trains of monophasic pulses, which deliver a linearly increasing charge density, eventually damage nerves, so the rate of charge delivery (i.e. the current) and the current density (determined by the surface area of the electrode) are clearly variables that should be explored systematically in future studies of nerve ablation.

In our first two acute experiments, monophasic stimuli ($2\ s^{-1}$) were used to elicit the test muscle twitches. We estimate the charge density per pulse to have been about $8\ \mu C\ cm^{-2}$ and the net charge $0.12\ \mu C\ cm^{-2}$. These values are orders of magnitude below damaging levels, so we assume that they did not contribute to the nerve blocks reported here.

In the chronic cat experiment, we proceeded cautiously, increasing DC amplitudes and durations in successive trials. The common peroneal nerve was evidently completely ablated after the sixth DC lesioning session. By week 5 post-lesioning, the TA and EDL muscles had atrophied and twitches could not be elicited with surface, direct or intramuscular stimulation. We saw a resumption of muscle responses at week 8 (56 days post-lesioning). This delay corresponds well to the classical data of Gutmann *et al* (1942) who either crushed or cut the common peroneal nerve in rabbits 40–50 mm proximal to their muscles and found that motor responses first appeared 38–40 days after crushing and 54–70 days after cutting. In other experiments, after cutting the same nerves closer to the muscles (11–16 mm, mean 13 mm), the delays ranged from 35 to 48 days (mean 42 days).

Our study suggests that controlled nerve lesioning with DC might provide an alternative treatment to chemo-denervation to reduce spastic hypertonus. Currently, the most common form of chemo-denervation comprises intramuscular injections of BtA. Nerve ablation with phenol provides a cheaper alternative, but this can be painful. The benefits of chemodenervation are reduced focal muscle over-activity, improved range of motion, improved hygiene and reduction in pain and caregiver time (McGuire 2001). These are all goals that could potentially be achieved by using DC to ablate nerves innervating muscles. BtA injections only take effect a week or two after injection, and so it is impossible to grade the amount of attenuation of muscle force during the injection procedure. Furthermore some side effects of chemodenervation including adjacent muscle weakness, transient fatigue, nausea and dry mouth as well as the difficulty in determining the best injection site (McGuire 2001) would be avoided with DC ablation.

DC could be delivered to a nerve by an implanted device. Once the desired attenuation of spastic contractions was achieved, the same device could be used to deliver trains of stimulus pulses to the nerve to control muscles in functional movements such as hand grasp. In this way, the device would serve two functions: that of an ablation device and that of a neuroprosthesis. This might overcome the well-known disadvantage of chemo-denervation, the reduction in functionality due to muscle weakness (O'Brien 2002; Esquenazi 2006).

Nerves recover after chemo-denervation, so BtA treatment needs to be repeated every few months. We found that nerve regeneration also occurred after DC ablation. However, once DC leads are implanted, repeated ablations would simply involve short applications of DC, which could be performed by medical technicians. Unlike cardiac pacemakers, implanted DC stimulators could potentially receive energy by inductive coupling prior to each use and they would therefore not need replacement every few years. It may even be possible to dispense with an implanted stimulator by delivering DC to the subcutaneous terminal of an implanted lead with a percutaneous needle (AP, patent pending). On the other hand DC implants would be unlikely to replace BtA in the cases in which hypertonus in multiple muscles needs to be treated, as this would require several nerve electrodes to be implanted.

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