University of Alberta

Reduction of spastic hypertonus by controlled nerve ablation with implanted devices

by

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Abstract

Spastic hypertonus is a sensorimotor disorder characterized by involuntary muscle over-activity. Hypertonus emerges in the days and months following upper motor neuron lesions such as spinal cord injury, stroke or multiple sclerosis and affects millions of individuals around the world. Hypertonus impedes activities of daily life including mobility and self-care and may result in muscle contracture and pain if it remains untreated or if treatment fails. Current treatments for spastic hypertonus include physiotherapeutic approaches, anti-spastic drugs, surgery and nerve ablation with neurolytic drugs such as Phenol or Botulinum toxin A. Studies have shown a limited efficacy of these treatments, and it is unclear whether they lead to improvements in function. This dissertation deals with a novel approach to the treatment of spastic hypertonus, namely the use of direct current (DC) delivered with implantable electrodes, to ablate muscle nerves in a controlled fashion. This leads to an attenuation of the neural drive to the innervated muscle. Once a desired attenuation is achieved, functional electrical stimulation may be delivered via the same electrode to activate the muscle for the purpose of functional movement.

The dissertation consists of three related studies. The first study (Chapter two) evaluates the feasibility of the suggested treatment. Proof of principle was

demonstrated in anesthetized animals by producing controlled reductions in muscle force by applying DC to muscle nerves. The second study (Chapter three) explored DC parameters (amplitudes and durations) that would be both effective and clinically acceptable. DC nerve ablation was tested in the absence of anesthesia, and possible mechanisms were explored. The third part of the dissertation (Chapter four) describes the effect of DC on nerves of chronically implanted animals in the anesthetized and conscious states. Nerve conduction abolished by DC was found to recover completely after three months, consistent with nerve regeneration. A subsequent DC application again abolished conduction, with a similar 3-month recovery. Differences between results observed in anesthetized and conscious animals are discussed. In the conclusion (Chapter 5), it is argued that controlled nerve ablation with DC may be a cost–effective method for treating spastic hypertonus.

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List of Abbreviations

Abbreviation	Definition
ADL	activities of daily life
BtA	Botulinum toxin A
CNS	central nervous system
CPN	common peroneal nerve
CRF	conventional radiofrequency
DC	direct current
ECF	extra cellular fluid
EDL	extensor digitorum longus
EPSP	excitatory post synaptic potential
FES	functional electrical stimulation
KCC2	potassium-chloride co-transporter
ITB	intrathecal Baclofen
JIH	joint interval histogram
MN	motoneuron
MS	multiple sclerosis
PIC	persistent inward current
PRF	pulsed radiofrequency
Pt-Ir	platinum iridium
ROM	range of motion
SCI	spinal cord injury
SINE	separate interface nerve electrode
TA	Tibialis anetrior
UMN	upper motor neuron

Chapter 1
Introduction

The use of nerve conduction block for the treatment of neurological disorders has been practised for many years. It was used to treat conditions such as neuropathic pain, trigeminal neuralgia, sympathetic disorders and, since the early 1900's, as a treatment for spastic hypertonus (Zafonte & Munin, 2001), a condition resulting from upper motoneuron lesions (UMNs), characterized by muscle over-activity.

Spastic hypertonus or spasticity often develops after UMN lesions resulting from spinal cord injury (SCI), stroke or multiple sclerosis (MS) and is characterized by over activity of muscles. This condition is manifested in increased resistance to muscle stretch, hyper-reflexia and in some cases tonic co-contraction of agonists and antagonists. It is most often associated with pain, contractures and abnormal posture. Spasticity has a negative impact on mobility and function, activities of daily lives (ADL) and delivery of personal care (Welmer *et al.*, 2006; Lundstrom *et al.*, 2008; Mayer, 2010).

Current treatments include conventional physiotherapy, anti-spastic medications, surgical interventions and nerve conduction blocks. Physiotherapeutic treatments have limited efficacy (Goldstein, 2001). Systemic drugs can reduce hyper-reflexia, but the evidence for their overall efficacy is surprisingly weak, and they nearly always have unwanted side-effects (Montane *et al.*, 2004; Rizzo *et al.*, 2004). Nerve blocking agents have been used for many years to reduce muscle over-activity. The most commonly used agent these days, Botulinum toxin A (BtA), is very costly (Horn *et al.*, 2010). BtA has largely replaced alcohol and phenol, which are difficult to administer and may result in unacceptable complications (Zafonte & Munin, 2001). Administration of nerve blocking agents is often suggested as an accompanying treatment to other interventions such as systemic anti-spastic drugs (Kofler *et al.*, 2009; Simon & Yelnik, 2010). Current spasticity treatments are often successful in reducing muscle tone, but there is some debate as to whether this necessarily results in improved functional outcomes (Elovic, 2001; Goldstein, 2001; van Kuijk *et al.*, 2002).

Considering the current status of spasticity treatment, there is a need for a cost-effective intervention that would address reduction of spasticity as well as improve motor function. Experiments with direct electrical current (DC) have demonstrated its ability to block nerve conduction (Kuffler & Gerard, 1947; Mendell & Wall, 1964; Fukushima *et al.*, 1975; Bhadra & Kilgore, 2004) resulting in nerve damage and diminished action potential propagation (Whitwam & Kidd, 1975; Hughes *et al.*, 1981).

Neural prostheses (NPs) have been used for the last 40 years to improve motor and sensory function by delivering electric stimulation to muscle nerves either

percutaneously via electrodes applied to the skin or via wire electrodes implanted close to or on the nerves (Prochazka *et al.*, 2001). Some examples include cochlear implants for restoration of hearing, peroneal nerve stimulators for improvement of ambulation and NPs that improve hand grasp and release such as the stimulus router system (Gan, 2009). In theory, an implantable NP with one or more electrodes placed directly on the nerve could provide the means for the precise delivery of DC to the nerve, enabling block or controlled ablation. The same electrode could also be used to deliver pulsatile functional electrical stimulation (FES) to axons not blocked or ablated by the DC treatment.

Although DC was originally deemed unsuitable for clinical application due to its damaging effect on nerve integrity and conduction (Whitwam & Kidd, 1975), It is this very effect that may enable its success as a novel means of partially ablating nerves in the treatment of spasticity.

This thesis investigates the feasibility of the use of DC, delivered via implantable electrodes, as a cost-effective means of blocking or ablating nerves in the treatment of spasticity. This introductory chapter provides a review of spasticity, its epidemiology, symptoms, underlying mechanisms and current treatments. It also includes a historical review of DC applications in the medical field and a current overview of DC nerve blockade research as well as a brief description of the considerations for the use of electrodes that could be used to deliver DC.

1.1 Spastic hypertonus

Spastic hypertonus was initially defined by Lance as “a motor disorder characterized by a velocity-dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks, resulting from hyper-excitability of the stretch reflexes, as one component of the upper motoneuron syndrome” (Lance, 1980). New evidence from spasticity research indicates this definition is no longer sufficient to describe the phenomenon. It has become apparent for example that pathways such as those involving cutaneous afferents, and not only stretch reflexes, are involved in generating spastic hypertonus. A new, broader definition was suggested by Pandyan in 2005 which describes spastic hypertonus as “disordered sensori-motor control, resulting from an upper motor neuron lesion, presenting as intermittent or sustained, involuntary activation of muscles”. (Pandyan *et al.*, 2005; SCIRE, Version 3.0b).

It is important to define spasticity in order to be able to measure and test the effects of any intervention aimed at treating this condition in a clinical setting. However, the first question that needs to be asked is: why is spasticity important?

1.1.1 The Importance of Spastic Hypertonus

In order to understand the importance of spastic hypertonus, one must look at two main factors: impact on the daily lives of the individual suffering from spasticity and the financial burden on the health care system which provides treatment for those who are affected by it.

1.1.1.1 Impact of spasticity on daily life

Spasticity becomes clinically relevant when it impacts the function and care of a patient (Brashear & Elovic, 2010). It is associated with reduced mobility and limitation of activity (Welmer *et al.*, 2006). Stroke survivors suffering from spasticity have lower scores on measures of activities of daily life (ADL) (Sommerfeld *et al.*, 2004; Welmer *et al.*, 2006; Lundstrom *et al.*, 2008). Beyond the limitations on function, a myriad of issues affect the everyday well-being of individuals with spasticity including skin irritation and breakdown (resulting from constantly clenched fists), fungal infection and resulting odor, shoulder subluxation, joint and muscle contractures, pressure on nerves such as that exerted on the median nerve by a flexed wrist, difficulty in completing personal care and hygiene and, of course, pain. Spasticity also has an impact on the individual's body image causing discomfort and embarrassment due to abnormal posture (Mayer, 2010). In a database of self-reported secondary complications following SCI, spasticity was found to be the most commonly reported complication (53%) preceding pain (44%) and pressure ulcers (38%) (Walter *et al.*, 2002).

1.1.1.2 Impact of spasticity on health care costs

In 2008 the overall cost of the treatment of stroke in the United States (US) was $34.3 billion (Roger *et al.*, 2012). This report states that stroke results in disability more often than mortality (19.4% death rate) and is reported as the 4th most common cause of death but as the leading cause of long term disability in the US (Roger *et al.*, 2012).This often means longer hospital stays, rehabilitation treatment and placement in long term continuing care facilities following discharge, all of which are considered a major expense to the health care system (Rothwell, 2001; Roger *et al.*, 2012). Compensation for post-acute stroke care in the US is one of the rapidly growing expenses in Medicare (Centers for Disease Control, 2003).

1.1.2 Epidemiology of spastic hypertonus

1.1.2.1 Stroke population

There are over 7 million stroke survivors living in the US today. Each year approximately 795,000 individuals will suffer a stroke, 610,000 of these incidents will be a first time event (Roger *et al.*, 2012). Six months following a stroke event, 50% of survivors will remain with some hemiparesis, 30% will be unable to walk independently

and 26% will require assistance in ADL and the same percentage will require admission into long term care facilities (Roger *et al.*, 2012). Spasticity develops in 18% to 38% of stroke survivors (Watkins *et al.*, 2002; Sommerfeld *et al.*, 2004; Welmer *et al.*, 2006; Lundstrom *et al.*, 2008; Kong *et al.*, 2012). The number of affected individuals ranges from 1,260,000 to 2,660,000 in the US alone.

1.1.2.2 SCI population

270,000 individuals are estimated to be living with the after-effects of SCI in the US today. Incidence is approximately 12,000 new cases each year (NSCISC, 2012) and numbers are on the rise (SCIRE, Version 3.0a). Sixty seven percent of SCI survivors developed spasticity before being discharged from hospital. This percentage increases to 78% by the first annual checkup. The prevalence of spasticity increases with the level of injury (Maynard *et al.*, 1990).

1.1.3 Pathophysiology of spastic hypertonus

Injury to the CNS has immediate detrimental consequences to independent function. But many changes that occur in the CNS, muscle and soft tissue following such an insult take time to evolve and present as further complications days and even months after the initial injury. Spasticity is one such change.

Changes that develop after CNS injury are classified into two categories: negative signs and positive signs. Negative signs include flaccid weakness and loss of dexterity due to lack of cortical drive. These usually appear immediately after injury. Positive signs that develop over weeks and months are attributed to a gradual withdrawal of cortical inhibition and other changes occurring in the CNS, as well as changes in the properties of muscle and soft tissue (Mayer, 2010). Positive signs include: co-contraction, clonus, spasms and what was defined by Lance as spasticity – increased tonic stretch reflexes with exaggerated tendon jerks (Lance, 1980).

There is no definition of spasticity that is universally accepted. It is often used to describe several of the positive signs mentioned above. Lance's definition of spasticity is still used by many, though it is considered narrow and incomplete for clinical purposes. In the context of this work, the pathophysiology of positive signs presented as muscle over-activity will be briefly reviewed and are shown in Figure 1.1.

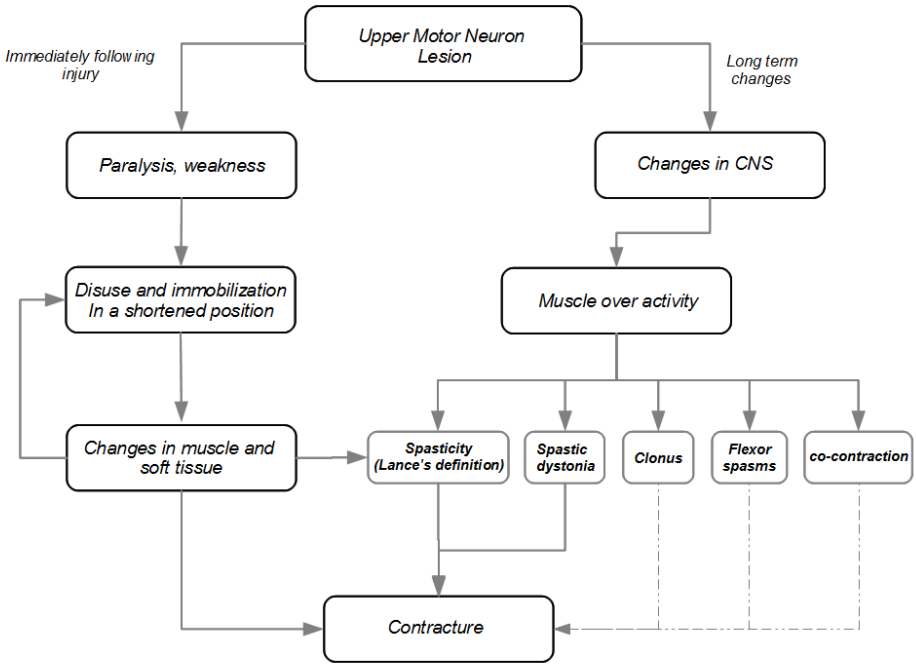

Figure 1.1: An upper motor neuron lesion results in a sequence of events that often lead to impaired function. Weakness appears immediately following the injury but long term changes in the CNS and soft tissue contribute to the eventual development of muscle over-activity, which can lead to contractures, further impeding function and often cause pain. Based on Gracies et al. 1997a.

1.1.3.1 Muscle over-activity in spastic hypertonus

Response to passive muscle stretch

An abnormal resistance to imposed muscle stretch is Lance's definition of spasticity. Lance attributed the increased resistance to an abnormal increase in the tonic stretch reflex. In healthy people, imposed stretch of a relaxed muscle results in very little or no EMG activity. In spastic individuals, imposed stretch elicits involuntary EMG activity that increases with stretch velocity (Lance, 1980; Sheean, 2002; Mayer, 2010). In the 1950s, it was posited that in spasticity, muscle spindles were overly sensitive due to elevated fusimotor drive and this caused exaggerated stretch reflexes. However, this was not supported by later human neurography recordings (Hagbarth *et al.*, 1973; Wilson *et al.*, 1999). A more likely explanation is that motoneurons have a reduced threshold to afferent input (Powers, Campbell et al. 1989) and this may be the result of persistent inward currents that develop after the loss of corticospinal synaptic inputs (Bennett *et al.*, 2004)

Spastic dystonia

The flexed posture of the upper extremity in stroke survivors is a hallmark of spastic dystonia. A similar phenomenon was studied in monkeys with brain lesions which resulted in a flexed posture without any voluntary activity or muscle stretch(Denny-Brown, 1980). The posture was maintained after transection of the dorsal roots which indicated the drive was of efferent origin (Denny-Brown, 1980). Spastic dystonia is believed to be a result of tonic drive to low threshold α-motorneurons from supraspinal pathways. The outcome is sustained firing resulting in the inability of the muscle to relax (Sheean, 2002; Gracies, 2005b)

Spastic co-contractions

Co-contraction is the simultaneous activation of the agonists and antagonists muscles observed during volitional movements. Muscle co-activation occurs during voluntary movements in healthy individuals, for example, when stabilization of a joint is required, but in individuals with UMN lesion the occurrence is uncontrolled and often excessive (Gracies, 2005b; Mayer, 2010). Abnormal co-activation of flexors and extensors was found to be highly correlated with the response to imposed muscle stretch (Dewald *et al.*, 1995). It is suspected that spastic co-contraction occurs due to inappropriate descending drive combined with impaired reciprocal inhibition which may be further augmented by stretch reflex activity (Sheean, 2002; Mayer, 2010; Kheder & Nair, 2012).

Clonus

Clonus is an involuntary, rhythmic contraction during or after a muscle stretch and is often seen in a distal joint such as the ankle, however, it can also be triggered by cutaneous stimuli such as cold or pain (Mayer, 2010). The occurrence of clonus is attributed to the oscillation of the hyper-excitable phasic stretch reflex (Sheean, 2002; Adams & Hicks, 2005). Reduced motoneuron (MN) thresholds and long pathway delays have also been implicated (Hidler & Rymer, 1999).

Flexor spasms

These are involuntary movements that may be caused by a disinhibited flexor withdrawal reflex, which is a polysynaptic, mediated response to painful stimuli causing activation of flexor muscles and inhibition of extensors. (Sheean, 2002; Adams & Hicks, 2005; Kheder & Nair, 2012).

1.1.3.2 Possible mechanisms underlying spasticity

The time taken for exaggerated reflex responses to develop after a stroke or SCI, which can be up to six weeks, indicates that they are not solely caused by the lack of cortical inhibition. Changes in the CNS following injury, discussed below, are believed to contribute to the emergence of an imbalance in inhibition and excitation (Brown, 1994; Ward, 2012).

1.1.3.2.1 Neuronal mechanisms

Axonal sprouting

Following an UMN lesion, injured descending axons innervating spinal MNs degenerate. Intact axons sprout and form synapses on the spinal MNs in the spaces left by the damaged axons. Sprouting can occur from residual descending corticospinal and brainstem fibers, interneurons and Ia afferent fibers. Reorganization of axons might contribute to motor recovery but is probably also responsible for the imbalance of excitatory and inhibitory inputs to the MN that results in spasticity (Brown, 1994; Gracies, 2005b, a; Elbasiouny *et al.*, 2010).

Afferent inputs

In the intact nervous system, inputs to spinal MNs from descending pathways and afferent pathways modulate MN activity. Figure 1.2 shows some of the neural connections that are implicated in altered MN excitability in spasticity.

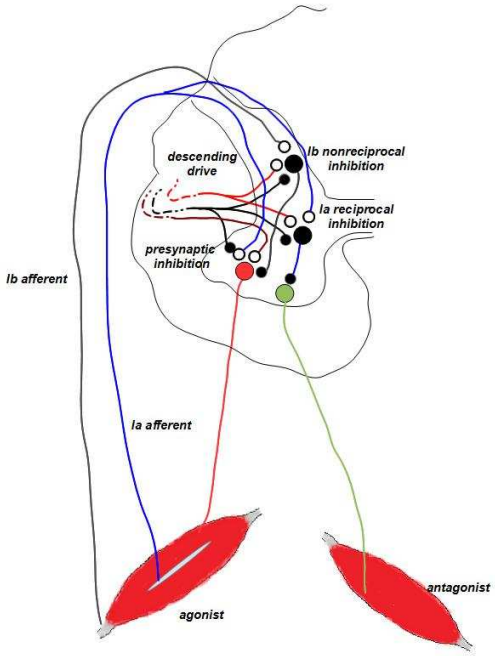

Figure 1.2: spinal pathways involved in the development of spastic hypertonus. Excitatory synapses are represented by white circles; inhibitory synapses are represented by black small circles. Inhibitory interneurons are represented by black large circles and α –motoneurons are represented by colored circles. Based on Kandel and Schwartz et al. 2000.

One of the mechanisms that has been implicated in spasticity is presynaptic inhibition. Axons descending from the brain and terminating on Ia afferent synapses has been shown to reduce Ia-evoked responses in α–MNs. Loss of this descending inhibitory control may result in increased Ia input, which in turn can bring MNs closer to firing threshold. Presynaptic inhibition was shown to be reduced in some individuals with spasticity but not all (Nielsen *et al.*, 2005). Another mechanism that was suggested to play a role in facilitating MN excitation was post-activation depression, the reduction of the amount of neurotransmitter release at Ia synapses with MNs due to repetitive activation (Elbasiouny *et al.*, 2010). Post-activation depression was shown to be consistently reduced in spastic individuals (Nielsen *et al.*, 2005).

Other inhibitory pathways implicated in spasticity are: reciprocal Ia inhibition and nonreciprocal Ib inhibition. When Ia afferents of a muscle are activated by muscle stretch, they activate Ia interneurons that inhibit MNs of the antagonist muscle, which prevents it from contracting at the same time. A reduction in reciprocal inhibition can result in undesirable co-contraction (Gracies, 2005b). A reduction in reciprocal inhibition was shown to be present in ankle plantarflexor MNs of individuals with spasticity resulting from MS, SCI and stroke (Nielsen *et al.*, 2007). Non-reciprocal Ib inhibition results from changes is muscle tension, signaled by Golgi tendon organs, which inhibit homonymous MNs via di- and tri-synaptic connections (Kandel *et al.*, 2000). This inhibition was not detected during ankle plantarflexion in spastic individuals, instead facilitation of soleus was observed (Gracies, 2005b; Nielsen *et al.*, 2007). This impaired mechanism could further exacerbate muscle co-contraction (Gracies, 2005b; Nielsen *et al.*, 2007).

Changes in α-Motoneuron excitability

Denervation hypersensitivity of α-MNs is seen after UMN lesions. It has been attributed to persistent inward currents (PICs). PICs are voltage sensitive currents that inactivate slowly and are under serotonin and norepinephrine control. Experiments carried out in spinal rats showed that PICs changed after SCI, initially exhibiting reduced amplitudes, but over time they became elevated, producing long-lasting plateau potentials. PICs had the effect of amplifying excitatory post-synaptic potentials (EPSPs) elicited by action potentials in sensory afferents responding to muscle stretch. This change in α-MN excitation was proposed as one of the mechanisms leading to spasms, hyper-reflexia and clonus (Bennett *et al.*, 2004; Heckman *et al.*, 2008).

A recent study showed that potassium-chloride co-transporter KCC2 is down-regulated in MN membranes below a spinal cord lesion in individuals with SCI but not in people with supraspinal lesions (Boulenguez *et al.*, 2010). KCC2 maintains a low intracellular chloride (Cl^-) by transporting Cl^- ions outside the cell. Once KCC2 is down-regulated, Cl^- can accumulate inside the cell, leading to membrane depolarization (De Koninck, 2007).

1.1.3.2.2 Non-neuronal mechanisms

After UMN injury paresis occurs, load-bearing muscles tend to stay immobile at a short length. Immobilization and disuse lead to muscle atrophy and loss of sarcomeres, as well as increases in connective tissue and fat content, all of which eventually results in contractures. If disuse and immobilization persists, reduction in ROM and muscle compliance may follow. It is suggested that changes to muscle properties may be one of the underlying mechanisms of spasticity (Gracies, 2005a; Dietz & Sinkjaer, 2007).

1.1.4 Current treatments for spastic hypertonus

There are several approaches to the treatment of spasticity including physiotherapy treatments such as stretching and vibration, anti-spastic medication, chemical neurolysis and surgical interventions.

1.1.4.1 Physical and occupational therapy

This approach includes treatments such as stretching, casting, strength and ROM exercises, vibration and cooling. Muscle stretch and ROM exercises require considerable time commitment and often have limited efficacy and duration (Bovend'Eerdt *et al.*, 2008). Muscle stiffness and spastic reflexes may be reduced for no more than a few hours after such treatment (Hinderer & Dixon, 2001). Spasticity and pain can be exacerbated by stretch and in extreme cases, muscle tears have been reported (Bovend'Eerdt *et al.*, 2008). A recent study found that whole body vibration was ineffective (Brogardh *et al.*, 2012) though another study suggested that repetitive focal muscle vibration might be beneficial (Caliandro *et al.*, 2012). Overnight splinting of the upper extremity may preserve wrist and finger extension but was not shown to contribute to improvement in ROM or reduction in pain (Lannin *et al.*, 2003). Local muscle cooling was reported to alleviate spasticity and this could last up to 20 minutes after cessation of treatment, thus this method may be useful as a rehabilitation treatment to train antagonist muscles (Gracies, 2001). Regardless of the limited efficacy of the treatments described above, rehabilitative approaches such as ROM exercises assist in optimization of results achieved by other interventions and as such are important in the overall treatment of spasticity (Goldstein, 2001).

1.1.4.2 Systemic anti-spastic drugs

Systemic anti-spastic medications targeting some of the central nervous mechanisms described above are widely used to alleviate and treat spasticity. Four drugs are approved for the treatment of spasticity: Baclofen, Diazepam, Tizanidine and Dantrolene.

Baclofen

Baclofen acts on pre- and post-synaptic terminals and inhibits mono- and poly-synaptic pathways. It has been shown to induce hyperpolarization of the presynaptic membrane in interneurons, resulting in reduced calcium influx, which, in turn, causes a reduction of neurotransmitter release (Davidoff, 1985). Post-synaptically, Baclofen in high concentrations hyperpolarizes the Ia afferent terminal, resulting in increased presynaptic inhibition. Oral administration of Baclofen was shown to be most effective in reducing spams in individuals with SCI and MS and less effective in treating post-stroke spasticity (Kita & Goodkin, 2000). Side effects of orally administered Baclofen include weakness and sedation, nausea, hallucination and reduced seizure threshold (Elovic, 2001) and occasionally confusion, hypotonia, ataxia and reduction in motor function (Gracies *et al.*, 1997b).

When oral Baclofen is ineffective, intrathecal delivery may be an option. A pump is implanted in the abdominal wall with a catheter inserted into the subarachnoid space, delivering the drug directly onto the spinal cord, allowing a higher concentration of Baclofen at lower dosages to be delivered locally, minimizing systemic effects (Gracies *et al.*, 1997b; Kita & Goodkin, 2000). Intrathecal Baclofen (ITB) delivery was successful in reducing muscle tone (Meythaler *et al.*, 2001; Schiess *et al.*, 2011) and improving strength as well as some function in individuals with post stroke spasticity (Schiess *et al.*, 2011), but it may also impede ambulation and is not effective for treatment of the upper extremities (Kofler *et al.*, 2009). Several complications can arise from ITB including infection and mechanical pump failure such as catheter breakdown (Awaad *et al.*, 2012). The ITB procedure is expensive and therefore recommended for individuals with severe limitations who do not respond to alternative treatments (Kita & Goodkin, 2000).

Diazepam

Diazepam, which increases presynaptic inhibition, has proved to be effective in treating spasticity both in SCI and post stroke populations (Kita & Goodkin, 2000). Side effects include addiction, intoxication, reduced motor co-ordination and, specifically, sedation and cognitive depression (Gracies *et al.*, 1997b), which may prove to be detrimental in stroke survivors (Elovic, 2001). These side effects have relegated the use of diazepam to an adjunct treatment (Gelber & Jozefczyk, 1999).

Tizanidine

Tizanidine is an α2-adrenergic agonists, which blocks excitatory neurotransmitter release from spinal interneurons (Elovic, 2001). It was shown to be effective in spasticity control, reducing tone and clonus as well as pain (Gelber *et al.*, 2001). Side effects may include sedation, dry mouth, potential for liver damage, and weakness. Tizanidine is recommended for patients where weakness is more of a concern than the effects of sedation (Gracies *et al.*, 1997b).

Dantrolene

Dantrolene blocks excitation-contraction coupling in muscle by the inhibition of calcium release from the sarcoplasmic reticulum via the ryanodine receptor (Lapeyre *et al.*, 2010). Dantrolene has been shown to improve muscle tone and ROM (Gracies *et al.*, 1997b). Side effects may include weakness, nausea and liver toxicity (Gracies *et al.*, 1997b; Elovic, 2001). It is often recommended for individuals with supraspinal lesions such as stroke and individuals with acute dystonia where weakening muscles can improve postural control without the side effect of sedation (Gelber & Jozefczyk, 1999).

General comments

Though systemic anti-spastic medications may help control spasticity to some extent, evidence of their overall efficacy is weak (Montane *et al.*, 2004; Rizzo *et al.*, 2004). Furthermore, quite often improvement in function was not noted and in some cases treatment impeded ADL and sensorimotor function (Gracies *et al.*, 1997b; Elovic, 2001; Esquenazi, 2006).

1.1.4.3 Surgical intervention

Following UMN lesions, muscle and soft tissue changes may occur, eventually resulting in contractures, (Dietz & Sinkjaer, 2007; Ward, 2012) that may lead to pain, reduced function and poor hygiene. The main goal of surgical interventions is to treat the outcome of such changes with the aim of pain relief and function restoration. Surgical interventions such as tendon lengthening, tendon transfer (Woo, 2001) and partial neurotomy (Bollens *et al.*, 2011) may be performed for this purpose. Superficialis to Profundus tendon transfer surgery in the hemiplegic hand resulted in improved hygiene and pain reduction at 6 weeks post-surgery (Keenan *et al.*, 1987) as well as at 19 months post-surgery (Heijnen *et al.*, 2008). Tibial neurotomy, although controversial, is used to treat spastic equinovarus foot (Bollens *et al.*, 2011). Surgical procedures are recommended only after conventional treatments, such as the use of orthotics, have failed (Heijnen *et al.*, 2008) and many surgeries result in poor outcomes due to common mistakes such as creating erroneous insertion points in tendon transfers (Woo, 2001).

1.1.4.4 Nerve conduction blocks

Nerve blocks are defined as the application of a chemical agent to a nerve for the purpose of impairing conduction along the nerve (Gracies *et al.*, 1997a). The primary goal of such treatments is the reduction of muscle over-activity in selective muscles without systemic effects (Frangiamore & O`Brien, 2004). There are three treatments that fall into this category: injections of alcohol, phenol and BtA.

Alcohol

Ethyl alcohol was used to treat spasticity since the early 1900's (Zafonte & Munin, 2001). It works by non-selectively denaturizing proteins when administered at concentrations above 10%, resulting in neuronal degeneration and fibrosis (Gracies *et al.*, 1997a). Adverse side effects include pain at the site of injection and dysesthesias (unpleasant abnormal sensations) (Horn *et al.*, 2010). Dysesthesias tend to be short-lasting and treatable with medications.

Phenol

Similar to alcohol, phenol (also known as carbolic acid) acts by denaturizing proteins in a non-selective manner when administered in concentrations above 3%. The resulting effect is axonal demyelination and muscle denervation which can last on average up to 6 months (Zafonte & Munin, 2001). The main side effects are pain during injection and dysesthesias that may subside with re-injection. The most dangerous complication of phenol is unintentional vascular injection (Frangiamore & O`Brien, 2004). Due to the non-selectivity of phenol, injection into a blood vessel may lead to thrombosis. Phenol is fast-acting and results can be observed within 2-60 minutes.

A disadvantage common to treatment with both phenol and alcohol is the technical difficulty of the injection procedure. Damage can result from exploration with the injecting needle (Frangiamore & O`Brien, 2004; Horn *et al.*, 2010) and the dosage required for a desirable outcome is difficult to predict (Zafonte & Munin, 2001). Furthermore, success of both the injection procedure and the results are highly dependent on the physician's expertise (Elovic *et al.*, 2009).

Botulinum toxin A (BtA)

BtA causes neuromuscular block by disrupting neurotransmitter release from the presynaptic terminals of motor nerves. It is injected into the target muscles where it is taken up by endocytosis at the neuromuscular junction and selectively blocks acetylcholine release (Dolly & Aoki, 2006). The onset of a BtA block is 24-73 hours from injection but the peak effect is not observed until 2-6 weeks later. The duration of the effect depends on factors such as dose, muscle size and injection technique (McGuire, 2001). Side effects of BtA injection include transient skin rash, pain at the injection site, flu-like symptoms and muscle weakness which can impede function especially when affecting the finger flexors (van Kuijk *et al.*, 2002). Further complications may arise from neurophysiological changes taking place after intramuscular injection. Animal experiments demonstrated that BtA can reach the spinal ventral roots and migrate to the contralateral spinal cord, altering central synaptic transmission (Gracies, 2004). Furthermore, it leads to reduced muscle mass and weakness on both the injected side and contralateral side (Fortuna *et al.*, 2011). Although rare, some individuals may develop immuno-resistance to BtA resulting in non-responsiveness to the treatment (Brashear *et al.*, 2002; Elovic *et al.*, 2008; Elovic *et al.*, 2009).

BtA was shown to be effective in treating spasticity (Esquenazi, 2006) both in upper extremity (Brashear *et al.*, 2002; Elovic *et al.*, 2008) and lower extremity (Kirazli *et al.*, 1998). It was effective in reducing tone and improving ROM, but no clear evidence was

demonstrated regarding its effect on functional outcome or pain reduction (van Kuijk *et al.*, 2002; Elia *et al.*, 2009; Esquenazi *et al.*, 2009).

When compared with phenol and alcohol injection, BtA administration is considered easier (Elovic *et al.*, 2009) but the use of electrical stimulation or ultrasound to ascertain needle placement is still recommended (Esquenazi, 2006). In general BtA injections are better tolerated than phenol, but phenol can be administered more often and is not dose-limited like BtA (Elovic *et al.*, 2009). Treatment outcomes demonstrated better results for BtA in weeks 2 and 4 post-injection but no difference between a group treated with BtA and a group treated with phenol were observed in weeks 8 and 12 (Kirazli *et al.*, 1998).

BtA treatment is extremely costly and must be repeated every time its effects wear off. It was suggested to be more cost-effective than oral medication (Ward *et al.*, 2005) but was estimated to cost twice as much as phenol administration, even after treatment of the adverse effects of phenol were taken into consideration (Horn *et al.*, 2010).

Table 1.1 lists the advantages and disadvantages of each treatment.

Treatment	Pros	Cons
Physiotherapy	May preserve muscle length Helps optimize results of other treatments.	Limited duration Time commitment Not very effective
Orally administered Anti-spastic drugs	Alleviates pain	Systemic effects such as weakness, sedation and dizziness, habituation. Can impede functional improvement
Intrathecal Baclofen	No systemic effect May help improve function	Infections, mechanical failure Expensive Can impede walking
Surgical intervention	Alleviates pain Can release contractures Improved hygiene in the hand	Complications due to surgery Poor functional outcome

Treatment	Pros	Cons
Phenol and Alcohol	Fast acting Affect sensory fibers that may reduce reflexes Low cost Can be repeated often	Pain during injection Dysesthesias Vascular damage Outcome depends on physician's expertise
Botulinum Toxin	Reversible effect Better patience acceptance than phenol and alcohol Easier to administer than phenol and alcohol	Costly Delayed effect Cannot be repeated often Effects non-target muscles Immuno-resistance

Table 1.1: Pros and Cons of common treatments for spastic hypertonus.

The many treatments offered for spasticity today emphasize the importance of defining spasticity and understanding the underlying causes of a patient's condition before prescribing any one treatment.

1.2 Historical review of the use of direct current (DC) in medicine

The following review is based in part on accounts in a book entitled *Electricity and Medicine: History of Their Interaction* (Rowbottom 1984).

Since the earliest documented account of electrical stimulation in the year 47 AD, electricity was harnessed for the service of medicine (Stillings, 1975). The first report was made by Scribonius Largus, a physician to the Roman emperor Claudius. The account was of a man being stung by an electric ray, resulting in alleviation of gout pains. Largus suggested that electric rays may be used to treat headaches (Stillings, 1975). The ancient Greeks were aware of the ability of amber to attract various substances when rubbed, but no real breakthrough in understanding electricity and its biological effects was made until the seventeen century (Geddes, 1984).

1.2.1 Electricity in medicine prior to Galvani's discoveries

Gilbert, a physician of Queen Elizabeth I, devised the first electroscope to detect weak electrostatic attraction (Rowbottom 1984). He placed a light metal needle on a pin, rubbed numerous substances and watched for the needle to turn. Substances such as crystal, opal, sealing wax possessed attractive powers, but substances such as metal did not (Rowbottom 1984). In 1600 Gilbert published his work and named the latter, *electricks*, the Greek name for amber, unknowingly naming a new science (Skilling, 1948). The use of the English word electricity began fifty years later (Skilling, 1948).

In late 1745 the first capacitor was invented and was known as the Leyden jar (Geddes, 1984). It was composed of an electrified conductor (nail or tin tube) placed in a container filled with water. When the container was held with one hand and the conductor was touched with the other, the person who held the jar felt an electric shock. The jar was simultaneously and independently invented by Kleist in Germany and by Musschenbroek in Holland (Heilbron, 1979).

A surgeon from Paris, Abbé Nollet, claimed that he along with other two surgeons were the first to use the Leyden Jar for therapeutic purposes on paralyzed limbs but were unsuccessful in their trials (Rowbottom 1984). Another Parisian surgeon, Antoine Louis, attempted to use the Leyden jar on three paralyzed patients but failed to restore any functional movement (Hoff, 1936; Rowbottom 1984). The first report of successful use of electricity to stimulate muscles using the Layden jar was in 1747 by Jean Jallabert, a professor of mathematics and philosophy at Geneva, who worked with the leading surgeon of Geneva, Guyot (Rowbottom 1984).

Jallabert and Guyot documented a set of experiments performed on a locksmith who suffered a head injury 14 years prior (Rowbottom 1984). After conventional treatment the locksmith remained with a limp and loss of sensation and movement of the right arm, except for vertical motion. Both wrist and fingers were in a constant flexed position and could not be moved. The arm was emaciated and fingers swollen. Jallabert found a way to stimulate groups of muscles rather than to simply shock the patient. After three months of treatment, complete use of the arm was regained and sensation was restored. Furthermore, Jallabert combined electrotherapy and physiotherapy by rubbing the patient's arm, which was placed on a warm stove, before and after treatment (Rowbottom 1984).

Following Jallabert's successful account, numerous attempts by several individuals, not necessarily physicians, to treat various medical conditions with electricity ensued, resulting in varying degrees of success (Rowbottom 1984). The literature describing the use of electricity in treating medical conditions was mounting (Hoff, 1936). Between 1750 and 1780, twenty six articles or book reviews on medical electricity were published in the Journal de Médicine alone (Hoff, 1936). Research on the use of electricity also continued in the field of physics (Baigrie, 2007). In the 1750's Franklin explained the concept of charge and in 1786 Coulomb published the law of electrical attraction. Ironically, the next breakthrough in the science of electricity came from the field of anatomy, not physics. In 1791, Galvani published his discoveries of 'animal electricity'. A publication that lead to Volta's invention of the battery, which dramatically changed the field of electricity (Baigrie, 2007).

1.2.2 Galvani, Volta and the invention of the battery

Luigi Galvani, a professor of obstetrics in Bologna, practised medicine and performed surgeries while doing extensive research in the field of anatomy (Skilling, 1948; Rowbottom 1984). During Galvani's studies in Bologna, several researchers experimented with electrical stimulation of nerves and muscles using electric rays and electric eels (Rowbottom 1984). During the 1780s, while making his famous discoveries Galvani was probably aware of those experiments as well as the controversy of whether electricity was the basis of muscle contraction (Hoff, 1936).

Galvani's initial discovery was accidental. He conducted experiments on frogs' muscle nerve preparation where the thighs were removed and the legs and feet were connected to a stump of the spine by the sciatic nerve (Hoff, 1936). The preparation was placed on the same table as an electrical machine used for other purpose. When an assistant touched the nerve with a scalpel, muscle contractions were observed (Skilling, 1948; Rowbottom 1984). It was discovered that muscle contraction occurred only when the machine was discharged and the assistant was touching the blade or the metallic rivets of the handle. Galvani substituted the scalpel for wire conductors placed close to the electrical machine achieving the same result (Rowbottom 1984).

In another set of experiments, based on Franklins experience with lightning and storms clouds, Galvani was able to cause frogs muscles to contract using atmospheric electricity (Rowbottom 1984). To his surprise he noticed infrequent contraction during days of good weather (Geddes & Hoff, 1971). In order to test this phenomenon the frog preparations were hung on an iron railing by a brass hook inserted into the spine and Galvani was waiting for muscles to contract. When he pushed the preparations into the iron railing, contractions occurred frequently (Rowbottom 1984).

Galvani then took the preparation into his laboratory and placed the frog on a metal surface, when the hook touched the surface muscle contraction occurred (Skilling, 1948). He went on to try the same experiments with different metals and several other non-conducting materials and found out that contractions were achieved only when metals were used (Geddes & Hoff, 1971). This led Galvani to believe that electricity was inherent in the animal itself. In 1791 Galvani published the work in *Commentary on the Forces of Electricity in Muscular Motion* (Rowbottom 1984). Alessandro Volta, a professor of physics in Pavia, read Galvani's manuscript and after conducting a set of experiments concluded differently (Skilling, 1948). Volta reasoned that the contractions were not due to animal electricity, as Galvani claimed, but due to the contact of dissimilar metals. He was able to show that electricity was present when two different metals were brought into contact (Skilling, 1948). Volta theory was widely accepted (Brazier, 1957) and animal electricity theory was pushed aside for the next 30 years (Rowbottom 1984).

Galvani made a dramatic impact on the world of electricity and in the mid-1790s the term *galvanism* was coined in honor Galvani's discoveries (Rowbottom 1984).The Galvani-Volta controversy eventually resulted in the invention of the voltaic pile in 1800 – what is now known as the first battery.

1.2.3 Direct current in the service of neurophysiology

The increased use of DC in the 19th century corresponded to the rise of electrophysiology. In the 1830s and 40s Carlo Matteuci, an Italian physicist and neurophysiologist, was the first to observe what is now recognized as the action potential (Moruzzi, 1996). He was also the first to demonstrate electrode polarization. In the 1940s Du Bois-Reymond recreated Matteuci's experiments in muscle physiology and continued to explore the electrophysiology of nerves (Rowbottom 1984). Many findings in this field are attributed to Du Bois-Reymond, including the discovery of action potentials in peripheral and central nerves and the capability of nerves to produce current in the brain (Brazier, 1957).

To further study the effect of DC on nerves, Du Bois-Reymond placed a section of a nerve between two platinum wedges connected to a galvanometer, applied DC and followed the deflection of the needle indicating the strength of the electrical current (Rowbottom 1984). He discovered that when the current flowed in one direction the deflection was increased while when the current flowed in the opposite direction the deflection decreased (Rowbottom 1984). This phenomenon was initially noticed almost 40 years earlier by Johann Wilhelm Ritter, a German chemist, but his discovery went unnoticed at that time (Geddes, 1984). Ritter passed electrical current from one hand to the other and discovered that the hand connected to the negative electrode became more stiff (Geddes, 1984). By the time Du Bois-Reymond discovered the effect of DC, Faraday published his work introducing terminology such as electrode, cathode and ion and Du Bios-Reymond used Faraday's terminology to name the phenomenon electrotonus (Rowbottom 1984).

In 1859, Pflüger, a student of Du Bois-Reymond further explored the electrotonus phenomenon with DC, and used a myograph to measure contraction (Rowbottom 1984). He demonstrated that the excitably increased under the cathode and declined under the anode, when the circuit was closed (Geddes, 1984). He further demonstrated that increased excitability under the cathode, upon closing of the circuit, constituted a more effective stimulus than the increase in excitability under the anode at the break of the circuit. Lastly, Pflüger was also successful in achieving anodal block: with strong current no contractions were observed (Rowbottom 1984).

Direct current was instrumental in discoveries made in the field of neurophysiology and the field of electrophysiology was on the rise during the 19th century. But in the field of electrotherapy a consensus was not reached regarding its beneficial contribution.

1.2.4 The use of direct current in electrotherapy

Early experiments with direct current from the voltaic pile were performed by Volta on himself (Rowbottom 1984). He discovered that muscles could be contracted and senses stimulated. He placed the two ends of the pile on his tongue to stimulate taste and excited the optic nerve by touching one end of the pile to the eyeball and the other to various areas of the face resulting in a perception of a flash. He noted that connection to the body is improved when the area is initially moistened. Volta mentioned that

shock could be felt only when the circuit was closed or broken. If contact was maintained after the circuit was closed, pain would ensue several minutes later. He attempted to stimulate the auditory nerve by placing the end of the pile to his ears, but found that the shock was too uncomfortable and resulted in noise sounds (Rowbottom 1984).

In the late 18th century and early 19th century the use of galvanism with two different metals and later with the voltaic pile was becoming common practice (Rowbottom 1984). In the early 1800s galvanism was reported successful in some cases of paralysis and in treatment of pain. Contact breakers were invented and used in the treatment of deafness (Rowbottom 1984). Resuscitation of individuals who drowned or asphyxiated and later during surgeries was explored (Geddes, 1984).

Two extensive accounts of the uses of DC were published. In 1804, Charles H. Wilkinson, an English surgeon and a pioneer in the use of galvanism in medicine, published *Elements of Galvanism in Theory and Practice,* where he devotes several sections to the application of galvanism in medicine, including a description of the equipment he used and developed (Thornton, 2006). In 1826 Michael La Beaume, a London surgeon published his work and recommendations on the use of galvanism (Geddes, 1984). This body of work was translated to French and influenced the development of electropuncture, the delivery of direct current via acupuncture needles (Rowbottom 1984). Chinese acupuncture was introduced in France at that time and the use of electropuncture to alleviate pain was published by Sarlandière, a French anatomist and physiologist, in 1825 (Stillings, 1975) and accepted as a treatment method. This method was later adopted by Magendie who used needles to deliver both direct and induced current in his experiments (Rowbottom 1984).

In the 1830s, Guillaume Benjamin Armand Duchenne, a Parisian medical doctor, conducted his first experiment with electrical stimulation on patient who suffered neuralgia (Rowbottom 1984). Familiar with Magendie's work on nerve stimulation with needles, Duchanne started his scientific exploration with electropuncture and later, introduced the methods of single muscle simulation using electrodes place on the skin. He opposed the use of direct current in muscle and nerve stimulation but suggested that it would be useful for cauterization during surgery and as a method to warm acupuncture point (Rowbottom 1984).

A contemporary of Duchenne, Robert Remak, a physician in Prussia, preferred the use of direct current and applied it on such cases as paralysis, contractures, rheumatism and arthritis (Rowbottom 1984). He discovered that better outcomes were achieved in cases of rheumatic conditions where inflammation was involved and concluded that galvanic current had a catalytic action. This would prove to be true in 1900 when Stéphane Leduc, a French Physician, demonstrated that Strychnine ions could penetrate the skin by means of galvanic stimulation (Rowbottom 1984).

In the mid 1840's the use of DC as a diagnostic tool commenced (Rowbottom 1984). The first to use galvanic current in this manner was Marshal Hall, an English physician and physiologist who used it to settle an argument of the time on whether paralyzed muscle could be stimulated. In the late 1860s, Wilhelm Heinrich Erb, a German physician, investigated the "reaction of degeneration" by means of direct and faradic

currents and demonstrated that the phenomenon occurred only when lesions were in the spinal cord and peripheral nerves (Rowbottom 1984).

One of the most famous experiments conducted with DC was carried out in 1870 by Hitzig and Fritsch, German neurologists, who used it to demonstrate the localization function of the brain (Hagner, 2012). Hitzig specialized in galvanic therapy, used it to alleviate pain and developed his own electrodes.

In the 1930s electroshock was introduced as a treatment for depression by Ugo Cerletti, an Italian neurologist (Rowbottom 1984). This was initially demonstrated by Aldini, Glavani's nephew, who explored the use of direct current stimulation on the brain and reported that it improved melancholy.

The most common uses of galvanic therapy since its invention in the mid-19th century has been to alleviate pain, where DC was being passed through the body with a very low current (Geddes, 1984). An electric bath has been used, ostensibly for the "removal of poison and metallic ions from the body" by immersing the individual in water and passing current through the bath water. Further uses of galvanism included resuscitation and cautery during surgery and the use of elecropuncture for the removal of warts and moles (Geddes, 1984).

Today direct current is most commonly used for wound and bone fracture healing (Friedenberg *et al.*, 1971; Kloth & McCulloch, 1996; Kuzyk & Schemitsch, 2009; Victoria *et al.*, 2009) and galvanic vestibular stimulation is used as a research tool for whole body balance (Fitzpatrick & Day, 2004; Carmona *et al.*, 2011; Curthoys & Macdougall, 2012). In the 1960's there was an interest in brain stimulation with DC and in the last 5 years this has been rekindled with the introduction of transcranial DC stimulation (tDCS). It is being evaluated as a treatment for different neurological conditions and is used as a research technique into mechanisms of excitability in the brain (Schulz *et al.*, 2012)

1.2.5 The use of direct current to block nerve conduction

As mentioned earlier it was Pflüger who demonstrated in 1859 that nerve block can be achieved using DC stimulation. By the early 20th century several methods of nerve block were known, such as crush, local anesthetics (e.g., ether vapors) and hypoxia (Grerard, 1931). It was recognized that the block could be reversible once the blocking agent is removed (Grerard, 1931) and that nerve integrity must be maintained in order for impulse propagation to occur (Adrian, 1914).

In the 1930s onwards the technique of large nerve fiber block using DC was used to study small nerve fibers (Kuffler & Gerard, 1947; Manfredi, 1970). In the 1960s research conducted on C fibers with this method contributed to the development of the gate theory of pain (Mendell & Wall, 1964). In 1975 Whitwam and Kidd studied the effect of DC on conduction in peripheral nerves. Their results indicated that after a certain duration of DC application the block could not be reversed. They concluded that nerve damage ensued and recommended that this technique should not be used in a clinical setting where complete recovery is necessary (Whitwam & Kidd, 1975).

Whitwam and Kidd's observations were supported by results of studies exploring the use of a DC stimulator for nerve explorations in clinical applications (Hughes *et al.*, 1981). In these studies myelin and axonal degeneration were observed when the stimulator came in contact with the nerve for long durations, though it was demonstrated that in surgical applications, damage is most likely avoided due to the short durations of the stimulator's use (estimated one second contact with the nerve) (Hughes *et al.*, 1981). Under appropriate conditions, the use of a DC stimulator for nerve exploration was considered safe (Chase *et al.*, 1984).

The technique of selectively blocking nerves with DC (Fukushima *et al.*, 1975; Petruska *et al.*, 1998) or with high frequency alternating current, whereby DC was used to block neuronal firing at the onset of the high-frequency current (Ackermann *et al.*, 2011b, a) was further explored with the intention of avoiding nerve damage. Recently, the mechanisms underlying DC conduction block have been studied (Bhadra & Kilgore, 2004; Tai *et al.*, 2009). It was found that DC block is most likely due to continuous depolarization of axons under the cathode.

1.3 Electrochemistry and considerations for electrodes used with direct current

An electrode is defined as an electronic conductor which makes contact with part of a circuit that is not metallic. When an electrode is placed in biological tissue and current is delivered, several processes take place. Initially an interface develops between the electrode and the extracellular fluid (ECF) and a charged double layer is created. The charge carriers in the electrode are electrons, while carriers in the ECF are ions (e.g. sodium, chloride). When current is carried by two different substances in two phases, chemical reactions at the electrodes are necessary in order to ensure continuation of charge flow. This process is called electrode reaction.

1.3.1 The interface between electrode and electrolyte

Placing an electrode in an electrolyte (whether biological or otherwise) brings two different phases into contact resulting in changes to their surface layers while an interface develops between the phases. Transient processes occur at the interface including charge transfer across the electrode, eventually resulting in a double layer (typically 0.1 to 0.4nm wide) with a potential difference due to redistribution of charged particles (Bagotsky, 2005). Charge redistribution in the metal results in electrons accumulating on the electrode's surface next to the interface, forming a tight layer of charged particles. In the solution, a concentration gradient is formed, ions of opposite charge are adsorbed and an opposite charged layer next to the electrode is created in the solution. While the electrons cannot leave the metal, the ions are able to move a small distance away from the surface. The entire double layer can be modeled as a

capacitor with high capacitance (10-20 μF/cm^2) due to the small distance between the charged layers (Brummer *et al.*, 1983; Bagotsky, 2005; Merrill *et al.*, 2005; Merrill, 2011).

Faraday's first law of electrolysis states that the mass of a substance produced in electrolysis is directly proportional to the quantity of electricity (charge, measured in coulombs) delivered at the electrode. Therefore, when transient currents occur resulting in charge accumulation on the interface, a deviation from Faraday's law is observed. The resulting current is called a non-faradic or charging current (Bagotsky, 2005).

1.3.2 The electrode reaction

When current is delivered through an electrode placed in an electrolyte, non-faradic as well as faradic currents occur where charge crosses the electrode into the electrolyte. The resulting electrode reaction ensures the current will flow across the interface. There are two types of electrode reactions: oxidization and reduction. The oxidization reaction occurs at the anode (anodic reaction) and involves withdrawal of an electron from a substance. Reduction occurs at the cathode (cathodic reaction) and involves electron addition.

Electrode reactions can be reversible or irreversible. Most reactions are reversible and will occur in the opposite direction once the direction of the current is reversed. When no new species are formed, the reaction is non-faradic and results in the redistribution of charge. If electron transfer does occur in a reversible reaction, the reaction products remain attached to the electrode surface. An example of this type of reaction would be oxide formation on an electrode. Irreversible reactions result in products that cannot revert to their original form when the current direction is reversed. This could be due to the solubility of the reaction product in the electrolyte or the evolution of a gas. An example of an irreversible reaction is electrolysis and formation of hydrogen gas at the cathode.

Irreversible reactions may result in changes to the electrode and surrounding biological tissue. In the case of reduction of water, the reaction results in the formation of hydrogen gas and hydroxyl ions. The latter contribute to change in the tissue's pH level (Brummer *et al.*, 1983; Bagotsky, 2005; Merrill *et al.*, 2005).

1.3.3 Electrode materials

There are two types of electrodes: reacting electrodes which are chemically involved in the reaction and non-consumable electrodes or inert electrodes that do not participate in the electrode reaction but are the source of the electrons for the reaction (Bagotsky, 2005). When choosing an electrode for neural tissue stimulation, aspects such as tissue reaction, electrode impedance and corrosion resistance should be taken into consideration. Electrode material should be biocompatible and result in minimal inflammatory response. Stainless steel and Platinum which are non-consumable electrodes, demonstrated a small tissue response (Geddes & Roeder, 2003). Stainless

steel contains nickel which makes it a potential allergen that quickly corrodes and hence is less suitable for long term implantation. Platinum and Pt-Ir are not allergenic, have a low electrode impedance and high corrosion resistivity and are therefore widely used in implantable systems such as cardiac pacemakers and cochlear implants (Geddes & Roeder, 2003; Merrill *et al.*, 2005).

1.3.4 Considerations for electrical stimulation of biological tissue

Since faradic reactions may result in damage to the electrode and surrounding tissue, much effort was made to describe stimulation parameters that are considered safe. Monophasic pulses tested on brain tissue of monkeys proved to result in damage presented as reduced neuronal excitability (Lilly *et al.*, 1952). Experiments carried out on cats showed that monophasic stimulation resulted in damage defined by the breakage of the blood brain barrier, while biphasic charge-balanced stimulation demonstrated no such result (Mortimer *et al.*, 1970). Agnew and McCreary showed that prolonged biphasic stimulation may still result in nerve damage, depending on the stimulation parameters (Agnew & McCreery, 1990). Increase in stimulation threshold of myelinated fibers, loss of nerve fibers and edema resulted when high frequency (50Hz) pulses were applied as opposed to low frequency pulses (20Hz), even when the total charge delivered was identical. The results indicated that several parameters were influencing the occurrence of nerve damage and it was shown that the relationship between charge density and charge per phase could indicate when damage would emerge (Agnew *et al.*, 1989; Agnew & McCreery, 1990; McCreery *et al.*, 1990). Their work, as well as others' work is summed up in Figure 1.3, reproduced with modifications from Merrill et al. 2005.

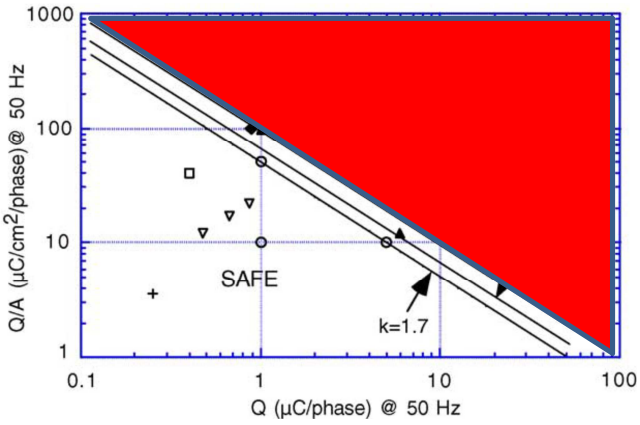

Figure 1.3: Charge density and charge per phase. This graph shows the relationship between the amount of charge delivered per phase to the charge density and the safe regions of stimulation. The red triangle shows the area where nerve damage was shown to occur. Reproduced with modifications from Merrill et al. 2005.

1.3.5 Mechanisms of damage

The exact mechanisms responsible for tissue damage created by electrical stimulation is still not fully understood but several mechanisms were identified including neuronal hyperactivity, burn due to heating and toxic effects of the products of the electrode reaction (Dymond, 1976; Scheiner *et al.*, 1990; Merrill *et al.*, 2005; Stecker *et al.*, 2006).

Neuronal hyperactivity occurs when the nerve is over-stimulated resulting in neurons firing for a prolonged duration. This theory is supported by Agnew and McCreary's work. They showed that only myelinated fibers were injured during damaging stimulation and suggested that this pattern of damage was likely due to hyper-activity rather than other mechanisms (Agnew & McCreery, 1990). Other investigators showed that damage is most probably caused by species produced during the electrode reaction (Ackermann *et al.*, 2011b).

Electrical burns may result from increased temperatures of the tissue immediately adjacent to the electrode during stimulation, though this issue is controversial (Scheiner *et al.*, 1990; Stevenson *et al.*, 2010). Heating can be considered as one of the effects of the electrode reactions. Several other products of the electrode reaction may contribute to nerve damage. Hydrolysis of water molecules resulting in shifts in pH can change the nerve environment to be more alkaline (Cathode) or acidic (Anode) (Brummer *et al.*, 1983; Scheiner *et al.*, 1990). Irreversible reactions occurring at the electrode surface can have numerous effects on the biological tissue during stimulation. These include changes to the electrode due to development of a film on the electrode itself which may result in higher current densities (Dymond, 1976) and dissolution of metal and electrode corrosion that may be toxic to the surrounding tissue (Brummer *et al.*, 1983). Lastly, oxidization of inorganic particles such as chlorine at the anode may contribute to the acidic environment created around the anode (Brummer *et al.*, 1983; Berendson & Simonsson, 1994).

1.4 Neurolytic blocks

The use of neurolytic agents is well documented and used for treatment of pain and neurological disorders such as spasticity. There are several neurolytic techniques applied to treat numerous conditions. Phenol, alcohol and BtA were previously described in this chapter, the other techniques are: conventional radio frequency (CRF), pulsed radiofrequency (PRF), and cryoablation.

CRF and PRF operate by creating a thermal lesion. They are applied to nociceptive pathways for the purpose of pain control. CRF in the range of 55^{o} to 70^{o} is used on dorsal root ganglia. PRF, considered safer since heat generation does not exceed 42^{o}, is often preferred to CRF (Khalid, 2011), but was shown to result in nerve structural damage (Erdine *et al.*, 2009) probably due to combined electrical and thermal injury.

Complications include nerve damage and infection from needle insertion as well as burns from erroneous placement of the grounding pad (Khalid, 2011).

Cryoablation is used mostly for the relief of post-thoracotomy pain. Localized freezing of intercostal muscles by application of a cryprobe, with its tip cooled to -50^{o} to -70^{o}, is achieved, resulting in reduced usage of post-operative analgesics. Cryoanalgesia may lead to nerve damage but is not associated with neuromas, since the epineurium and perineurium remain intact. Side effects may include: respiratory depression, nausea (Moorjani *et al.*, 2001) and possibly neuropathic pain (Myers *et al.*, 1996).

Neurolytic treatments, specifically radiofrequency and alcohol, were shown to be effective for the treatment of cancer pain. There is still controversy regarding its usefulness in treating benign chronic pain and it is advised to exhaust other treatments first (Jackson & Gaeta, 2008). Neurolytic treatments such as phenol and alcohol work by causing a temporary or permanent conduction block. Treatment with phenol for example is considered safe, simple and effective (Copp *et al.*, 1970). The side effects of neurolytic treatments and the benefits to be gained from them such as elimination of clonus, improved range of motion (ROM), improved gait, reduction of pain, reduced cost and the ability to repeat the treatments more often than BtA were reviewed in detail in Horn et al. 2010.

1.5 Dissertation objectives and outline

The purpose of the work presented in this thesis was to explore the feasibility of the use of DC as a novel treatment for spastic hypertonus in order to cause a partial nerve lesion . Three studies were conducted and are presented in the following sections. This report concludes with a final chapter in which the results are discussed and future research directions are suggested.

Chapter 2: This chapter describes proof of principle experiments in which DC was used to cause a controlled nerve lesion resulting in graded attenuation of muscle force. Acute and chronic experiments are described with various current amplitudes and durations, as well as histological results.

Chapter 3: Further exploration of DC amplitudes and durations are presented for the purpose of determining parameters that would be clinically suitable and effective in achieving force gradation and could be clinically suitable. Furthermore, acute experiments conducted for the purpose of elucidating possible mechanisms of damage are presented.

Chapter 4: DC nerve block and ablation in chronically implanted animals are described. Experiments were carried out on anesthetized as well as awake animals. Detailed descriptions of electrodes developed for the purpose of DC delivery in the conscious animal are presented.

Chapter 5: This chapter includes a summary of the results of my thesis work with a discussion regarding the implication of the findings and the possibility for clinical applications. Future directions are discussed.

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Chapter 2

Nerve Lesioning with Direct Current[1]

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 SCI in the U.S. 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 over-activity 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'Eerdt *et al.*, 2008). Oral medications include benzodiazepines, Dantrolene and Baclofen (Kita & Goodkin, 2000; Goldstein, 2001; O'Brien, 2002). These drugs have adverse side effects such as drowsiness, fatigue, weakness, sedation and dizziness (Kita & Goodkin, 2000; Montane *et al.*, 2004), and 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 & 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

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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 two 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. Previously DC has been used in acute physiological studies to selectively block large nerve axons (Mendell & Wall, 1964; Whitwam & Kidd, 1975; Bhadra & 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.

2.1 Methods

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

2.1.1 Animals and surgical procedures

2.1.1.1 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

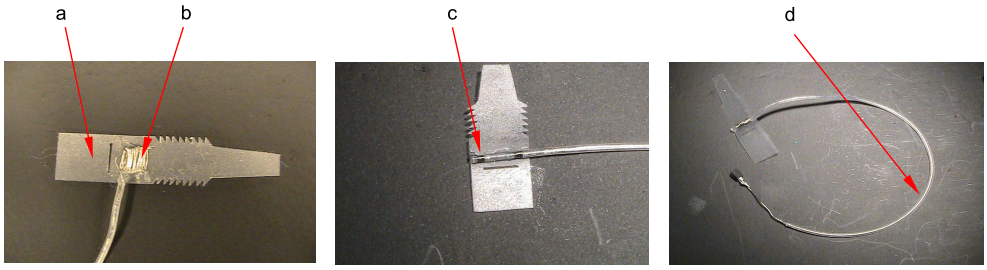
terminals (Figure 2.1A). The strip functioned like a "tie-wrap," which was used to fix electrodes to the nerve (Kowalczewski, 2009). The nerve cuff leads were made of insulated multi-stranded, stainless steel wire (AS632, Cooner Wire, Chatsworth, CA) coiled inside a silicone tube (508-003, Dow Corning 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 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 cm and 0.5 cm (Figure 2.1A). 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 2.1B). Each terminal was 1mm in length and the distance between them was 4mm. A third cuff, similar to the proximal cuff but with only a single bared terminal 5mm in length, was implanted on the tibial nerve of the contralateral leg, to serve as a control (Figure 2.1C). Finally, an indifferent (anodal) electrode comprising an insulated lead terminating in a bare stainless steel disc 3cm in diameter was placed under the skin over the paravertebral muscles of the lower lumbar region.

2.1.1.2 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 im), hydromorphone (0.05 mg/kg im) and glycopyrrolate (0.01 mg/kg SC) 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. 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 iodine solution.

The distal cuffs in the chronic implant had 5mm Pt-Ir terminals as in Figure 2.1C 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 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 (0.5-1 mg/kg 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 (0.05-0.1mg/Kg SC) 6 hours after extubation. The antibiotic Clavamox (10-20 mg/Kg) was administered for 7 days after surgery.



(A) (B) (C)

Figure 2.1: 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 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.

2.1.2 Experimental procedures

2.1.2.1 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 2.2).

Pulses of electrical current (200 μs, 2 Hz) 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 (1.5T) 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 enabling either 20 s 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 (Euthanyl).

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

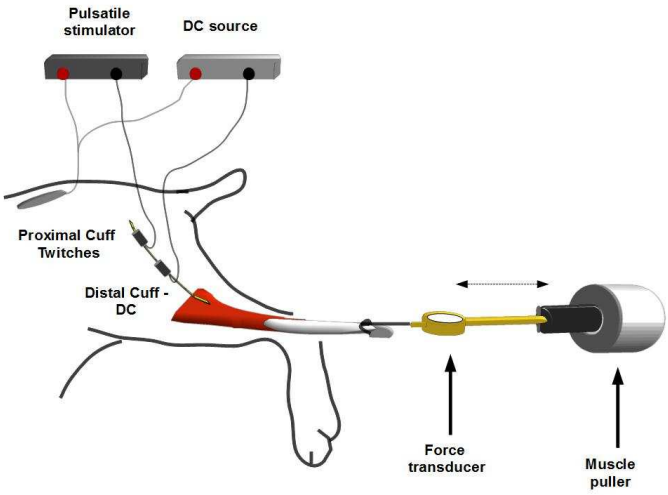


Figure 2.2: Acute experiment setup. The tendon of the triceps surae muscles was exposed and dissected free through a skin incision, detached with a small portion of the calcaneus and connected to a force transducer. The bony origin of the muscle at the knee was immobilized by a clamp attached to a metal 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 15mm.

2.1.2.2 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.

2.1.2.3 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 1cm wide band of webbing material was looped around the metatarsals 60mm 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 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 μs, 2 Hz) 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 1.5T. At the beginning of each experiment 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 two 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 (45V), 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 3cm 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 0.3-0.4mA for durations of 10 to 40 min according to the lesioning effect. The twitch force responses to 1.5T 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 continued for up to one hour 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 Results). 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 made as above.

2.1.3 Data analysis

2.1.3.1 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 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 (1Hz, 5^{th} order Butterworth) and subtracted from the unfiltered signal to leave just the twitch forces. Finally, a low pass filter (100 Hz, 5^{th} order Butterworth) was used to attenuate high-frequency noise. Figure 2.3 (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).

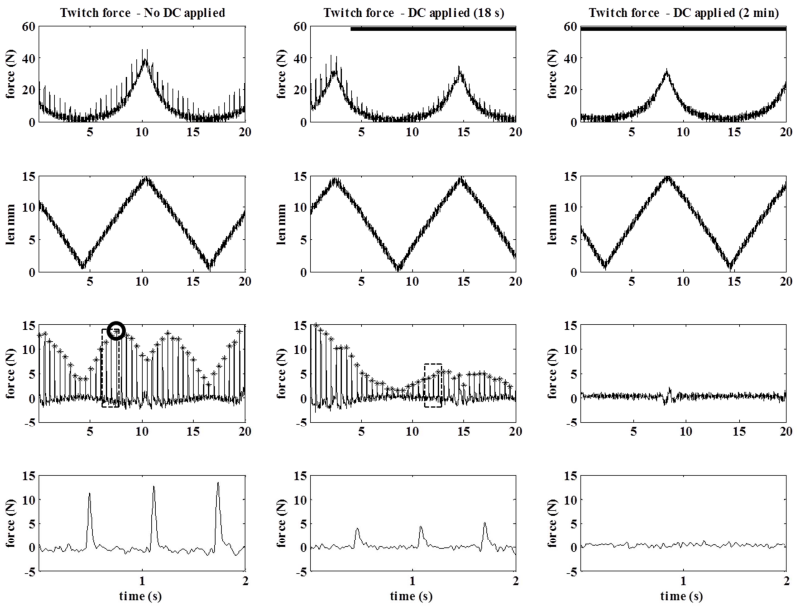


Figure 2.3: Muscle twitches were generated by pulsatile stimulation at 2Hz through the proximal nerve cuff. Top row: force transducer signal during triangular changes in muscle length. Black bars represent the time DC was applied through the distal nerve cuff. Second row: imposed displacement. Third 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.

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. Normalized twitch forces are plotted in Figures 2.4-2.7.

2.1.3.2 Chronic experiments

As in the acute experiments, the force signal was digitally band-pass filtered (1Hz, 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.

2.1.3.3 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 experiments had an estimated surface area of 0.3*0.5*□/2 = 0.236 cm^2. The cuff used in the chronic implant in the cat had an estimated surface area of 0.093 cm^2. In Figures 2.4, 2.5, 2.6, 2.7 and 2.9 we provide values of total charge density based on the level and duration of DC, and the above estimates of surface area.

2.2 Results

2.2.1 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 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: Period following the cessation of DC delivery. Test stimuli continued to be applied through 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.

2.2.2 Acute experiments

Details of animals used in these experiments, DC application parameters and summary of results can be found in Appendix A, table A.1.

2.2.2.1 Effect of DC amplitudes in the range 0.1 – 0.4mA

DC at 0.1mA 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 0.2mA had little attenuating effect. It was not until DC reached 0.3mA and above that attenuation of peak twitch forces became obvious. This is shown in the experiment of Figure 2.4. 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 2.5, this time for three repeated applications of DC at 0.3mA. 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.

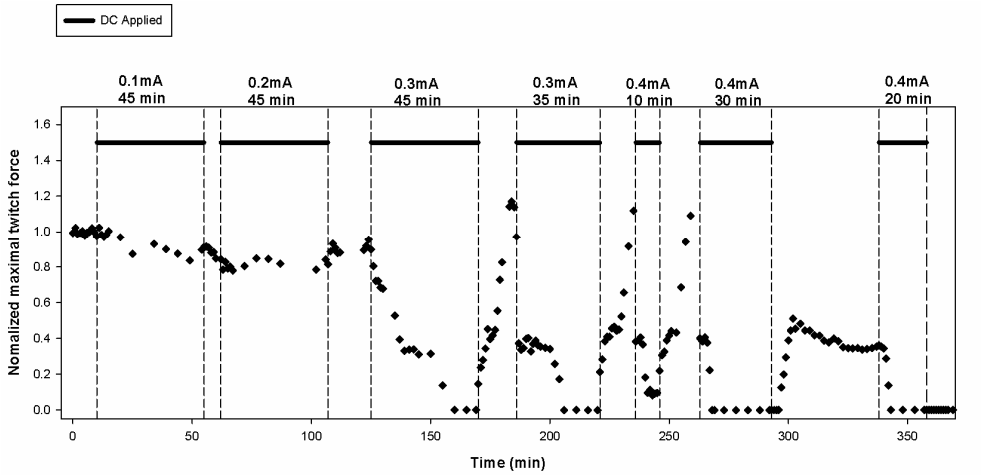


Figure 2.4: Changes in twitch force during episodes of DC application over a 6-hour experiment. Top: amplitudes and durations of DC. Horizontal bars: duration of DC application. Data points: normalized maximal twitch forces in stretch cycles measured at intervals of 1 to 5 min throughout the experiment. Peak twitch forces were normalized to the baseline value: the mean of 5 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.

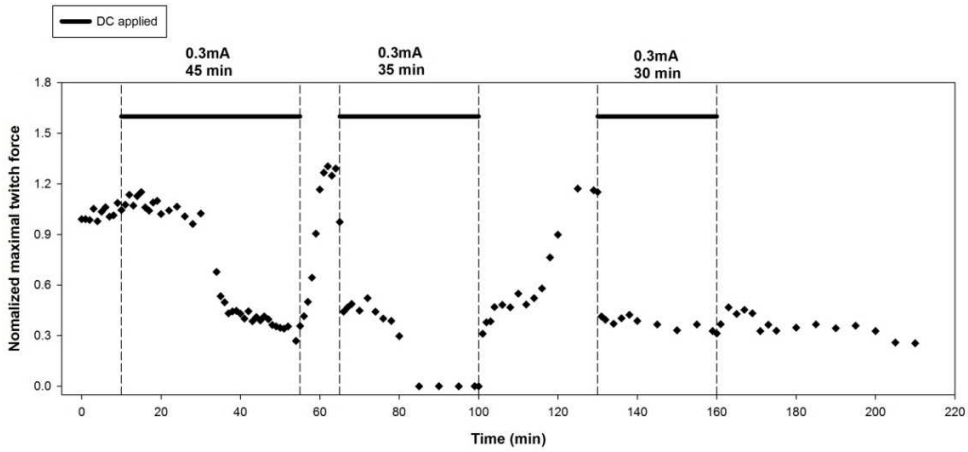


Figure 2.5: Changes in twitch force during episodes of DC application over a 4-hour experiment, data displayed as in Figure 2.4. Clear examples of rebound and increased efficacy and speed of attenuation with repeated DC applications at the same amplitude (0.3mA). The estimated charge densities for each consecutive DC application in this figure were: 3.43, 2.67 and 2.29 C/cm^2.

2.2.2.2 Effect of DC amplitudes in the range 0.5-1mA

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 2.6), 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 2.7 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.

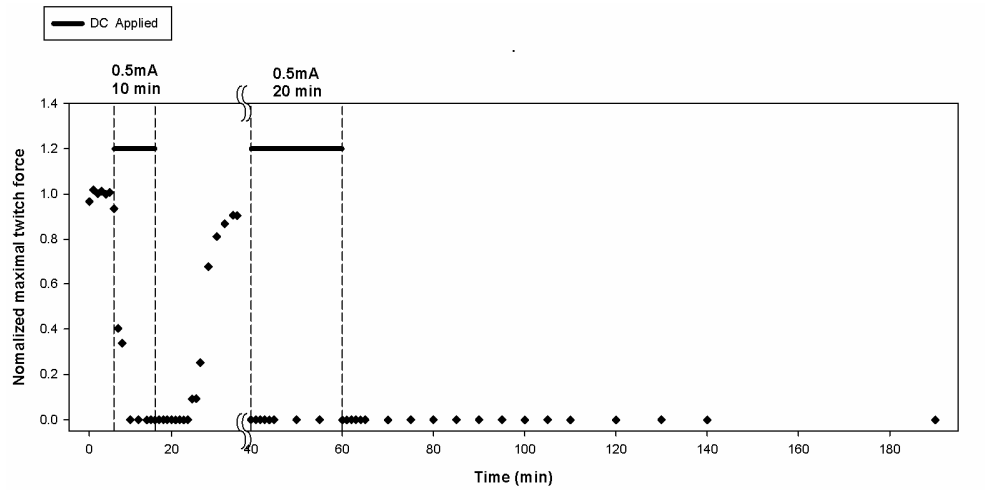


Figure 2.6: Changes in twitch force during episodes of DC application at an amplitude of 0.5mA. Data displayed as in Figure 2.4. In this experiment after a first application of DC (not shown) we discovered that the test nerve stimuli were not supramaximal. This was corrected and the experiment was restarted. 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 hours 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.

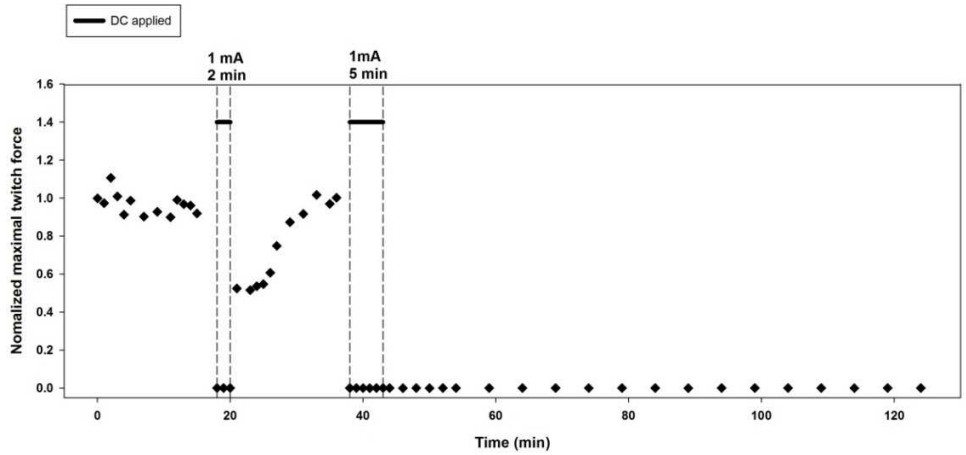


Figure 2.7: Changes in twitch force during episodes of DC application at an amplitude of 1mA. Data displayed as in Figure 2.4. As in Figure 2.6, force attenuation by DC was rapid and complete, after the second application, it was maintained until the end of the experiment. The estimated charge densities for each consecutive DC application in this figure were: 5.08 and 12.7 C/cm^2.

2.2.2.3 Force Overshoot

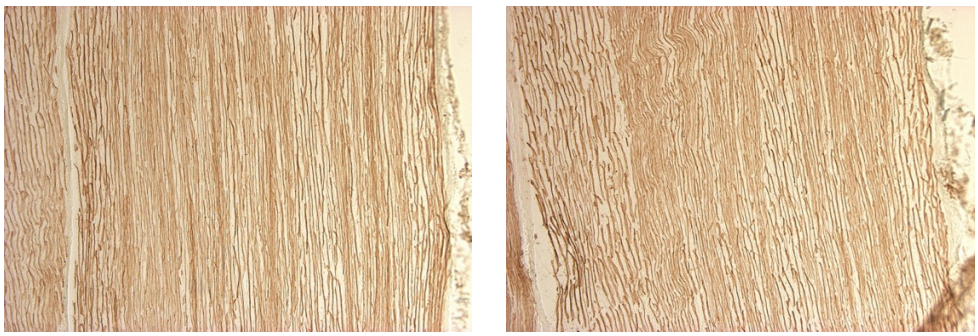
Because force recovered beyond the baseline levels in some of the DC applications, especially after having been abolished or strongly attenuated, in some experiments we verified that proximal test stimuli were supramaximal by increasing their amplitude without observing an increase in twitch forces.

2.2.2.4 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.

2.2.2.5 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 2.8A 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 2.8B, taken from under a DC cuff, most of the discontinuities have curly ends, indicating axonal damage.



(A) (B)

Figure 2.8: 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.

2.2.3 Chronic experiments

Details of animals used in these experiments, DC application parameters and summary of results can be found in Appendix A, table A.3.

2.2.3.1 DC delivery in the anaesthetized cat

As mentioned in Methods, 6 DC lesioning sessions took place at intervals of 7 to 14 days over a period of 8 weeks. On each occasion the cat (cat 1) was anesthetized for approximately 2 hours 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 2.9 shows 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 Methods, so the results of these experiments are not included in the Figure. Twitch forces were progressively reduced by DC in each experiment in Figure 2.9, culminating in a complete abolition of force at the end of the sixth session.

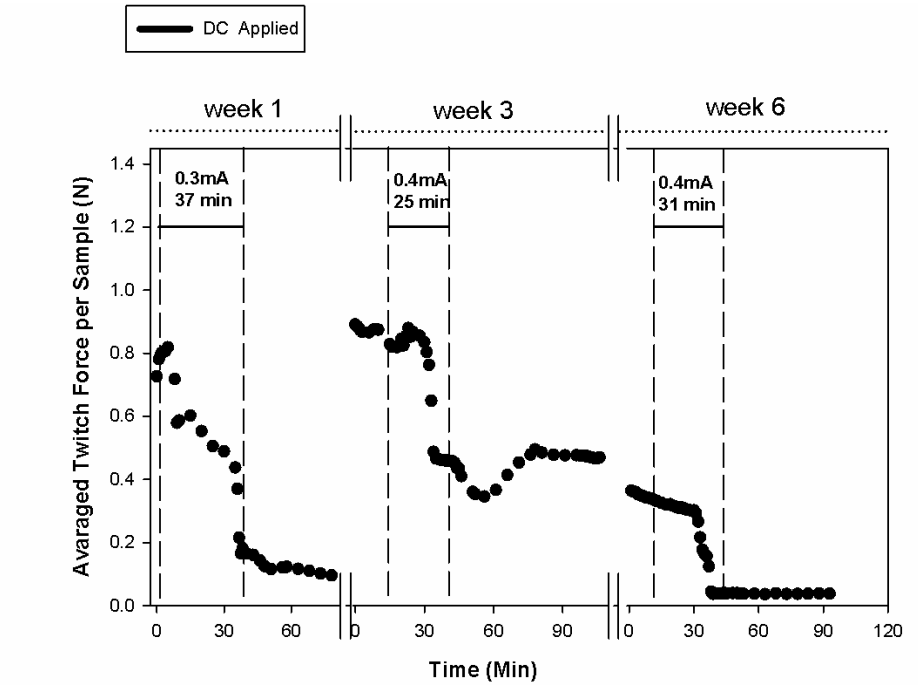


Figure 2.9: Three separate DC lesioning sessions in an anesthetized cat chronically implanted with test and DC cuffs on the common peroneal nerve innervating the pretibial muscles extensor digitorum longus and tibialis anterior. The force measurements were performed by immobilizing the leg just above the ankle with a retort clamp attached to a frame and attaching the transducer of Figure 2.2 via a loop of canvas webbing to the foot close to the metatarsophalangeal joint. Data displayed as in Figure 2.4. In the first session the DC amplitude was 0.3mA. In sessions 3 and 6 it was 0.4mA 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

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 μs at 40 Hz) was 0.12 mA, while in the right leg tetanic stimulation via the R_p cuff at amplitudes up to 1.5mA 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 5mm bared ends. In the left leg, tetanic intramuscular stimulation with 2mA 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 3mA whereas on the right side stimulation at up to 6mA 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: 0.1mA, 0.3mA, 0.7mA; week 12: 0.1mA, 0.3mA, 0.6mA; week 16: 0.1mA, 0.2mA, 0.3mA; week 20: 0.1mA, 0.2mA, 0.4mA. Because thresholds at week 20 had returned close to pre-lesioned values, twitch forces were measured with a force transducer as described in Methods (Chronic Experiments). 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.

2.2.3.2 DC delivery in the awake cat

As described in Methods, on one occasion DC (0.4mA) 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 2.5 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.

2.2.3.3 Condition of electrodes

An attempt to re-lesion the nerve following the 20 weeks recovery period using the same DC parameters (0.4mA 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.

2.3 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 et al. (Whitwam & Kidd, 1975).

DC amplitudes of 0.1mA or 0.2mA 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 0.3mA and 0.4mA 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 1mA 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 2.9) 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 ($1mC/cm^2$/phase) 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 pulses/s) 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 Tibialis anterior (TA) and Extensor digitorum longus (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 (Gutmann *et al.*, 1942) who either crushed or cut the common peroneal nerve in rabbits 40-50mm 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 (11-16mm, mean 13mm), 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 (Prochazka, A., patent pending). On the other hand DC implants would be unlikely to replace BtA in 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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Chapter 3

Nerve lesioning with direct current (DC): Investigation of DC parameters and possible mechanisms of DC induced nerve ablation

One of the most debilitating outcomes of stroke and spinal cord injury (SCI) is the loss of motor function. The initial insult may be further exacerbated by spasticity, a chronic over-activity of muscles. This condition can result in fatigue, reduced range of motion (ROM), pain and contractures which impede activities of daily life (ADLs) (Rizzo *et al.*, 2004; Taricco *et al.*, 2006). Several studies have shown that spasticity is associated with reduced function and that survivors who developed spasticity had lower scores in tests of ADLs (Sommerfeld *et al.*, 2004; Welmer *et al.*, 2006; Lundstrom *et al.*, 2008). Furthermore, a lesser degree of impairment was associated with better chances of recovery for the upper extremities (Coupar *et al.*, 2012) .

During the 6 to 18 month period following a stroke event, spasticity develops in approximately 20% to 30% of survivors (Watkins *et al.*, 2002; Sommerfeld *et al.*, 2004; Lundstrom *et al.*, 2008; Kong *et al.*, 2012). Although this is sometimes regarded as a low percentage, the actual number of individuals requiring management of spasticity is high when the prevalence of stroke and SCI is considered. Stroke prevalence in the United States (US) alone is estimated at 7 million individuals (Roger *et al.*, 2012). Of the estimated 270,000 individuals living with SCI (NSCISC, 2012), over 60% develop spasticity following injury, of whom more than half are medically treated. The incidence of spasticity is greater in those with cervical or high thoracic SCI (Maynard *et al.*, 1990). With a decreasing incidence of mortality resulting from a stroke event (Roger *et al.*, 2012) and the high prevalence of individuals with SCI requiring care, the burden on the health care system is increasing. Ten years ago, 250,000 disabled stroke survivors were reported in the United Kingdom (UK) and £2.3 billion per year were spent on stroke alone (Rothwell, 2001). In the US, a reported $34.3 billion were used for the treatment of stroke in 2008 (Roger *et al.*, 2012).

Current treatments for spasticity include physical and occupational therapy, anti-spastic medications and chemodenervation agents (Goldstein, 2001). Physiotherapeutic methods such as stretching, Range of Motion (ROM) exercises and casting require a

considerable time commitment, and are of limited efficacy and duration (Bovend'Eerdt *et al.*, 2008) .Whole body vibration has not lived up to its initial promise (Brogardh *et al.*, 2012; Lau RW *et al.*, 2012) though a new study indicates that focal muscle vibration may have a beneficial effect (Caliandro *et al.*, 2012). Common oral anti-spastic drugs such as Dantrolene, Tizianidine and Baclofen can cause adverse side effects such as muscle weakness, hepatotoxicity, dizziness and sedation (Gracies *et al.*, 1997; Kita & Goodkin, 2000; O'Brien, 2002) and they do not reduce spasticity in all patients (Maynard *et al.*, 1990; Taricco *et al.*, 2006). A recent review concluded that while Baclofen and Dantrolene reduced muscle tone, there was no evidence that this had a functional impact (Simon & Yelnik, 2010). Baclofen can be delivered intrathecally via a surgically-placed pump, allowing a lower dosage than oral medication, which reduces systemic side-effects, however, other complications such as infections around the implants may occur (Barker, 2008).

Chemodenervation agents such as phenol and Botulimum toxin type A (BtA) are used to block nerve conduction. Phenol injections have a rapid onset but often have painful side effects (van Kuijk *et al.*, 2002; Elovic *et al.*, 2009). BtA treatment is costly, may take up to two weeks to take effect and usually lasts only a few months (Dolly, 2003), necessitating repeated costly administration (Ward *et al.*, 2005). BtA has proven effective in reducing muscle tone and managing spasticity, but treatment did not necessarily improve motor function (Elia *et al.*, 2009; Teasell *et al.*, 2012; Wolf *et al.*, 2012). Furthermore, recent research shows that continuous use of BtA results in muscle atrophy and loss of muscle tissue in both the target muscle and muscles elsewhere in the body (Fortuna *et al.*, 2011).

Direct current (DC) has been used in the past to block conduction in peripheral nerves, but was deemed unsuitable for clinical use since it resulted in nerve damage (Whitwam & Kidd, 1975). We suggest that this effect of DC on nerves can in fact be useful clinically. In a previous publication (Ravid *et al.*, 2011) initial results of complete and partial nerve ablation were presented. Here we explored stimulation parameters, current amplitude and duration of stimulation with the goal of describing a stimulation profile that would be effective and clinically acceptable. The following is our presentation of the results of application of DC in anesthetized and decerebrate animals and the outcome of investigations into possible mechanisms of nerve ablation.

3.1 Methods

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

3.1.1 Definitions

Baseline: Test period at the beginning of each experiment prior to delivering DC.

DC application: Time duration when DC is applied to the nerve. Several DC applications may be carried out in a single experiment.

Nerve Recovery: Time duration following a DC application when the nerve is allowed to recover. DC is not applied during this period.

3.1.2 Animals and surgical procedures

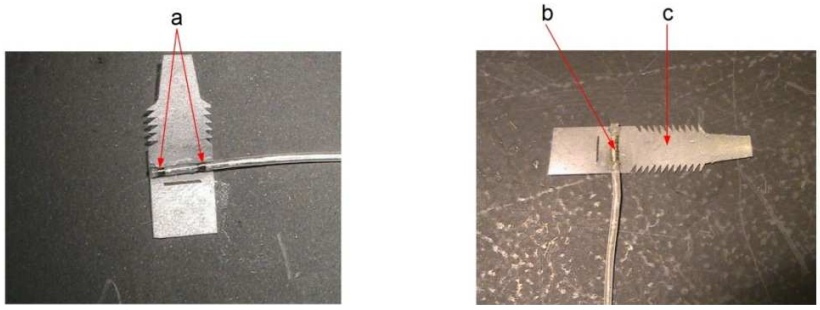
3.1.2.1 Non-recovery experiments in rabbits to explore DC parameters

Seven white New Zealand rabbits were used in single, non-recovery experiments. Surgical-plane anesthesia was induced with gaseous Isoflurane delivered through a mask. A tracheotomy was performed, allowing anesthesia to be maintained via an endotracheal tube. The left sciatic nerve was exposed through an incision overlying the hamstrings muscle. The common peroneal nerve (CPN) was separated from the tibial nerve and transected. Two silicone nerve cuff electrodes were attached to the tibial nerve branch: a proximal nerve cuff to deliver test stimuli and a distal cuff to deliver DC. The cuffs each comprised a strip of silicone in the form of a "tie-wrap," which fixed the cuff to the nerve (Figure 3.1). The leads were made of insulated, multi-stranded stainless steel wire (AS632, Cooner Wire, Chatsworth, CA) coiled inside a silicone tube, which was then filled with silicone sealant (RTV 118, Momentive Performance Materials, Waterford, NY). A more detailed description of the surgical procedure and leads can be found in a previous publication (Ravid *et al.*, 2011).

The proximal nerve cuff, which was used to elicit test muscle twitches, contained a pair of terminals formed from the stainless steel lead wires. Where they emerged from the silicone tube, these wires were stripped of insulation and tightly coiled back around the tube to form a bipolar terminal, which was then attached to the silicone tie-wrap with silicone adhesive (Figure 3.1A). The distal nerve cuff, which was used to deliver DC, contained a Platinum Iridium (Pt-Ir) terminal. In the first of the present experiments, this terminal was formed as previously described (Ravid *et al.*, 2011), by sewing a length of Pt-Ir wire, soldered to the end of a single stainless steel lead wire, back and forth on one side of the tie-wrap. In the remaining six experiments, the terminal was formed in a similar way to those in the proximal cuff, by tightly coiling the length of Pt-Ir wire back around the silicone tube containing the lead wire. As in the proximal cuff, this terminal was then attached to a tie-wrap with silicone adhesive (Figure 3.1B).

At the other end of each of the lead wires, two types of connector were used, those external to the body and those implanted under the skin of the animal's back. The external connectors were conventional 2-mm plug and socket connectors. The

connectors implanted under the skin were prototypes of insulated, conductive, subcutaneous ports suitable for human clinical applications, where repeated sessions of controlled nerve ablation are to be anticipated. The idea was to develop a port into which a hypodermic needle, insulated except for its tip, could be introduced through the skin, providing a temporary electrical connection to the nerve ablation lead (Figure 3.2).



(A) **(B)**

Figure 3.1: The nerve cuff electrodes used in the study. A) Proximal cuff which delivered pulses to the nerve to generate test muscle twitches. *a)* Two insulated, multi-stranded stainless steel wires that emerged from inside the silicone tubing of the lead were stripped of insulation and tightly coiled back around the tubing to form a bipolar terminal. Cuff is shown open, prior to placement on nerve. B) Pt-Ir nerve cuff, which delivered DC to cause controlled nerve ablation. *b)* A single strand of Pt-Ir wire was soldered to the stainless steel wire emerging from the silicone tubing of the lead. The Pt-Ir wire was coiled back around the silicone tubing to form a 5mm monopolar cuff. *c)* Silicone strip in the form of a tie-wrap.

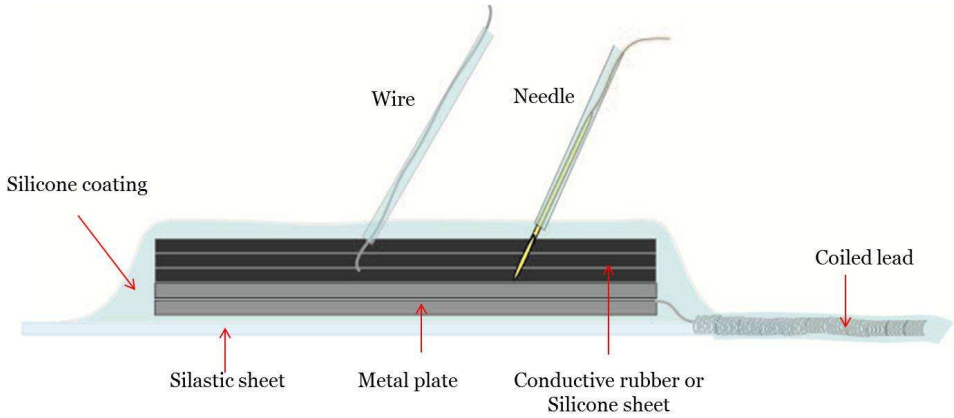


Figure 3.2: Subcutanous Port Schematics. The port is composed of two stainless steel plates. One of the plates is bonded with silicone sealant to a silastic sheet. A layer of conductive rubber or conductive silicone is glued to the second metal plate with conductive epoxy. The two plates are glued together with conductive epoxy as well. The entire port is coated with silicone sealant. There are two possible methods to connect to the port: 1) An insulated hypodermic needle with only the tip exposed, may be inserted into the conductive rubber or silicone layers. 2) A wire, insulated except for the tip, is inserted into the conductive layers via a hypodermic needle. The needle is then withdrawn, leaving the uninsulated tip of the wire in the conductive material.

In this study the port was composed of two thin 2x2 cm stainless steel plates (SS316 flat pack, 0.010", Mauldin products, Kemah, TX). An electrode lead was soldered to one of the plates and the plates were glued together with silver conductive epoxy (8331-14G, MG chemicals, B.C.), sandwiching the solder joint between them. A 2x2 cm sheet of conductive rubber (C5020PF Canadian Medical Products Ltd, Scarborough ON) was stuck to one of the plates with the same epoxy. The other plate was glued to a non-conductive silastic sheet (0.010"+/- .002, material: SSF-METN-750, Speciality Silicone Fabricators, Paso Robles, CA) with silicone sealant. Finally, the entire port and emerging section of lead were coated with a layer of silicone sealant (Figure 3.3).

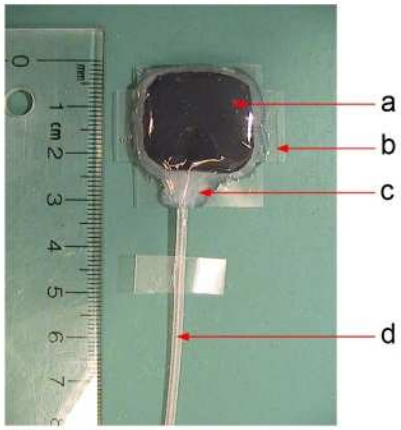


Figure 3.3: Subcutaneous port and lead. *a)* Conductive carbon layer under silicone sealant layers. *b)* Silastic sheet comprising the backing of the port. *c)* Silicone sealant layers. *d)* Coiled Pt-Ir wire inside a silicone tube filled with silicone sealant.

3.1.2.2 Non-recovery experiments in rabbits with Lidocaine blockade of common peroneal nerve (CPN) to elucidate mechanism of DC ablation

Two rabbits and one cat were anesthetized as described in section 3.1.2.1. The animal was placed prone on a table; the CPN was exposed in both legs. Two nerve cuff electrodes (distal Pt-Ir, proximal stainless steel, external connectors as described in section 3.1.2.1) were placed on each CPN. The incisions were closed with Michel clips to keep the nerve from drying.

3.1.2.3 Non-recovery experiments in decerebrate cats to demonstrate abolition of rigidity by DC nerve ablation

The surgical procedure described in 3.1.2.1 was performed on two cats with isoflurane anesthesia. A catheter was inserted into a jugular vein to enable intravenous drug administration. The top of the skull was trefined and an intercollicular decerebration was performed, after which anaesthesia was discontinued. Decerebrate rigidity developed in one of the cats an hour later.

3.1.3 Experimental procedures

3.1.3.1 Exploring DC parameters

The procedure is described in detail in previous publications (Ravid *et al.*, 2011), so only a summary is presented here. The rabbit was suspended prone in a stereotaxic frame. Its left knee was stabilized with a clamp. The tendon of the triceps surae muscle was exposed through a skin incision at the ankle and the calcaneal tuberosity was detached. The tendon was secured to a force transducer with a short length of strong silk suture. The force transducer was part of an electromagnetic, servo-controlled muscle puller that was configured to stretch the muscle back and forth through its full physiological range (Figure 3.4). 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. Pulses of electrical current (200 μs, 2 Hz) were applied through the proximal cuff throughout the experiment. The signals from the force transducer and a displacement transducer attached to the muscle puller, along with current and voltage signals from a custom, feedback-controlled, DC generator were sampled at 500 samples/s with a digital 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 20 s segments of data. These segments were recorded at intervals of 1 to 5 min throughout the experiment. DC was delivered intermittently through the distal cuff. DC amplitudes used in current experiments were 0.5mA, 0.75mA, 1mA, 1.5mA, 2mA, 2.5mA.

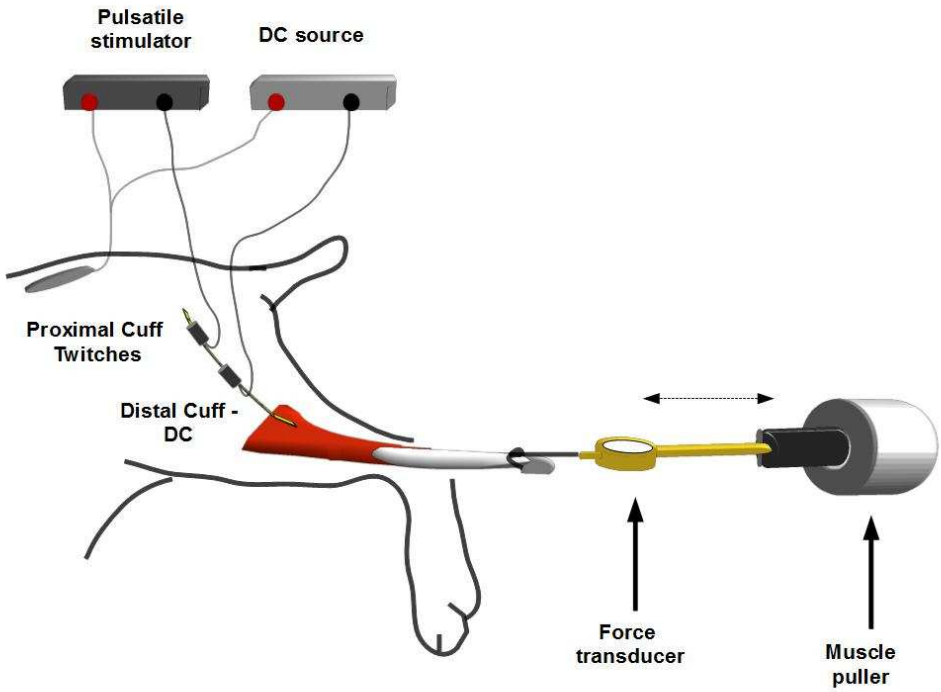


Figure 3.4: Arrangement in the experiments exploring DC parameters and decerebrate rigidity. The tendon of the triceps surae muscles was exposed and dissected free through a skin incision, detached with a small portion of the calcaneus and connected to a force transducer. The bony origin of the muscle 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 15mm.

3.1.3.2 Lidocaine blockade of nerve conduction to elucidate mechanism of DC ablation

The purpose of these experiments was to test the hypothesis that sodium channel blockade by Lidocaine protects nerves from DC induced damage. After the surgical procedures described in 3.1.2.1 and 3.1.2.2, the animal was placed prone on a table, its leg was clamped at the ankle and a strap around the metatarsals was used to attach the foot to a force transducer (Figure 3.5). Supramaximal pulsatile stimulation (200 μs, 2Hz) was applied through the proximal nerve cuff to elicit test muscle twitches. Force, DC and voltage signals were sampled and displayed as described in 3.1.3.1. A length-tension curve was performed to determine the optimal muscle length, by measuring twitch force amplitudes at different ankle angles. The force transducer was then magnetically clamped to the table at the position corresponding to optimal muscle length. Twitch responses were recorded for several minutes until their amplitudes settled to a stable baseline. An initial dose of 0.2ml of 1% Lidocaine solution (Xylocaine, AstraZeneca) was

injected into the distal cuff with the use of a 28G hypodermic needle over a period of 2-3 min. This initial dose was applied to the nerve to establish the time T_1 from the complete abolition of twitch force to near-complete recovery. As soon as the Lidocaine had abolished muscle twitches, the nerve was flushed with 10ml of isotonic saline in and around the cuff. When muscle twitch forces had recovered to 60%-80% of baseline values, a second identical quantity of Lidocaine solution was injected into the nerve cuff. When muscle twitches were abolished, the nerve was again flushed with 10ml isotonic saline and DC was applied through the distal nerve cuff (3mA for 4 min). Twitches were then monitored for at least $2*T_1$.

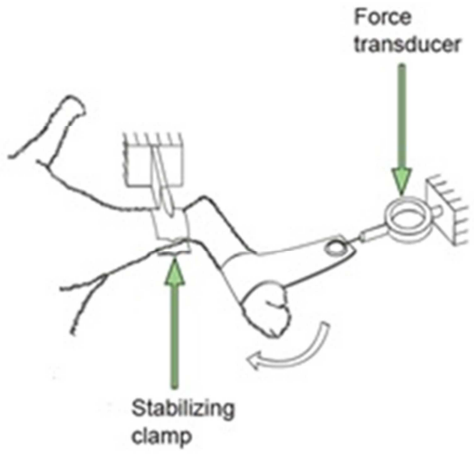


Figure 3.5: Arrangement in the experiments in which nerve conduction was blocked with Lidocaine during the application of DC. The purpose of these experiments was to test the hypothesis that sodium channel blockade by Lidocaine protects nerves from DC. The rabbit was placed prone, its leg was clamped and a strap was looped around the foot and connected to a force transducer. Test twitches of ankle flexor muscles were elicited by electrical pulses applied to the common peroneal nerve via a proximal nerve cuff. Lidocaine was applied to the nerve under and around a more distal cuff, with a fine needle. When the Lidocaine had abolished twitches by blocking nerve conduction at the distal cuff, DC was delivered to the nerve via the distal cuff.

3.1.3.3 Decerebrate rigidity experiments

After the surgical procedures described in 3.1.2.1 and 3.1.2.3, the cat was suspended in a stereotaxic frame as described in section 3.1.3.1. Intramuscular electromyogram (EMG) fine wire electrodes were implanted in the medial and lateral triceps surae muscles and connected to an amplifier (Iso-DAM8A, World Precision Instruments Inc., Sarasota FL) which was configured with the following settings: highpass filter at 10 Hz, low pass filter at 3Khz and Gain at 1000. The resulting EMG signal was passed through a custom-built amplifier (high pass 10Hz). The signal was full-wave rectified and low passed filtered at 3Hz with gain set to 56. The fully rectified EMG signal was displayed

and sampled at 5000 samples/s along with the applied force and length signals as described in 3.1.3.1. Each sampled segment was 2 s long. When rigidity had developed, stable baseline responses were recorded and DC was then applied at 3 mA for 1.5 min to ablate the nerve. EMG, force and displacement were monitored for an additional 40 min following the cessation of DC.

3.1.3.4 Nerve injury potentials

In the decerebrated cat that did not develop rigidity, EMG electrodes were placed as in 3.1.2.3. DC was applied as described in section 3.1.3.1. The EMG signal was sampled at 50,000 samples/s. Each sampled segment was 0.2 s long. Following application of DC to the tibial nerve, nerve injury potentials were recorded.

3.1.3.5 pH testing during DC delivery in saline

An identical electrode to the one used to deliver DC (made from Pt-Ir wire), described in section 3.1.2.1, and an anode made from stainless steel, 1 cm in diameter, were placed in a beaker containing 10 ml of isotonic saline. DC was delivered through the electrodes at 1mA, 2mA and 3mA for 1 min and 3mA for 4 min. pH test paper (EMD chemicals Inc, Gibbstown, N.J) was placed in close proximity to the cathode immediately after the cessation of DC.

3.1.4 Data analysis

3.1.4.1 Exploring DC parameters

Data analysis methods for this experiment were described in detail in previous publications (Ravid *et al.*, 2011), so only a brief description is provided here. Force, displacement, current and voltage signals were sampled and stored as described in 3.1.3.1. A custom Matlab program was used to detect the maximal twitch force occurring within each muscle stretching cycle (Figure 3.6). Twenty s segments of data were sampled every 0.5-10 min and the maximal twitch force for each stretch cycle was determined.

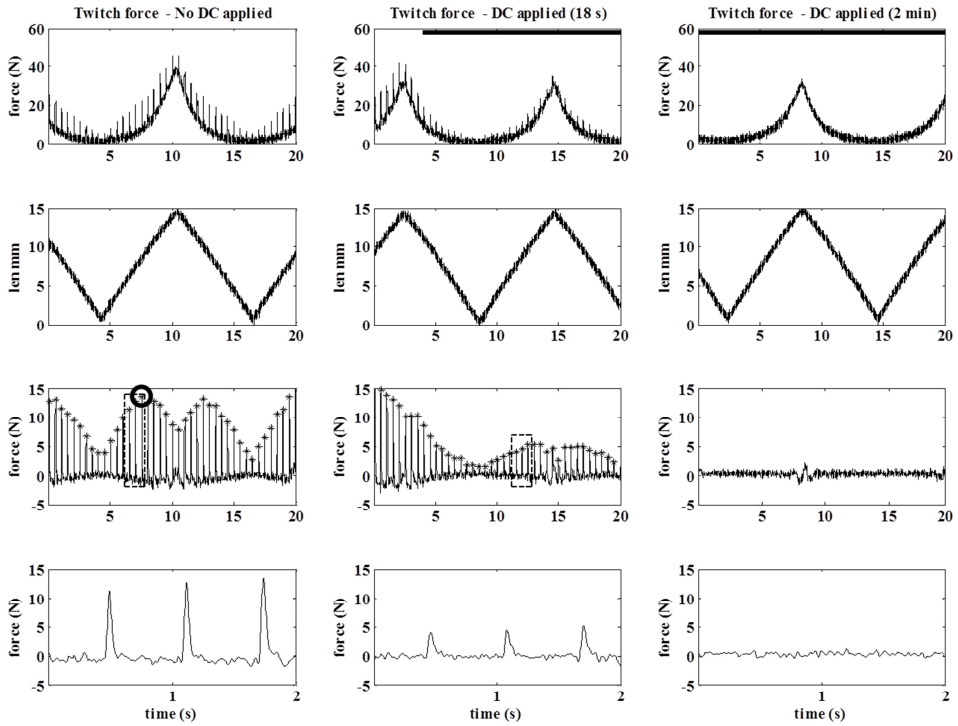


Figure 3.6: Muscle twitches were generated by pulsatile stimulation (200 µs, 2 Hz) through the proximal nerve cuff. Top row: Force transducer signal during triangular changes in muscle length. The black bars represent the time DC was applied through the distal nerve cuff. Second row: Imposed displacement. Third row: Twitch forces after the passive force was removed by filtering. The peak force in each twitch, (identified with asterisks) and the maximal peak force within each stretch cycle (circled asterisk) was automatically identified by a software program.

The maximal twitch force for each sample was then presented on a graph with the x axis representing the timeline of the experiment.

3.1.4.2 DC application during conduction block with Lidocaine

The force transducer signal and DC source were sampled and downloaded to a desktop computer as described in section 3.1.3.1. The force signal was then digitally low-pass filtered (50Hz) and the mean peak twitch force was calculated for each 20 s sample. The maximal twitch force for each sample was then presented on a graph with the x axis representing the timeline of the experiment.

3.1.4.3 Charge density calculations

In previous studies examining safe stimulation of nerves, the charge density per phase was found to be the most relevant variable (McCreery *et al.*, 1990). To calculate charge density, we estimated the surface area of the exposed wire inside a given nerve cuff in contact with the nerve, taking into account the semi-circular profile of the exposed wires. The DC cuff used in the first acute rabbit experiment had an estimated surface area of $0.3*0.5*\pi/2 = 0.236$ cm^2. The DC cuff used in the rest of the experiments had an estimated surface area of 0.093 cm^2. In Figures 3.7 to 3.11 we provide values of total charge density based on the level of DC and the above estimates of surface area.

3.2 Results

3.2.1 Exploration of DC parameters

Details of animals used in these experiments, DC application parameters and summary of results can be found in Appendix A, table A.1.

In previous published experiments on DC nerve ablation (Ravid *et al.*, 2011), the DC amplitudes tested were in the range 0.1-0.5mA except in one experiment in which 1mA of DC was applied for durations of 2 min and more. In the present experiments, we explored the possibility of applying higher levels of DC (up to 2.5mA) for shorter durations, a protocol that might be more suitable for clinical applications. Seven white New Zealand rabbits were tested with the following DC amplitudes: 0.75mA, 1mA, 1.5mA, 2mA and 2.5mA. The duration of application ranged from 20 s to 2.5 min.

In two experiments, 0.75mA DC was delivered to the nerve for durations of 2 min. In the first experiment (Figure 3.7), during the time DC was applied, twitches were abolished, but they recovered quickly upon the cessation of DC. Attenuation of the maximal twitch force was graded, with a reduction to 50% of baseline value after four applications. Little further attenuation followed the fifth application, but complete abolition was produced by the sixth application. Figure 3.8 shows a second experiment with the identical profile of current-duration simulation parameters. In this experiment, maximal twitch force reduction to ~50% of the baseline force value was seen immediately after the first DC application. Thirteen additional DC applications of identical amplitude and duration did not result in significant further attenuation. Note that in this experiment too, twitches were abolished completely during DC applications.

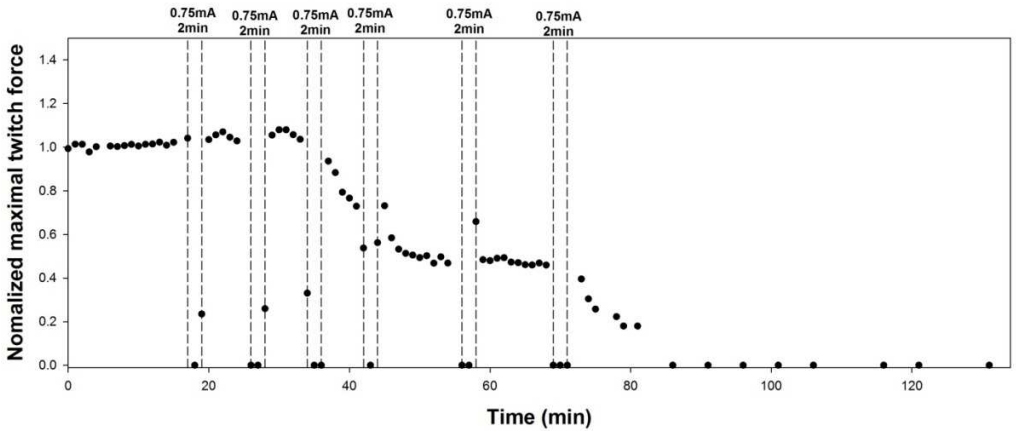


Figure 3.7: Changes in twitch force during and after six episodes of DC (horizontal bars at top) applied at an amplitude of 0.75mA for periods of 2 min. Twitch force was abolished during each DC application, but recovered quickly when DC ceased. Force between DC applications only began declining after the third DC application. An additional three applications were required to abolish force completely for the final 40 min of observation. In this experiment, the nerve cuff had an estimated contact area of 0.236cm^2, so that 0.75mA for 2 min corresponded to a charge density of 0.38 C/cm^2.

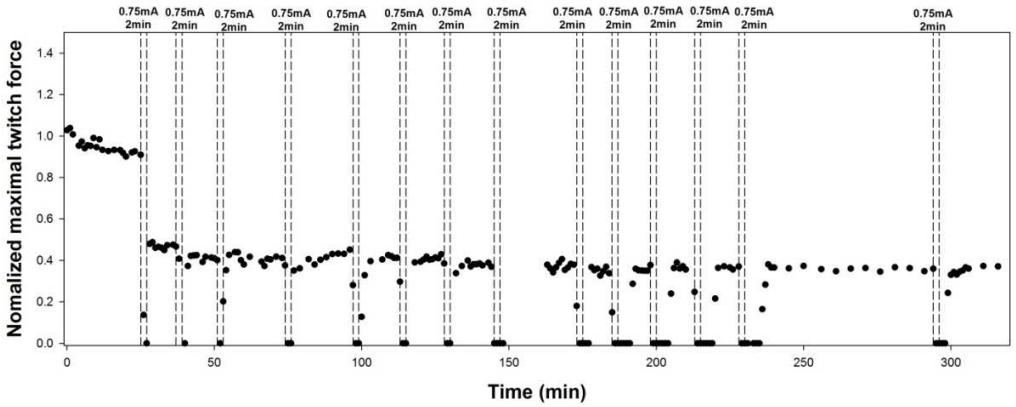


Figure 3.8: Changes in twitch force during and after 14 2-min applications of DC at 0.75mA, as in Figure 3.7. A reduction of the maximal twitch force to 50% of baseline value was evident immediately after the first DC application. No further substantial reduction in maximal twitch force was noted following 13 additional identical DC applications. As in the experiment of Figure 3.7, during each DC application, twitches, and by implication, nerve conduction through the DC cuff, were completely blocked. Recovery of force after each DC application became prolonged as additional DC applications were delivered (min 180 onwards). In this case the nerve cuff had an estimated contact area of 0.093cm^2 so that in each DC application, 0.75mA for 2 min corresponded to a charge density of 0.97 C/cm^2.

The inability to further completely abolish the twitches in the experiment of Figure 3.8 led to a strategy in subsequent experiments where either the amplitude or the duration of the DC application was increased if no force reduction was observed after two to three applications.

3.2.1.1 Constant DC amplitude and variable duration

In three experiments, the duration of DC was increased while the amplitude remained constant (1mA and 2mA). In two of these experiments, one of which is shown in Figure 3.9, after an initial substantial reduction in maximal twitch force, only small reductions occurred in several subsequent DC applications. However, the twelfth DC application completely abolished the twitches, which was unexpected, given that the DC amplitude and duration had not changed.

Another noticeable feature of the responses was an increased duration of recovery time each time DC ceased. This was also observed in other trials in this study (e.g. in Figure 3.8). Once the force was completely abolished, no further DC applications were carried out. In the third experiment, a more gradual reduction of maximal force was observed compared with the first two experiments.

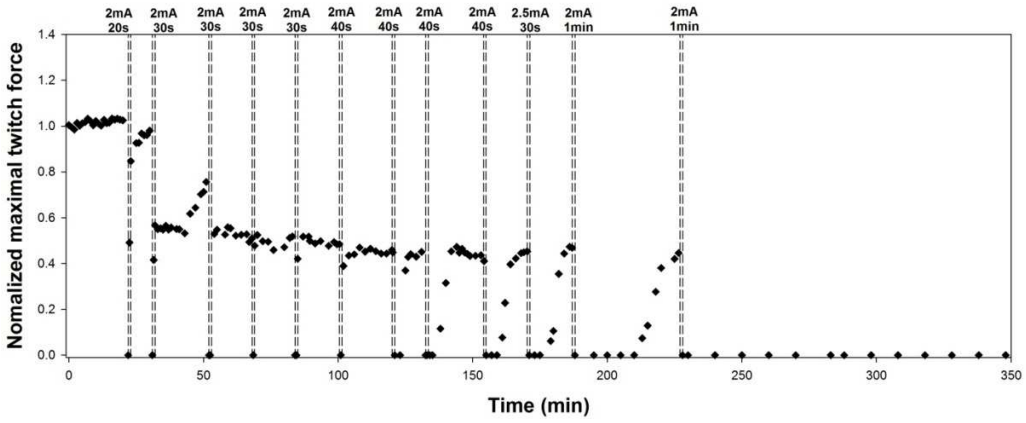


Figure 3.9: Changes in twitch force during and after large amplitude DC applications of various durations. In all but one case (2.5 mA), the DC amplitude was 2mA. Maximal twitch force was attenuated to about 55% of the baseline force after the third DC application. After each of the next 8 applications, force recovered progressively more slowly and to slightly lower levels, indicating increases in duration of nerve conduction block with increasing repetitions and durations of DC. After the 12th DC application, the force was completely attenuated and did not recover for the remaining 120 min of observation. Estimated charge densities were as follows. First application: 0.43C/cm^2; applications 2-5: 0.65 C/cm^2; applications 6-9: 0.86C/cm^2; application 10: 0.81C/cm^2; application 11-12: 1.3C/cm^2.

3.2.1.2 Constant DC duration and increasing amplitude

In a fourth experiment, the duration of DC application remained constant and the amplitude was progressively increased. A gradual controlled reduction of the maximal twitch force was observed (Figure 3.10)

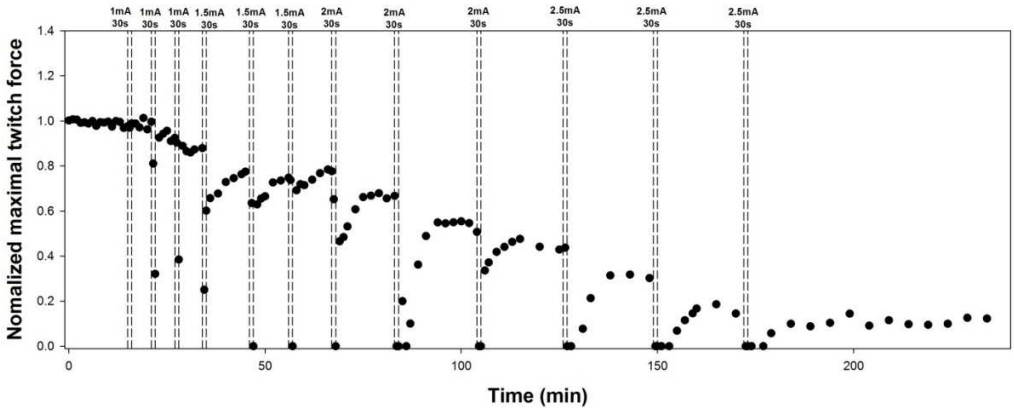


Figure 3.10: Changes in twitch force during and after episodes of DC at progressively increasing amplitude. In this experiment, 1mA of DC was initially applied for 30 s. A graded reduction in twitch force was observed as the DC amplitude was increased. Twitch force was eventually reduced to ~10% of baseline values after 12 applications. The estimated charge densities were as follows: applications 1-3: 0.32C/cm^2; applications 3-6: 0.48C/cm^2; applications 7-9: 0.64C/cm^2; applications 10-12: 0.8C/cm^2.

3.2.1.3 Testing the subcutaneous port

In the experiments described above, DC was applied through the Pt-Ir wire leading from the distal nerve cuff through the skin. In a clinical application, a subcutaneous connector would be used. We therefore tested in 4 rabbits whether DC applied through subcutaneous ports such as the one shown in Figure 3.3, gave similar nerve ablation results. Figure 3.11 shows the results of one such trial in which 1.5mA of DC was applied for periods of 30 and 60 s. A gradual reduction in force was observed with successive DC applications.

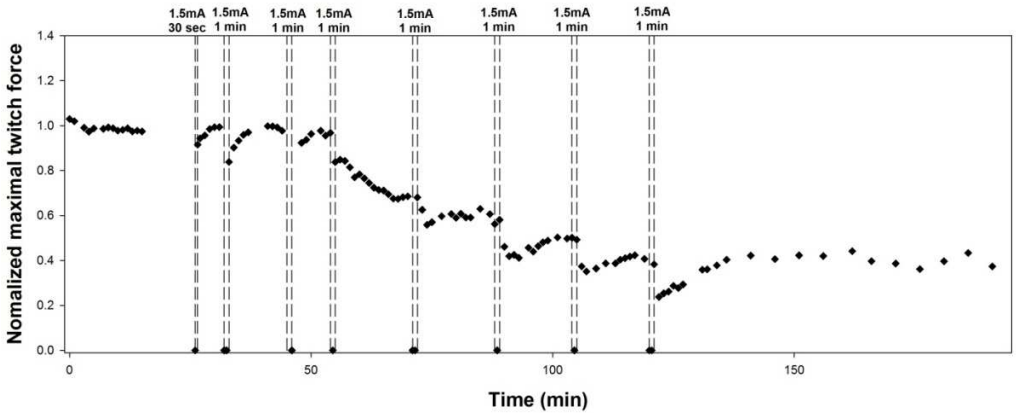


Figure 3.11: Changes in twitch force during episodes of DC delivered through a sub-cutaneous port connector. Initially, 1.5mA of DC was applied for 30 s. Consecutive applications were each 1 min in length. Similar results to those in Figures 3.9 and 3.10, but in this case, a gradual reduction was evident without increases in DC duration or amplitude. After 8 DC applications, the force was reduced to ~40% of baseline value. The estimated charge densities calculated in this experiment were as follows. First application: 0.48C/cm^2; applications 2-8: 0.97C/cm^2;

3.2.2 Decerebrate preparation

Decerebrate rigidity appeared about an hour after the procedures described in 3.1.3.3. Rigidity subsequently remained stable, as judged by reflex EMG responses of the test muscle to repeated stretching prior to DC application, and after this, the responses of the contralateral leg to sensory stimulation of the foot. Baseline values of force and EMG were recorded for 4 min followed by DC application for 1.5 min and then continued observation for 40 min to detect nerve recovery. Figure 3.12 shows the rectified EMG responses to muscle displacement prior to DC onset (A) at the onset of DC (B) and 40 min after DC was turned off (C). Figure 3.13, shows superimposed single stretch cycles before onset of DC, 1 min after onset and 40 min after cessation of DC. At the onset of DC (Figure 3.12B), EMG activity additional to the stretch-evoked responses (Figure 3.12A) occurred, indicating that DC had elicited sensory input. These additional responses quickly subsided and by 1 min after DC onset (Figure 3.13), even the stretch-evoked response disappeared. They remained absent 40 min after DC ceased (Figure 3.12C and Figure 3.13).

In the decerebrate cat that did not develop rigidity, there was also a brief EMG response during the DC ramp-up that was additional to the stretch-evoked responses (Figure 3.14).

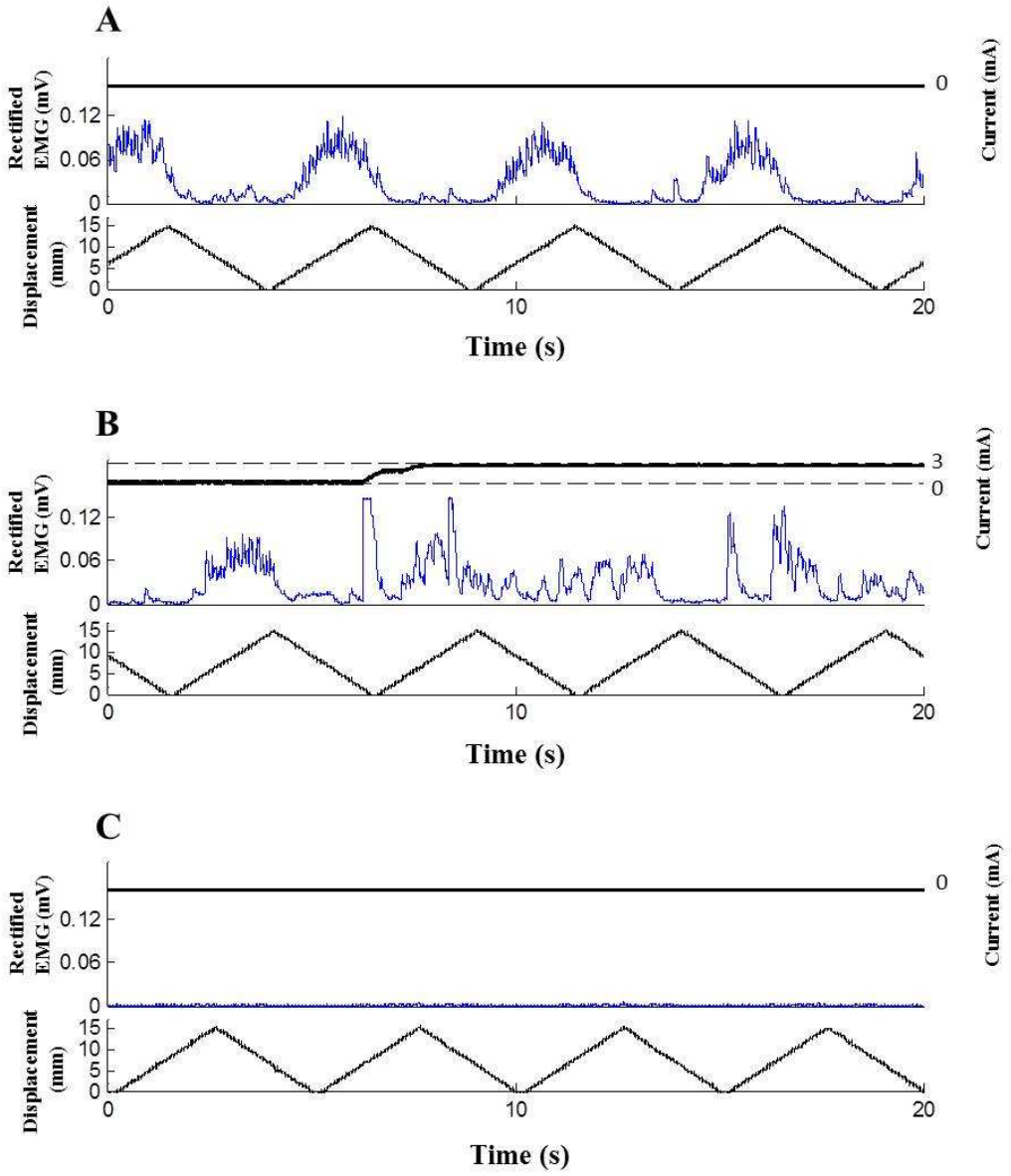


Figure 3.12: EMG responses to muscle displacement (positive incline indicates stretch) in the decerebrate cat that developed rigidity. A) Before DC onset the EMG response was correlated with displacement. B) At DC onset (3mA) the EMG activity became uncorrelated, indicating an additional component of EMG elicited by the DC. C) EMG response to stretch 40 min after cessation of DC at which time, EMG activity was virtually absent.

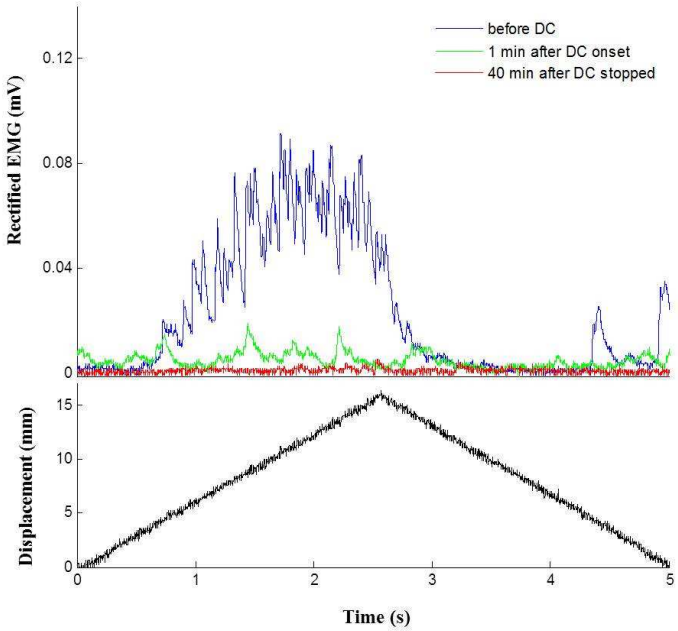


Figure 3.13: Superimposed EMG traces: Before DC onset (blue), during DC (green) and 40 min after the cessation of DC (red). One min after DC onset the additional EMG responses shown in Figure 3.12B subsided and stretch-evoked EMG activity disappeared. EMG responses to muscle displacement remained absent and could not be detected even 40 min after cessation of DC.

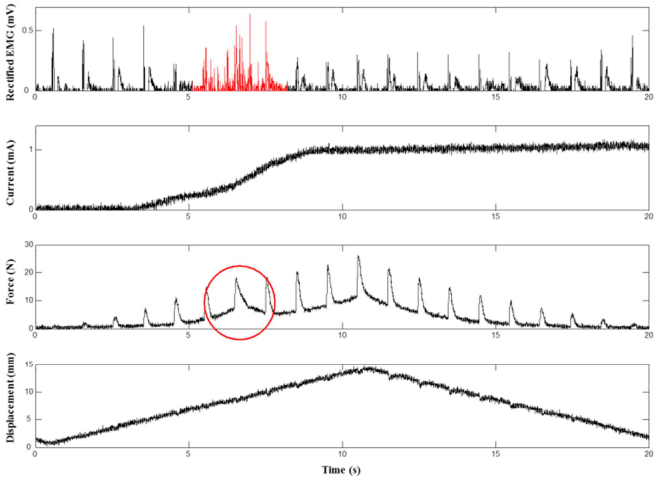


Figure 3.14: Triceps surae EMG and twitch forces during a slow ramp-up of DC and a slow muscle stretch and release in the cat that did not develop decerebrate rigidity. In this experiment the triceps surae was stretched slowly back and forth through 15mm (row 4). Twitches were delivered through the proximal cuff. DC was slowly increased to 1 mA over 5 s (row 2). During this ramp-up, EMG activity additional to that associated with muscle twitches was observed (row 1 red portion of EMG signal), and this was correlated with an elevation in force (third panel red circle).

Figure 3.15 shows the rectified EMG and force responses to muscle displacement before DC (A) and 40 min after DC (B). The muscle force in Figure 3.15A comprised an active EMG-related component plus a passive component. Only the passive component remained in Figure 3.15B.

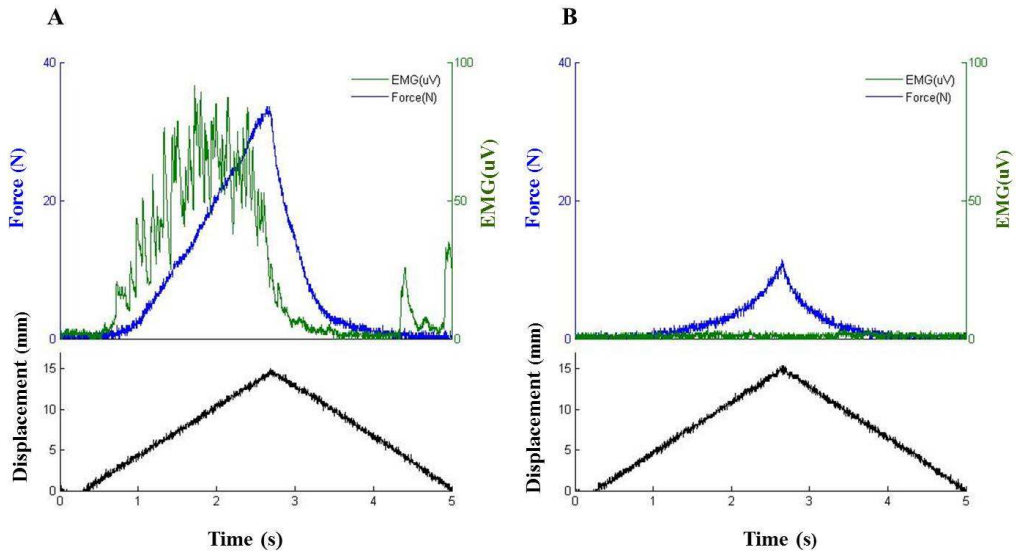


Figure 3.15: EMG (green) and force (blue) responses to muscle displacement (black, stretch upward) before (A) and 40 min after DC application (B). At 40 min, EMG responses were absent and only the passive force resulting from the stretch of the inactive muscle was observed.

Figure 3.16 shows the time course of mean rectified EMG and peak active muscle force in each stretch cycle before, during and after DC was applied to the nerve. The active force in each cycle was calculated by subtracting the average time course of three cycles of passive force (Figure 3.15B, blue signal). The peak active forces in each cycle throughout the experiment were then plotted in Figure 3.16. The mean EMG in each cycle was calculated from the area under the rectified EMG signal and plotted in Figure 3.16. EMG and force were greatly attenuated during DC application but not entirely abolished, as they had been in some of the DC applications in the anesthetized rabbits. During the 40 min post-DC period, no EMG responses to stretching were apparent and only a very small passive force response was observed.

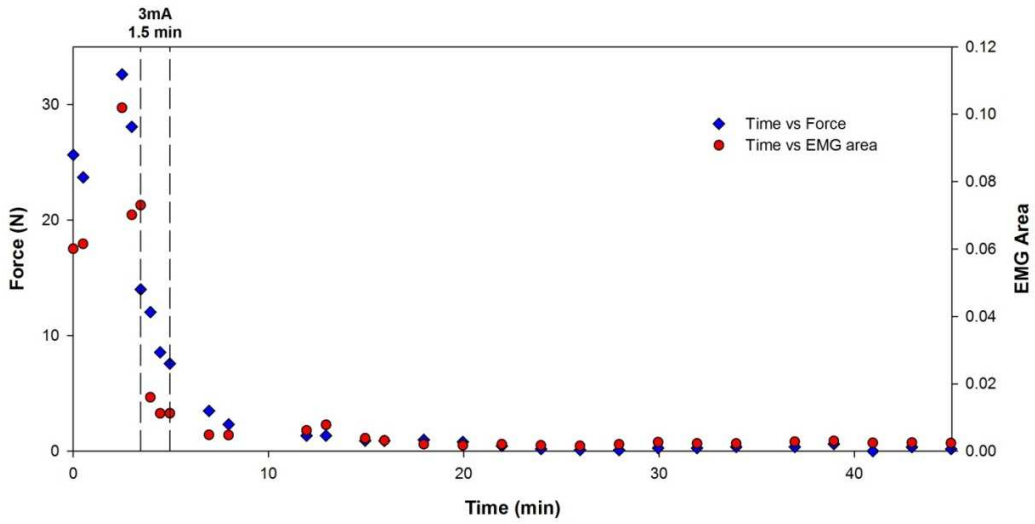


Figure 3.16: Time course of mean rectified EMG (red) and peak active force (blue) per stretch cycle in a decerebrate cat. The two dashed vertical lines indicate DC application (3mA for 1.5 min). Both the EMG and active force responses to stretch diminished to zero over a period of 15 min after DC was applied to the nerve, and remained absent for the following 25 min of observation, indicating that DC had abolished decerebrate rigidity in this muscle.

3.2.2.1 Nerve injury potentials in the absence of decerebrate rigidity

In the cat that did not develop decerebrate rigidity, DC nerve ablation resulted in spontaneous EMG activity that had the characteristics of denervation (fibrillation) potentials. It was previously shown (Conrad *et al.*, 1972) that unlike motor unit action potentials, denervation potentials have a very low variance between consecutive inter-spike intervals, along with a slow change in the mean of these intervals. This behaviour can be seen in Figures 3.17 and 3.18. Raw EMG recorded from the left triceps surae of the decerebrate cat after DC delivery is presented in Figure 3.17. The intervals between the EMG spikes were calculated and are presented in Figure 3.18. Figure 3.18A shows a plot of the inter-spike intervals versus time. Note the very small differences between consecutive inter-spike intervals and the slow trend, first to smaller intervals, then to larger intervals. We calculated the average proportional consecutive interval difference to be 18, which is well below the value of 100, proposed as separating fibrillation potentials from motor unit action potentials (Conrad *et al.*, 1972) Figure 3.18B shows the joint interval histogram where interval I_i is plotted against the next interval I_{i+1} for n values. This emphasizes the very low variance in consecutive intervals, confirming that the DC had resulted in nerve injury.

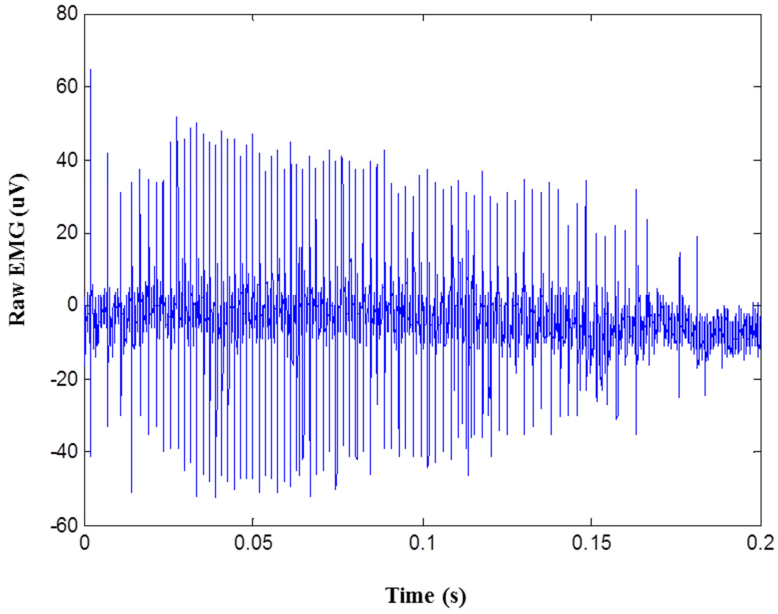


Figure 3.17: nerve injury potentials after DC ablation in a cat that did not develop rigidity after DC nerve ablation. Raw EMG data from medial and lateral triceps surae as recorded from the left leg of the decerebrate cat after ablation of twitch force with DC.

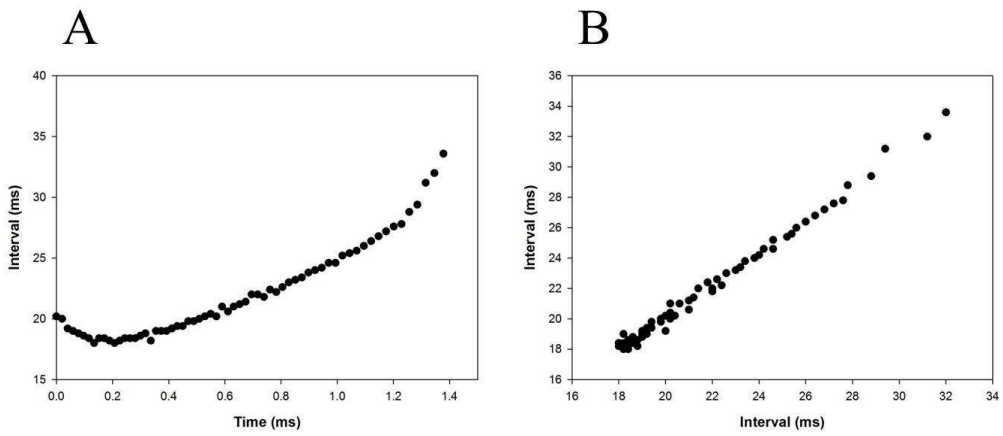


Figure 3.18: Nerve injury potentials analysis. A) Interval versus time histogram. This graph shows the very low difference between successive inter-spike intervals and the slow drift in these intervals that are characteristic of denervation potentials (Conrad et al, 1972). B) Joint interval histogram. The small variation between consecutive inter-spike intervals and the slow drift in these intervals result in a plot in which the points are closely aligned to a straight line with a 45 degree angle.

3.2.3 DC application during conduction block with Lidocaine

Details of animals used in these experiments, DC application parameters and summary of results can be found in Appendix A, table A.4.

It has been posited that high-frequency electrical stimulation of nerves damages them by over-activating voltage-gated sodium channels in the neuronal cell membrane (Agnew & McCreery, 1990). Lidocaine blocks sodium channels (Hille, 1966), the activation of which is required for the generation of action potentials at nodes of Ranvier and therefore the propagation of nerve activity. It was found that when Lidocaine was applied to nerves, this protected them from high-frequency stimulation (Agnew *et al.*, 1990). We reasoned that if Lidocaine also protected nerves from damage caused by DC, this would suggest that DC might ablate nerves by over-activating sodium channels. Accordingly, we performed experiments in 2 rabbits and 1 cat to test this hypothesis.

At the beginning of the experiment (Figure 3.19), the peak forces of muscle twitches elicited by supramaximal stimulation through the proximal nerve cuff were recorded for several minutes to establish the baseline twitch force. A 1% solution of Lidocaine was then slowly injected over 2-4 min into the distal nerve cuff until muscle twitches started to rapidly decline. T_1, the time from complete abolition of twitches to the time twitch forces recovered to 60%-80% of baseline was established (see section 3.1.3.2). Following the recovery of twitch force, an identical dose of Lidocaine was then re-applied to the nerve. Once nerve conduction was again blocked, DC (3mA) was delivered for 4 min. Following this, the nerve was allowed to recover for a time greater than $2*T_1$. In the two rabbits, the contralateral leg served as a control, where only DC (at identical amplitude and duration) was applied. In the cat, Lidocaine was used on both the right and the left leg. The experiment carried out on the right leg was identical to the one described above in the rabbits' right legs. In the cat's left leg, after Lidocaine was injected into the distal nerve cuff, 1mA of DC was applied for 10 min (Figure 3.20). These alternative parameters had previously been shown to result in nerve block that lasted for several weeks in a chronically implanted cat (described in Chapter 4).

In all three animals, after the initial dose of Lidocaine to establish T_1, twitch forces in response to the test stimuli returned to 60%-80% of baseline values within an hour or two. In one of these cases, the amplitude of the proximal test stimuli was increased, which returned the twitch forces close to baseline values. In all three experiments, twitch forces abolished by DC application to the nerve, did not recover, regardless of whether Lidocaine had been applied to the nerve or not.

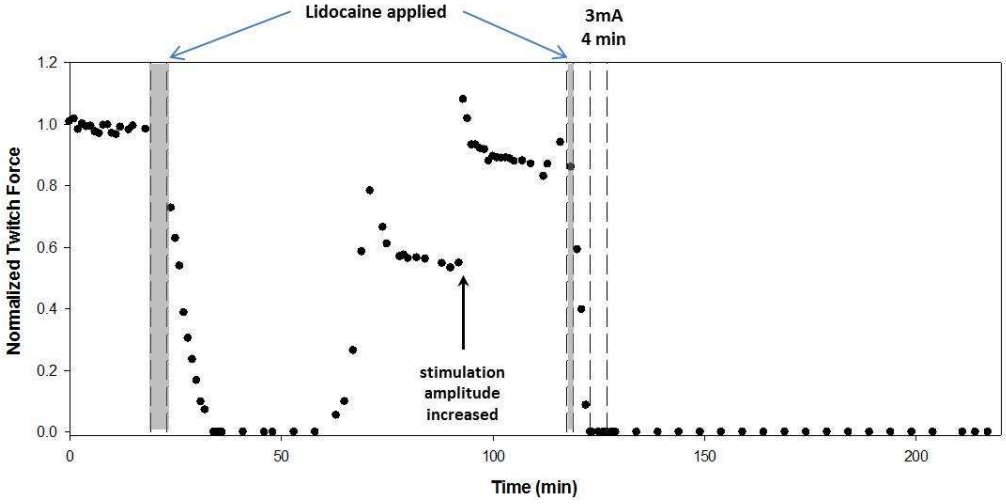


Figure 3.19: Lack of neuroprotective action of Lidocaine during DC delivery. Lidocaine was administered twice, the first time (left arrow) to establish the time course of nerve block and recovery, the second time (right arrow) to cause nerve block during which DC was delivered. After the first Lidocaine application, nerve conduction was completely blocked within 14 min. T_1, the time required for the force to begin to recover from the Lidocaine block was 29 min. At the vertical arrow, the amplitude of the test stimulus pulses was increased at 91 min after the beginning of the experiment and resulted in increase in twitch force. Lidocaine was re-administered for a second time and complete conduction block was achieved 5 min later. One minute after this, 3mA of DC was delivered for 4 min. Force did not recover for the remaining 2 hours of monitoring, indicating that Lidocaine had not protected the nerve from damage.

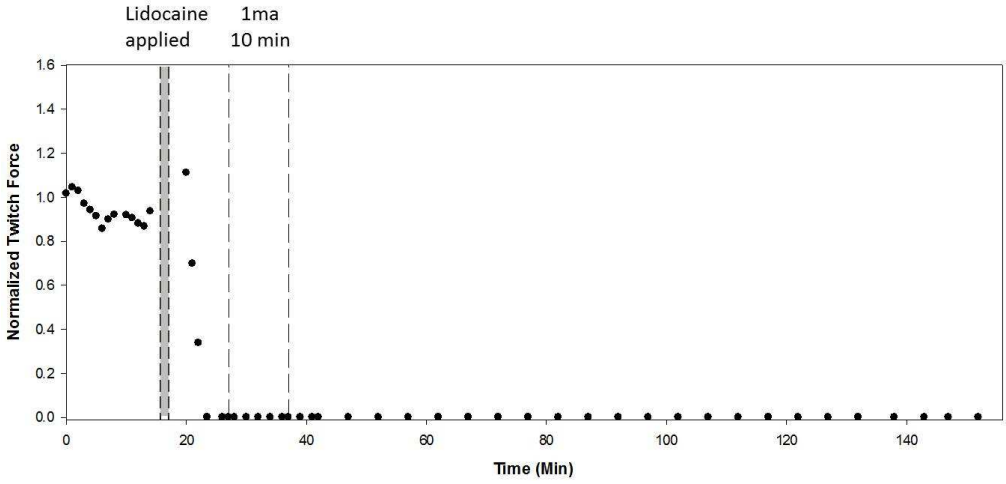


Figure 3.20: Same as in second half of Figure 3.19, performed in contralateral leg. Lidocaine was applied as indicated and 10 minutes later, once the nerve was completely blocked, 1mA DC was delivered to the nerve for 10 min (parameters previously shown to produce a block that lasted for several weeks: see chapter 4). Force did not recover for the remaining 2 hours of monitoring, again indicating that Lidocaine had not protected the nerve from damage.

3.2.4 pH testing in saline

In order to determine whether changes in pH may contribute to the conduction block and damage following DC application, pH testing was conducted in vitro. Two electrodes designed to deliver DC in vivo were placed in a 100 ml beaker of isotonic saline and connected to a DC generator. Initial testing in which a few drops of phenol red were added to the saline, showed that during the application of DC, there was a rapid and dramatic change in colour of the saline to purple, first around the cathode and then above it, eventually filling the entire top half of the beaker. This indicated a significant increase in pH. To obtain more accurate values, thin sections of pH test paper were placed in the vicinity of the cathode. For the durations and amplitudes of DC tested, which were similar to those employed in the animals, the pH close to the cathode changed from a physiological value of about 7.4 to values between 8 and 9.

3.3 Discussion

In this study we explored a range of DC amplitude and duration parameters that provided controlled amounts of nerve ablation. We then demonstrated that DC abolished hyperactive stretch reflexes in the absence of anesthesia in a decerebrate animal model of spasticity. Finally, a possible mechanism of DC nerve ablation was tested with the use of a Lidocaine blockade of nerve conduction.

The present results confirmed previously reported cumulative effects of DC application. In the present study, we tested DC amplitudes of 0.75mA and above, applied for relatively short durations. In the anesthetized animals, complete and immediate conduction block was apparent in the majority of DC applications. The only times this did not occur were at the first application, at instances where the DC duration was brief (< 1min) and where DC amplitude was less or equal to 1mA (e.g. Figure 3.10).

In some of the experiments, one of the parameters (either DC amplitude or duration) was kept constant while the other was increased. In Figure 3.9, DC amplitude was kept constant (with the exception of a single DC application). Twitch force did not show a graded attenuation. Similar results were observed in another animal (not shown). Figure 3.10 depicts the former case, where duration was kept constant and the amplitude was increased over several applications. A more controlled attenuation can be seen in this case. In some of the experiments, graded attenuation was observed after repeated DC applications even though the DC amplitude and duration were constant. More experiments are required with a larger number of animals to provide more clarity on the optimal choice of parameters.

The attenuation or complete block of nerve conduction observed in all experiments was presumably due to a “cathodic block” (Bhadra & Kilgore, 2004) in which a constant

depolarization of the membrane prevents action potentials from propagating. In the experiment in the decerebtrate cat illustrated in Figure 3.12, a short period of EMG activity was recorded when DC was turned off (not shown in the figure). This indicates that a virtual anodic block may also have contributed to conduction block in this case.

Charge densities used in the set of experiments presented in this paper were less than 1.5C/cm^2, which is lower than the charge densities used in previous experiments (Ravid *et al.*, 2011) but still in the range that was defined as damaging (Merrill *et al.*, 2005). Charge density was shown to be one of the major predictors of nerve damage caused by charge-balanced pulsatile stimulation at 50 pulses/s (McCreery *et al.*, 1990). Our results indicate that charge density may not be a reliable predictor of nerve damage caused by DC. In the experiments of Figures 3.7 and 3.8, an identical net charge was delivered (0.75mA for 2 min). However, the electrode surface areas, and therefore the charge densities, differed in the two experiments (0.38C/cm^2 in Figure 3.7 and 0.97C/cm^2 in Figure 3.8). Complete ablation was reached after 6 DC applications in the experiment of Figure 3.7, whereas even after 14 DC applications at the higher charge density (Figure 3.8), nerve ablation was still incomplete.

As was mentioned in chapter 2, cumulative effects of DC conduction block were observed after multiple consecutive applications of DC: force was attenuated faster and remained attenuated longer after successive applications. This is seen in Figures 3.8-3.10. In Figure 3.8 each DC application was 2 min in duration. In applications 1-7 complete conduction block was noted during DC delivery only. Further applications (8-14) resulted in conduction block persisting with increasing durations after the cessation of DC (except for application 14). In Figure 3.9 and 3.10 increased durations of conduction block were noted from the 7th DC application onwards. One interesting observation was the change in the duration of conduction block as the intervals between DC applications were changed. In Figure 3.8, the interval between DC applications 9 and 13 was 10 min and cumulative effects were noted (progressively longer conduction blocks). The interval between applications 13 and 14 was 60 min. The duration of conduction block seen after the 14th application was shorter than that after the 13th application (2 min and 5 min respectively). This suggested that the longer the interval between DC applications, the more the nerve recovers, reducing the duration of conduction block (and presumably the amount of nerve damage) resulting from a subsequent DC application. However, a different result was seen in Figure 3.9. The intervals between applications 8-11 were 20 min, 15 min and 15 min respectively and as in Figure 3.8, longer conduction blocks were observed with each application. However, the interval between the penultimate and final applications was 38 min yet the final application produced a complete and lasting conduction block. In a previous study, longer DC "off" durations resulted in faster nerve recovery from DC block (Ackermann *et al.*, 2011). These results were obtained using a SINE (separate interface nerve electrode), discussed below, which did not result in nerve damage. Though the results in Figure 3.8 also suggest that longer "off" intervals allow more nerve recovery, the results of Figure 3.9 show that this cannot be relied upon.

What is the mechanism by which nerves are damaged by electrical current? One possibility is that the damage is caused by electrochemical processes associated with the injection of large currents across the metal-tissue interface. In a study that tested

this hypothesis, it was shown that Lidocaine or Procaine administered to nerves and blocking conduction in them, protected them from the otherwise damaging effect of charge balanced, high-frequency stimulation (Agnew *et al.*, 1990). Nerves treated with the local anesthetics showed no sign of damage and were histologically similar to un-stimulated nerves, while nerves that were stimulated but not treated with anesthetics showed signs of axonal degeneration. Agnew et al. concluded that the nerve damage in the absence of local anesthetics did not result from electrochemical reactions, but rather from some process (e.g. hyperactivity of the damaged nerves or activation of sympathetic nerve axons, reducing the oxygen supply) which was suppressed by the local anesthetics. In our experiments, DC abolished muscle twitches, which is inconsistent with nerve hyperactivity. Furthermore, nerves treated with Lidocaine did not recover from the application of DC. In Figure 3.19 after the initial control Lidocaine application, an increase in stimulation amplitude was needed to restore the twitch forces to pre-Lidocaine baseline values, indicating a persistent effect of the Lidocaine or perhaps of the low-pH saline used to wash out the Lidocaine. It could be argued that the second dose of Lidocaine had an even longer lasting effect, obscuring an eventual recovery from the DC application. In order to rule this out, Lidocaine was applied only once to the nerve in the contralateral leg of the cat, followed by an application of DC using current and duration parameters previously shown to produce ablation (Figure 3.20). Lidocaine was not neuroprotective in this experiment either, so the mechanisms suggested by Agnew et al. are unlikely to explain DC nerve damage.

This is further supported by work done by Ackerman et al. (Ackermann *et al.*, 2011) in which DC was delivered either using a Pt-Ir electrode to deliver DC to a nerve or an electrode in which the metal electrode and the nerve cuff interface were physically separated with a column of electrolyte inside a silicone tube (separate interface nerve electrode: SINE). When the Pt-Ir electrode was used to deliver DC, there was evidence of rapid, irreversible nerve damage, whereas DC delivered with the SINE caused nerve conduction block during DC, but conduction was completely restored within seconds to minutes after DC ceased. These findings point to a mechanism at the metal-tissue interface.

Nerve damaging electrochemical reactions at the electrode-tissue interface might persist even after the cessation of DC delivery. Evidence of this may be seen in the results of several experiments. In Figure 3.7 for example, continuing attenuation in force was noted following the third, fourth and sixth applications of DC. In Figure 3.11 continuing force attenuation was noted after the 4^{th} application of DC (the second application of 1.5mA for 1min). In the decerebrate preparation continuing attenuation of force after the cessation of DC was also noted (Figure 3.16). It is unclear at this point why the force continued to decrease after the cessation of DC on some occasions and not others.

At high concentrations, Lidocaine itself can cause nerve degeneration (Kroin *et al.*, 1986). In the present study, 1% Lidocaine was injected twice onto nerves. Previous studies have shown that at this concentration Lidocaine produced temporary nerve blocks that did not result in a lasting loss of motor function. Furthermore, Kroin et al. showed that repeated Lidocaine administration at this concentration inside a nerve cuff 3 times a day over 3 days did not result in nerve damage (Kroin *et al.*, 1986). Considering

these data, we can safely assume that the nerve damage caused by DC during a Lidocaine-induced conduction block cannot be attributed to the Lidocaine.

Another possible factor that may contribute to nerve damage is a change in pH in the extracellular fluid at the electrode-nerve interface. The results of experiments described in 3.1.3.5 showed that pH levels rose to between 8 and 9 near the cathode when DC at amplitudes and durations similar to those delivered in the animals was delivered in an isotonic electrolyte solution. Similar results were seen in a previous study in which color pH indicators were used to test pH changes when DC was delivered in a solution that simulated the electrolytic composition of blood (Stevenson *et al.*, 2010). Mortimer et al. suggested that the rate of change in pH was responsible for tissue damage by electrical stimulation and not the change in pH itself (Mortimer *et al.*, 1980). These researchers found that current densities above 50μA/mm^2 damaged muscle tissue. This is an order of magnitude less than the current densities in the experiments described in this thesis, so the pH changes in our experiments may have been much greater. However, another study (Huang *et al.*, 2001) showed that pH shifts observed in vivo following unbalanced electrical stimulation were smaller than ones measured in vitro, probably due to the buffering action of proteins. Results from neurofilament H staining (chapter 2) showed that nerve damage was localized to an area inside the cuff. It is conceivable that the cuff restricted the spread of alkaline reaction products and those that escaped were physiologically buffered by extracellular fluid. The damage seen within the cuff could thus still have been a result of a large increase in pH. Further pH testing during DC application to peripheral nerves in vivo should be conducted in order to determine the true shift in pH that occurs during such stimulation.

In relation to this, one unexpected feature of our Lidocaine experiments was that twitch forces did not recover fully after the application of the Lidocaine solution alone (Figure 3.19). Furthermore, in the experiment of Figure 3.19, even though the test stimuli (0.6mA pulses) prior to the Lidocaine infusion were supramaximal (increases did not elicit larger twitches), during the recovery period, this was no longer the case: an increase of pulse amplitude 0.8mA pulses was needed to elicit maximal twitch responses. During the 90 min following DC application to the nerve, we increased the amplitude of the test stimuli again, to check that that the abolition of twitches was not simply due to a further increase in the threshold to the test stimuli. Previously nerves have been shown to recover conduction fully from a complete Lidocaine block. One factor in our experiments that could account for this was the use of isotonic saline from intravenous drip bags to flush the distal nerve cuff after Lidocaine injection. The quoted pH of these solutions is 5.5. It is likely that during the flushing procedure, some of the saline entered the proximal cuff, which was only 10mm or so proximal to the distal cuff. The ensuing shift in pH may have increased the threshold of the nerve in the proximal cuff. In several classical experiments, it was shown that reductions in pH in the extracellular fluid around mammalian nerves significantly increased their thresholds to electrical stimulation (Lehmann, 1937; Koenig & Groat, 1945).

In the present study there was evidence of nerve injury potentials after DC ablation in the decerebrate cat that demonstrated similar characteristics to denervation potentials and nerve injury discharge. Recordings from chronically injured afferents (Matzner & Devor, 1994) as well as denervation potentials in EMGs recorded in

individuals with peripheral nerve injuries (Conrad *et al.*, 1972), show a characteristically very low variability in the durations of consecutive intervals. It was found that the axons chosen in Matzner's study discharged at fixed intervals (Matzner & Devor, 1994), while denervation potentials demonstrated a slow drift in the mean interval duration (Conrad *et al.*, 1972). Figure 3.18 shows denervation potentials features in an EMG recording from a decerebrate cat following nerve ablation with DC. However, the maximal individual discharge frequency measured from the EMG data (Figure 3.17) was 45 impulses/s. This frequency is more in line with discharge frequencies measured from chronically injured afferents (13-60 impulses/s) (Matzner & Devor, 1994) than denervation potentials frequencies (< 20 impulses/s) (Conrad *et al.*, 1972). This indicates that DC was causing immediate nerve damage.

It is difficult to perform functional muscle tests during DC application to nerves in conscious animals. In order to test whether DC causes enough nerve ablation to reduce spasticity when applied in the absence of anesthesia, we conducted an experiment in which DC was delivered to the tibial nerve of an unanesthetized, decerebrate cat that had developed decerebrate rigidity. The results, presented in Figures 3.12 and 3.15, showed a complete reduction in force and EMG. This provides initial evidence that DC could reduce spastic hypertonus.

The work presented in this report demonstrates that nerve ablation with relatively short durations of DC is effective in reducing muscle force in a controlled manner. The durations and currents we used are within a clinically acceptable range. Further studies of the mechanism of DC nerve ablation are needed. Before a clinical trial could be considered, potential adverse effects such as pain during DC and the development of neuropathic pain need to be evaluated in chronically implanted animals.

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Chapter 4

Nerve lesioning with direct current (DC): delivery of DC in chronically implanted animals in the anesthetized and conscious state

Spastic hypertonus is a condition resulting from upper motoneuron lesions such as spinal cord injury (SCI), stroke, multiple sclerosis (MS) or cerebral palsy (CP), and is characterized by muscle over-activity. It is defined as "disordered sensori-motor control, resulting from an upper motor neurone lesion, presenting as intermittent or sustained involuntary activation of muscles" (Pandyan *et al.*, 2005). Spastic hypertonus can lead to reductions in range of motion and limitations in activity. Individuals with spasticity achieved lower scores in tests of activities of daily life (ADL) (Sommerfeld *et al.*, 2004; Welmer *et al.*, 2006; Lundstrom *et al.*, 2008) and were found to be more likely to be placed in long term care facilities (Watkins *et al.*, 2002). Other reported complications include: clenched fists resulting in skin breakdown and infection, contractures, pressure on nerves and negative body perception (Mayer, 2010). Such complications can impede personal care including grooming and dressing. In SCI populations, spasticity was the most commonly reported secondary complication, ahead of pain and pressure ulcers (Maynard *et al.*, 1990).

Over seven million individuals currently living in the United States (US) have experienced a stroke, with 710,000 events occurring every year (Roger *et al.*, 2012). Over 30% of victims experience motor deficits. The incidence of spasticity after stroke ranges from 17% (Lundstrom *et al.*, 2008) to over 38% (Watkins *et al.*, 2002) in stroke survivors. The prevalence of SCI is estimated at 270,000 affected individuals in the US (NSCISC, 2012). The incidence of spasticity after SCI is reported at over 60% (Maynard *et al.*, 1990).

Current treatments of spasticity include physiotherapeutic methods such as stretch and vibration, anti-spastic drugs, surgical interventions and injections of neurolytic agents. Though conventional physiotherapy is considered indispensable for the treatment of spasticity, approaches such as stretching (Bovend'Eerdt *et al.*, 2008), whole body vibration (Lau RW *et al.*, 2012), splinting (Lannin *et al.*, 2003) and cooling (Gracies, 2001) have not proven to be effective. The primary importance of physiotherapy lies in its contribution to outcomes when combined with other treatment modalities (Goldstein, 2001). Anti-spastic drugs such as Baclofen, Dantrolene and Tizanidine may

help control spasticity but evidence for their efficacy is weak and incomplete (Montane *et al.*, 2004). Side effects of these drugs such as sedation, dizziness, nausea and weakness are common (Rizzo *et al.*, 2004). Surgical intervention is recommended when contractures occur and all other treatment avenues are exhausted (Heijnen *et al.*, 2008). Chemodenervation agents such as phenol and alcohol have been used to treat spasticity for decades (Zafonte & Munin, 2001) but injections are painful and a favorable outcome highly depends on the expertise of the physician (Elovic *et al.*, 2009). Botulinum toxin A (BtA) is considered effective for the control of spasticity (Esquenazi, 2006), but is costly (Horn *et al.*, 2010). Its effect is only observed approximately two to four weeks after injection (McGuire, 2001). Although better tolerated than phenol (Kirazli *et al.*, 1998), BtA can migrate in the body and affect other muscles that are not in the vicinity of the injection site (Fortuna *et al.*, 2011).

Despite the variety of treatments offered for spasticity, functional benefits have not been clearly demonstrated, and in some cases, the treatments can evidently even impede the desired improvements in function (Elovic, 2001; van Kuijk *et al.*, 2002). Furthermore, the large number of individuals affected by stroke and the extensive care required to treat them, places a heavy burden on the health care system (Rothwell, 2001).

The application of direct current (DC) to partially ablate nerves using implantable electrodes was suggested as a novel, cost-effective treatment for spasticity (Ravid *et al.*, 2011). In that report we showed that careful application of DC, in an acute experimental setting, resulted in a controlled amount of reduction in muscle force. Preliminary experiments in chronically implanted animals, conducted under anesthesia, were described as well. In this chapter, we further explore the use of DC to lesion the nerve in a controlled manner in the chronically implanted animal, both under anesthesia and in the conscious state. Electrodes designed for use in conscious animals are described.

4.1 Methods

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

4.1.1 Electrode design for conscious animal experiments

In the design of an implantable electrode for DC nerve ablation, several issues had to be addressed, including the design of a robust nerve cuff, an easily accessible port that would be placed under the skin preventing the animal from damaging or removing it and potential human clinical applications.

Each electrode was composed of a nerve cuff terminal (Figure 4.1), a lead and a subcutaneous port (Figure 4.2). The leads that delivered current pulses to nerves to test conduction were made of multi-stranded stainless steel wire (AS632, Cooner Wire, Chatsworth, CA). The leads that were used to deliver DC to ablate nerves were made of Platium-Iridium (Pt-Ir) wire (0.003" bare, or 0.0055" coated, A-M Systems Inc, Carlsborg, WA).

4.1.1.1 Nerve Cuffs

The lead wires, (stainless steel or Pt-Ir), were coiled inside a silicone tube (508-006, Dow Corning Corporation, Midland, MI). The leads terminated in a nerve cuff comprised of a strip of silicone with a serrated tongue at one end, designed to be wrapped around a nerve and inserted into a slot in the silicone strip, close to the conductive terminal (Figure 4.1). The strip functioned like a "tie-wrap," which was used to fix electrodes to the nerve (Kowalczewski, 2009). Inside each cuff there was a single conductive terminal 5mm in length. The terminal was formed from the lead wire, which emerged from the silicone tube and was coiled tightly around the outside of a second, smaller silicone tube (508-003, Dow Corning Corporation, Midland, MI). Both tubes were filled with silicone sealant (RTV 118, Momentive Performance Materials, Waterford, NY).

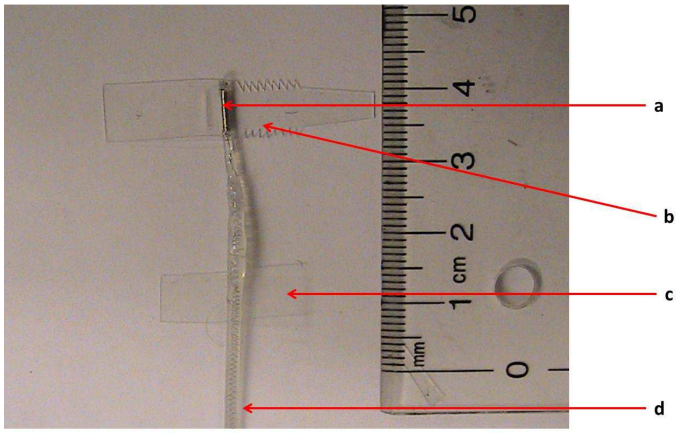


Figure 4.1: Nerve cuff and lead. *a)* Conductive terminal comprised of coiled Pt-Ir wire. *b)* Silicone "tie wrap". *c)* Tie down used to secure the electrode to the fascia. *d)* Coiled lead inside a silicone tube.

4.1.1.2 Subcutaneous ports

The connectors implanted under the skin were prototypes of insulated, conductive subcutaneous ports suitable for human clinical applications, where repeated sessions of controlled nerve ablation were anticipated. The idea was to develop a port into which a hypodermic needle or fine wire, insulated except for its tip, could be introduced through

the skin, providing a temporary electrical connection to the nerve ablation lead. We experimented with both needles and wires. The needles were those of intravenous catheter sets (see below). Alternatively, a length of AS631 Cooner wire, insulated except for its tip, was loaded into a 21G hypodermic needle. The uninsulated end of the wire was folded back from the tip of the needle to form a small (~5mm long) hook. The needle was then inserted into the conductive material as described above and then pulled back, leaving the uninsulated end of the wire securely embedded in the conductive material of the port. The other end of the wire was then connected to a DC or pulse generator. A schematic of the port and methods of insertion are shown in Figure 4.2.

The port was composed of two thin 2x2 cm stainless steel plates (SS316 flat pack, 0.010", Mauldin products, Kemah, TX) or Beryllium copper (0.1" in width). The electrode lead was soldered to one of the plates and the plates were then glued together with silver conductive epoxy (8331-14G, MG chemicals, B.C.), sandwiching the solder joint between them. A 2x2 cm sheets of conductive rubber (C5020PF Canadian Medical Products Ltd, Scarborough ON) or 2x2 cm sheets of conductive silicone (71-ECD-70D-0.32, CS Hyde, Lake Villa, IL) were glued to one of the plates with the same epoxy. In a later design two or three layers of the same conductive material were used (Figure 4.2). The second plate was glued to a non-conductive silastic sheet (0.010"+/- .002, material: SSF-METN-750, Specialty Silicone Fabricators, Paso Robles, CA) with silicone sealant. The silastic sheet was 2x3 cm in size, accomodating space to secure the coiled lead to the sheet in order to provide stress relief. In an early design a single silastic sheet was used. In a later design two silastic sheets glued together with a very thin layer of silicone sealant were employed. This alteration was made because we suspected that the silastic sheet was permeable and had allowed the ingress of extracelular fluid. The entire port and emerging section of lead were coated with a layer of silicone sealant. A photograph of a subcutaneous port is shown in Figure 4.3.

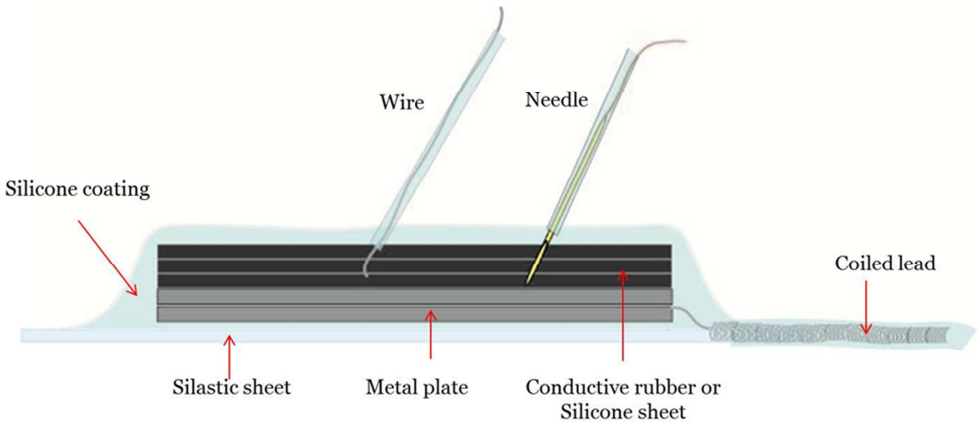
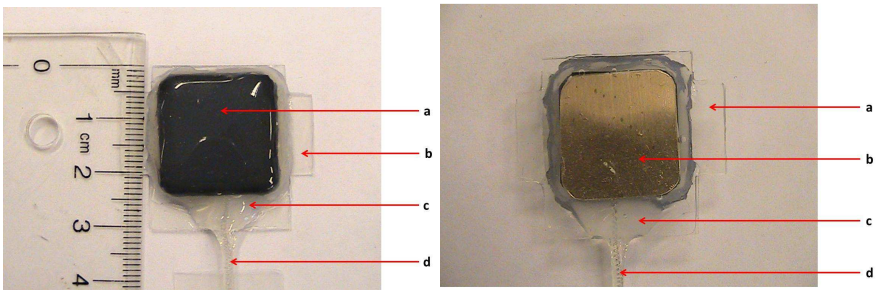


Figure 4.2: Schematic drawing of the subcutaneous port. A metal plate was secured to a silastic sheet on one side, and to a second metal plate on the other side. The solder joint between the lead and one of the plates was sandwiched between the plates. Layers of conductive rubber or conductive silicone were secured to the second plate and the entire port was coated with

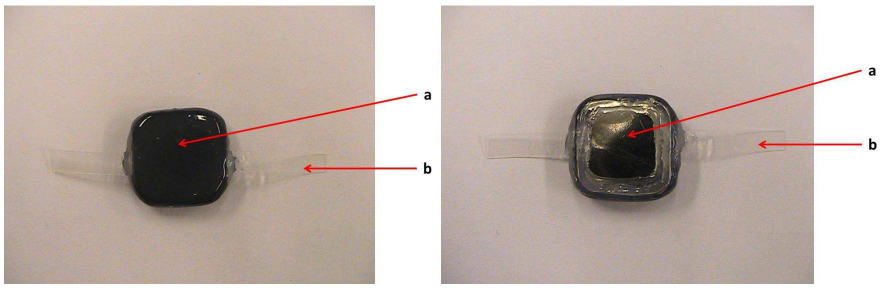
silicone sealant. Two methods were used to connect to the subcutaneous port. One method involved the insertion of a hypodermic needle, insulated except for the tip and connected to a wire lead. In the other method a wire lead, insulated except for the tip, was inserted into the conductive material via a hypodermic needle. The needle was retracted and the wire was left in the conductive material.



(A) (B)

Figure 4.3: Subcutanous port: top and bottom view. A) Top view. *a)* Conductive silicone covered with silicone sealant. Three layers of conductive silicone were used. *b)* Two silastic sheets glued with a very thin layer of silicone sealant. *c)* Silicone sealant coating. *d)* Coiled lead inside a silicone tube secured to the silastic sheet for stess relief. B) Bottom view *a)* Silastic sheets. *b)* Beryllium copper plate. *c)* Silicone sealant coating. *d)* Coiled lead inside a silicone tube.

In animals that were assigned to be stimulated without the use of anesthesia an anode was implanted under the skin of the back near the tail. This was done in order to avoid attaching large surface electrodes to the skin of the animal during DC applications. The implantable anode was composed of two stainless steel plates connected with conductive epoxy. A 2x2 cm sheet of conductive rubber or conductive silicone was glued to one of the plates with the conductive epoxy. Two layers of conductive material were used. The anode was covered with silicone sealant except for an area on the bottom stainless steel plate (Figure 4.4). During experiments, the conductive ends of insulated wires were introduced percutaneously into the conductive rubber or silicone layers as described above. Current was delivered through the wires and transmitted to the stainless steel plate by the conductive rubber or silicone. The exposed underside of the plate delivered the current to the tissues.



(A) (B)

Figure 4.4: Implantable anode: top and bottom view. A) Top view. *a)* Conducive silicone covered with silicone sealant. *b)* A strip of silastic sheet used to secure the anode to the fascia. B) Bottom view. *a)* Stainless steel plate *b)* Strip of silastic sheet to be used as tie down.

The initial design used a single sheet of conductive rubber which proved to be too thin for the needle insertion method. The needle used was a 24G intravenous injection needle having a Teflon sleeve (BD Insyte-W shielded IV catheters, BD medical, Utah). A lead wire was soldered to the needle close to its hub. The needle was inserted through the skin, into the port at a slanted angle, to maximize the amount of conductive rubber contacted by the tip. However, we found that the single layer of conductive rubber did not secure the needle in place sufficiently. Furthermore, the sleeve tended to get crumpled back during insertion. Skin movement when the animal was awake, caused the needle to be displaced from the port. The addition of a second layer of conductive rubber did not improve the stability of the needle connection. The use of the wire as connector was more reliable than the use of the needle, but the wire had to be inserted deep into the conductive rubber in order to be secure and avoid pull-out while the animal was awake and mobile. For these reasons, the conductive rubber was abandoned for conductive silicone, which was softer and allowed for easier insertion, though even in this case needles tended to pull out. Wire insertion proved to be much easier, more dependable and secure with conductive silicone than with conductive rubber. One of the problems encountered with this port configuration (regardless of the conductive material used), was the appearance of tears in the silicone sealant coating, after multiple needle insertions. This raised the suspicion that the tears might have allowed leakage of current from the port. Susceptible areas for tears were the sides of the ports, when insertion was imprecise. However, preliminary testing in saline did not confirm leakage of current when a port with previous multiple needle insertions and tears was tested.

4.1.2 Surgical procedures

4.1.2.1 Chronic surgical procedure in rabbit

In two rabbits, a pair of monopolar nerve cuffs was implanted on the common peroneal nerve of one leg. Implanted electrodes are described in section 4.1.1. The proximal cuff was made of a stainless steel terminal and lead, the distal cuff was made

of a Pt-Ir terminal and lead. The conductive material used in the subcutaneous port was conductive rubber. In one additional rabbit, only a single monopolar nerve cuff was implanted on the common peroneal nerve of one leg. Implanted electrodes were as described in section 4.1.1. The nerve cuff terminal and lead were composed of Pt-Ir wire and conductive silicone was used in the subcutaneous port. The omission of one electrode in the latter implant procedure was done in order to reduce possible damage to the common peroneal nerve due to multiple cuff placements and to allow easier recovery from surgery. An anode was implanted on the lower back of all three rabbits approximately 2 cm rostral to the base of the tail. The implant surgery was performed in a fully equipped operating room with sterile equipment and procedures. The following pre-operative medications were administered: Acepromazine (0.05 mg/kg SC), Ketamine (10 mg/kg SC) and Glycopyrrolate (0.01 mg/kg SC). The rabbits were anesthetized with isoflurane (Forane, Baxter Corp., Toronto, ON, Canada). Anesthesia was maintained with 2-3% isoflurane in carbogen at 1.5 L/min. An intravenous catheter was inserted in the marginal ear 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 iodine solution.

Tie downs (as shown in Figure 4.1) were secured with a 3-0 Silk suture. Muscle incisions as well as skin incisions on the back were closed with 4-0 Vicryl sutures. Skin incisions on the rabbits' legs were closed with 3-0 Prolene sutures. Thirty minutes before the rabbit was taken off anesthesia, ketoprofen (0.5-1 mg/kg SC) was administered to minimize discomfort. During post-operative recovery, the rabbit was kept warm in a heated cage and was also provided with blankets. Analgesia was maintained by giving ketoprofen (0.5-1mg/Kg SC) up to three days post-surgery. Baytril (5 mg/Kg SC) was administered for seven days after surgery.

4.1.2.2 Chronic surgical procedure in cat

A detailed account of this procedure was previously published (Ravid *et al.*, 2011) and is briefly reviewed here. In two cats (cats 2 and 3, table A.3 Appendix A), pairs of nerve cuff electrodes were implanted on the common peroneal nerve of each leg. The distal cuff had a 5mm long terminal, formed from a Platinum Iridium (Pt-Ir) wire wound around a silicone tube and secured to a zip tie cuff, as in Figure 4.1. The proximal nerve cuff terminal was identical in design to the Pt-Ir nerve cuff terminal but was made from stainless steel wire, as was the lead. The lead wire from the nerve cuff terminal in the distal electrode differed in the two cats. In the first cat, the lead was made of stainless steel and the terminal was made from Pt-Ir, requiring a stainless steel-Pt-Ir solder joint as previously described (Ravid *et al.*, 2011). In the second cat, the lead wire was made from Pt-Ir, as was the terminal. No solder joint was necessary since the bare end of the lead wire was coiled around the silicone tube to create the terminal. This change in design occurred due to failure of the stainless steel-Pt-Ir joint several months after implantation. The leads from the proximal and distal nerve cuff terminals were tunneled under the skin of the animal's back to a connector secured in a percutaneous, acrylic headpiece attached to the skull with screws.

Both cats were anesthetized with isoflurane and anesthesia was maintained with 2-3% in carbogen (1.5L/min). At extubation, ketoprofen (0.5-1 mg/kg sc) was given to minimize discomfort. During post-operative recovery, the cats were kept warm in a heated cage and were also provided with blankets. Analgesia was maintained by giving Hydromorphone (0.05-0.1mg/Kg SC) six hours after recovery. A single injection of Convenia was administered to prevent infections.

4.1.3 Experimental procedures

Experiments were performed on 2 cats and 3 rabbits. Two types of procedure were carried out.

Procedure 1: Force measurements done under anesthesia. The twitch forces elicited by test stimuli applied through the proximal nerve cuff were measured. This was done weekly for one to 5 weeks prior to DC nerve ablation and for several weeks thereafter.

Procedure 2: DC nerve ablation. This was performed either under anesthesia (2 cats) or in the awake animal (1 cat, 3 rabbits). Force or acceleration were monitored before and after DC delivery and in 2 cats and 1 rabbit, during DC delivery.

4.1.3.1 Experimental setup and procedures in the implanted cat during brief periods of anesthesia

After the animals had recovered from the implantation surgery, two procedures were performed during brief periods of isoflurane anesthesia performed at weekly intervals. In both procedures, the cat was placed in a prone position. The hind limb 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 potentially damage tissue. 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 force transducer were secured to a heavy metal frame, which in turn was clamped to a table (Figure 4.5).

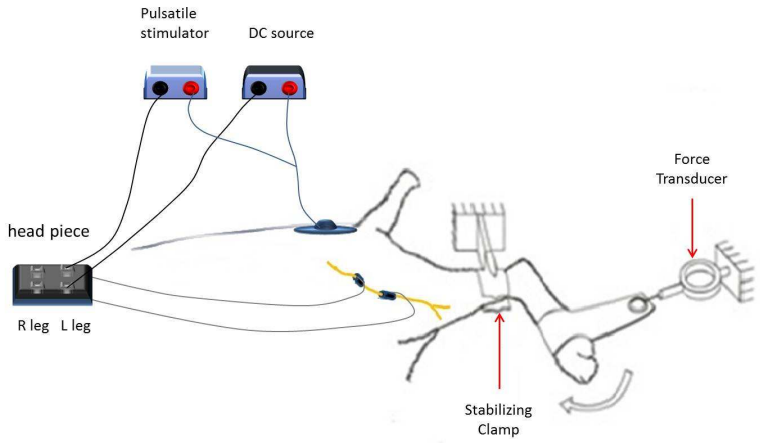


Figure 4.5: Experimental arrangement to partially ablate the common peroneal nerve with DC, while testing conduction with pulses applied proximally. The anesthetized cat was prone, its leg held by a clamp and its foot connected to a force transducer with a looped strap. Test pulses (200 µs, 2Hz) and DC were delivered through the nerve cuffs of leads previously implanted and tunneled subcutaneously to a headpiece connector. The test pulses were delivered throughout the experiment. Force was measured before, during and after DC delivery.

Test muscle twitches were generated by connecting the cathodic output of a custom-built, biphasic, charge-balanced, constant-current pulse generator to the proximal lead via the headpiece connector. The anodic output of the pulse generator was connected to a self-adhesive gel electrode (Kendall ES40076) on the back of the animal (Figure 4.5). Continuous trains of pulses (200 µs, 2Hz) elicited test twitches of the tibialis anterior and extensor digitorum longus muscles. Dorsiflexion force was measured using a force transducer. At the beginning of each of these procedures, muscle twitch thresholds were monitored by applying pulsatile stimulation through each of the electrodes. This was done to check on the viability of the electrodes.The optimal ankle angle for muscle force production was determined for each animal by making a series of measurements at different ankle angles of the twitch forces elicited by stimulation through the proximal nerve cuff. An angle-force curve was constructed, from which the optimal angle was determined.

The signal from the force transducer, along with current and voltage signals from the DC generator during DC delivery were viewed on a digital 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, with the use of Tektronix Visa software. The oscilloscope was set to sample at 500 samples/s enabling 20 s segments of data to be stored as digital files. Pulsatile stimulation was delivered through the proximal cuff and five 20 s segments of data were recorded 30 to 60 s apart.

In procedure 2 (DC nerve ablation), a custom DC source was used to deliver DC. The cathode of the DC stimulator was connected to the appropriate pin on the head connector that led to the distal cuff. The anode of the DC stimulator was connected to a wettable pad surface electrode 3cm in diameter, similar to those used in functional electrical stimulation garments, placed on the back of the animal. The biphasic pulse stimulator was connected to the proximal cuff through the appropriate pin on the head connector leading to the proximal cuff, and the anode was connected to the same wettable pad electrode on the back of the animal. Muscle twitch force was first measured for approximately 10 min to provide a baseline. DC was then delivered via the distal cuff for several min. Muscle twitch force was recorded for approximately 10 min prior to DC delivery, during DC delivery and for about 20 min after DC delivery at intervals of 1 to 5 min.

In cat 2, both procedures were performed during one session of anesthesia. After procedure 1 was completed, 0.5mA DC was delivered for a duration of 10 min. Twitch forces were monitored before, during and for 30 min after DC delivery. The cat was then allowed to recover from the anesthetic. Procedure 1 was subsequently performed weekly for 6 weeks as well as at weeks 9 and 10 after DC ablation.

In cat 3, procedure 1 (force measurements) was performed weekly for five weeks. Procedure 2 (DC ablation) was then performed twice, with an intervening period during which nerve function recovered. The first DC ablation session (1mA for 10 min) occurred 13 weeks post-implant. Twitch forces were monitored before, during and for 1 hour after this DC delivery. The cat was then allowed to recover from the anesthetic. Procedure 1 was carried out on nine different occasions between weeks 14 and 30 post-implant, 1 to 3 weeks apart with the exception of weeks 17 to 22 post-implant. A second DC ablation session (1mA for 9 min) was carried out at week 30 post-implant. Twitch forces were monitored before, during and for 1 hour after this DC delivery. As before, the cat was then allowed to recover from the anesthetic. Force measurements were conducted ten times between weeks 31 and 50 post-implant, 1-4 weeks apart with the exception of weeks 39 to 45.

4.1.3.2 Experimental setup and procedures in the implanted, awake cat

Cat 2 was used to test whether DC ablation could be performed while the cat was awake. After the DC ablation experiment described above, it took about 10 weeks for twitch forces to return to pre-lesioning values. Starting 8 months after this, procedure 1 (baseline force measurements) was performed during brief periods of anesthesia on 4 separate occasions. On three subsequent occasions, the cat was brought to the laboratory for procedure 2 (DC delivery in the awake animal). In these cases, the cat was allowed to move freely in a padded open crate placed on a table and was offered treats. Connections to the headpiece from the biphasic electrical stimulator and DC source were as described in section 4.1.3.1. Long leads were used in order to allow the cat to move freely.

4.1.3.3 Experimental setup and procedures in the implanted, awake rabbit

Three rabbits were implanted as described in section 4.1.2.1. One rabbit was implanted with a single nerve cuff and two were implanted with two nerve cuffs (table A.6 appendix A).. In one of the rabbits (rabbit 3) implanted with two nerve cuffs, the distal cuff (Pt-Ir DC electrode) failed. In all three rabbits, leads from the external current sources were connected to the implanted nerve cuffs via the subcutaneous ports as described in section 4.1.1. Two types of procedure were conducted. Procedure 1 involved the measurement of muscle twitch forces as described in section 4.1.3.1. Procedure 2 was the application of DC when the animal was conscious. In the rabbit implanted with only one (Pt-Ir) electrode, this electrode was used in procedure 1, to apply test pulses, and in procedure 2 to apply DC.

Procedure 1: measurements of muscle twitch force to establish baselines were performed during brief periods of anesthesia (see 4.1.3.1), for several weeks before and after procedure 2. Leads were connected to the implanted electrodes via the subcutaneous ports. Signals from the force transducer and the DC source were viewed on the Tektronix oscilloscope and downloaded to a desktop computer at intervals ranging from 30 to 60 s (see 4.1.3.1).

Procedure 2: The animal was briefly anesthetized with isoflurane. Cooner lead wires or needles were inserted percutaneously into the subcutaneous ports. The leads were looped and secured to the animal's body to minimize the event of wire pullout during movement once the animal was awake. A 3-axis accelerometer (ADXL335, Analog Devices) was fixed to the foot, 60mm from the pivot point of the ankle joint. The animal was wrapped in a blanket except for the test leg, which was held by the experimenter at the shank while slightly elevated from the table in order to prevent the foot from touching the table during dorsiflexion (Figure 4.6). The animal was securely held with its head tucked under the experimenter's elbow to ensure that the eyes were covered.

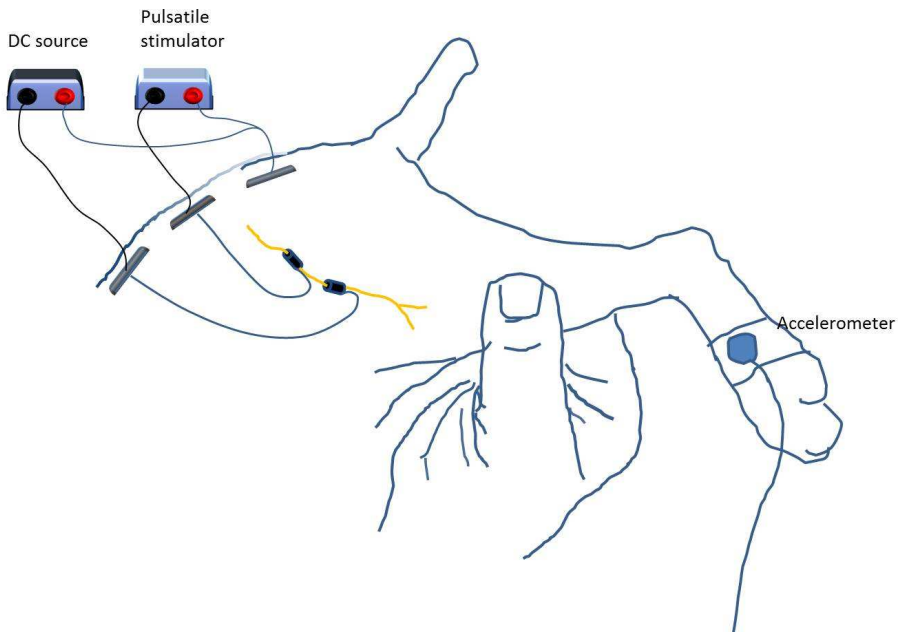


Figure 4.6: Experimental setup for delivery of DC in the chronically implanted rabbit. Initially, the animal was anesthetized and stainless steel needles or wires were inserted into the subcutaneous ports as described in section 4.1.1. The animal was wrapped in a blanket, allowed to wake up and the test leg was then held as shown. Test pulses or DC were delivered via needles or wires inserted percutaneously into implanted subcutaneous ports.

In all three rabbits procedure 1 was performed in the weeks before and after DC was delivered to the awake animal (procedure 2). DC was applied to the nerves in all three rabbits when they were conscious. It was not possible to measure force when the animals were awake, so instead, in two of the animals, an accelerometer was fixed to the foot. Signals from the accelerometer and the DC source were viewed on the Tektronix oscilloscope and downloaded to a desktop computer at intervals ranging from 30 s to 120 s.

In rabbit 1, in which only one electrode had been implanted, pulsatile stimulation and DC could not be delivered simultaneously. For this reason, accelerometer recordings of twitches were only carried out during brief periods of isoflurane anesthesia before and after DC delivery. During the first period of anesthesia, leads were inserted percutaneously into the subcutaneous ports as described in section 4.1.1. The accelerometer was placed on the foot and pulsatile stimulation was delivered to the nerve. After about 5 min of accelerometer recordings of twitches, the animal was allowed to recover from anesthesia, as judged by its ability to hold its head up and make exploratory movements. The leads were transferred from the pulse generator to the DC source. Full recovery took about 5 min. Ten min after anesthesia was discontinued, DC (5 mA for 3 min) was delivered to the nerve. After the cessation of DC the animal was again briefly anesthetized. The leads were transferred back to the pulse generator. Pulsatile stimulation was resumed along with accelerometer recordings of twitches.

In rabbit 2, implanted with two nerve cuffs that remained viable throughout, after the baseline force measurements had been performed, DC was applied on four occasions, separated by 9 weeks, 1 week and 1 week respectively (Appendix 1, Table A.6). On each occasion the animal was first anesthetized with isoflurane for a few minutes. Leads were inserted into the subcutaneous ports. On the last three occasions an accelerometer was placed on the foot and pulsatile stimulation (200 μs, 2Hz) was delivered via the proximal cuff (section 4.1.3.3). DC (3mA) was applied for 5-6 s via the distal cuff to confirm nerve blockade, as evidenced by the abolition of muscle twitches . Anesthesia was discontinued and the animal was allowed to recover. During this recovery, which lasted about 10 min, DC (3mA for 5-6 s) was again applied to confirm nerve blockade. Once the animal was fully awake, as judged by its ability to hold its head up and make exploratory movements, DC was delivered for longer durations.

Rabbit 3 had been implanted with two nerve cuffs, distal (Pt-Ir) and proximal (stainless steel). DC delivery was carried out on two occasions. On the first occasion DC was delivered through the Pt-Ir electrode, but prior to the second DC application muscle twitch thresholds via the Pt-Ir electrode had risen from 0.2mA to more than 0.6 mA. Furthermore, local pressure applied over the cuff reduced the threshold. This indicated that the Pt-Ir electrode had migrated and was no longer reliable. Consequently, DC ablation (procedure 2) was carried out on the second occasion with the use of the stainless steel electrode. The animal was first anesthetized with isoflurane for a few minutes. Wires were inserted into the subcutaneous ports. Anesthesia was discontinued and the animal was allowed to recover. Once the animal was fully awake, as judged by its ability to hold its head up and make exploratory movements, DC was delivered.

Delivery of DC was terminated if and when the animals showed signs of discomfort.

4.1.3.4 Transducer calibrations

At the end of each force measurement experiment, the force transducer was calibrated with a spring balance. The accelerometer was calibrated by rotating it rapidly through 90 degrees with the use of a custom hinged support.

4.1.3.5 Data analysis

A Matlab (The MathWorks, Inc., Natick, MA) program was written to detect the peak amplitude of the muscle twitch force generated during pulsatile stimulation. From each session prior to and after DC lesioning that twitch forces were measured under anesthesia in both the cat and the rabbits, five data segments, each 20 s in length, were collected and the mean twitch amplitude was computed. The overall mean twitch amplitude from all individual baseline sessions was then computed and used to normalize the means of the individual sessions (Figure 4.8).

For the sessions in which DC was delivered in the anesthetized cat, the mean twitch force was calculated for each 20 s of sampled data (one data segment) and normalized

to the mean baseline value (the mean of the first five twitches in the plot, as shown in Figure 4.9).

For the sessions in which DC was delivered in awake rabbits, the mean peak-to-peak value of acceleration was calculated for each 20 s of sampled data (one data segment) and normalized to the mean baseline value.

4.2 Results

4.2.1 DC delivery in the chronically implanted cats during brief periods of anesthesia

Details of animals used in these experiments, DC application parameters and summary of results can be found in Appendix A, table A.3.

In the first cat (cat 2 table A.3 appendix A), as described in 4.1.3.1, procedure 1 (force measurement) and procedure 2 (DC delivery) were performed in a single session of brief anesthesia. After procedure 1 was completed, 0.5mA DC was delivered for 10 min. Twitch forces were monitored before, during and for 30 min after DC delivery. The cat was then allowed to recover from the anesthetic. In the 5 weeks following DC delivery, the mean twitch force was 40% to 50% of the mean baseline value. At week 6 it began recovering to ~60%. In week 9, the mean force had actually increased to ~130% of baseline value and remained at this level in week 10.

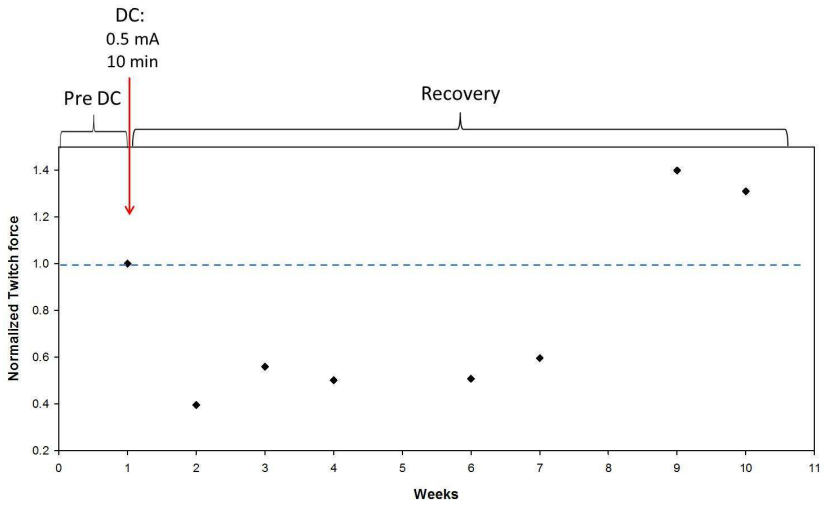


Figure 4.7: Mean twitch forces in a chronically implanted cat (cat 2 table A.3 Appendix A) in the weeks before and after two sessions in which DC was delivered to the common peroneal nerve. Each dot represents the mean twitch force derived from five data segments recorded in a single force measurement experiment as described in 4.1.3.5. The DC application resulted in 60% decline in force as was measured in the following week. Force recovered over the next 8 weeks.

In the second cat (cat 3 table A.3 Appendix A), five force measurement experiments were performed, starting 2 weeks after the implant surgery. The mean twitch forces increased during this period, probably reflecting a recovery from the effects of surgery. Figure 4.8 displays the normalized mean twitch forces recorded over 40 weeks. The mean twitch force just prior to DC delivery had increased from the previous two baseline values. DC (1mA for 10 min) was then delivered. No force could be detected in the week following DC application. The first sign of recovery started 3 weeks later. Force gradually recovered to baseline values over the following 13 weeks. A second DC application (1mA for 9 min) was delivered in week 30 post-implant. Twitches were again abolished. The mean twitch force then gradually recovered over the subsequent 20 weeks and reached the baseline value in week 50.

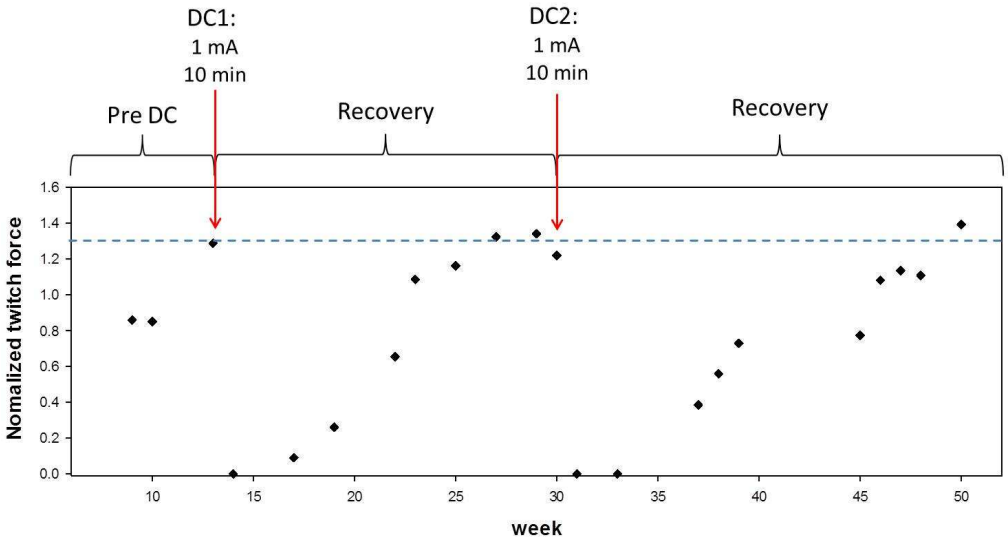


Figure 4.8: Mean twitch forces in a chronically implanted cat (cat 3 table A.3 Appendix A) in the weeks before and after two sessions in which DC was delivered to the common peroneal nerve. Each dot represents the mean twitch force derived from five data segments recorded in a single force measurement experiment as described in 4.1.3.5. Forces were measured in the weeks prior to the initial DC application. Although the force seemed stable from measurements conducted in weeks 9 and 11 it increased was increased when measured just prior to DC application, which proved to be the true baseline value. Therefore the line indicating the baseline is not at 1.0 (the average of the 3 measurements taken prior to DC) but at the true baseline value measured just prior to DC1. The first delivery of DC (DC1) caused a rapid decline in twitch force to zero (see Figure 4.9) and this was maintained in the following week. Force gradually recovered to the baseline value over the subsequent 13 weeks. A second application of DC (DC2) resulted in a similar time course of twitch decline followed by recovery.

Figure 4.9 shows the results of the first DC application (DC1) on an expanded time scale. Twitch force amplitude was reduced gradually over the 10 min DC was applied. DC application was discontinued once twitches completely disappeared. Force did not recover for the following 54 min.

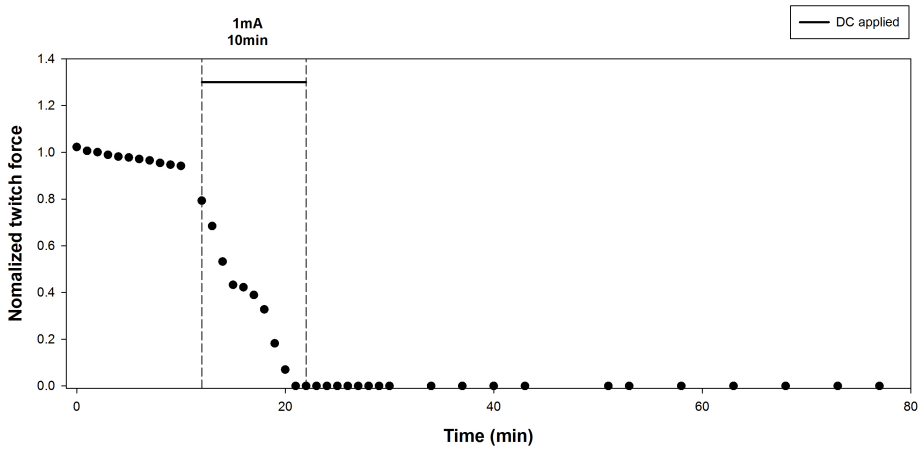


Figure 4.9: DC delivery (DC1 of Figure 4.8) on an expanded time scale. Mean twitch forces are shown for 10 min prior to DC delivery. DC caused the force gradually to decline over a further period of 10 min. DC was discontinued when twitches disappeared. Force continued to be monitored for a further 54 min to ensure that nerve conduction had been completely blocked.

4.2.2 DC delivery in chronically implanted, awake cat and rabbits

Details of animals used in these experiments, DC application parameters and summary of results can be found in Appendix A table A.6.

4.2.2.1 Cat

As mentioned earlier, after the DC ablation experiments performed under anesthesia in cat 2, it took about 10 weeks for twitch forces to return to pre-lesioning values. On three separate occasions, starting 8 months after this recovery, DC was delivered while the cat was awake (see section 4.1.3.2). On the first occasion, DC was applied twice (0.5mA for 3.5 min and 4 min respectively with a 4 min break). On the second occasion, DC was delivered three times for (0.75mA for 6 min, 3 min 40 s and 2 min 40 s respectively) and on the third occasion DC was delivered once (0.75 mA for 5 min 40 s). Procedure 1 (force measurements under anesthesia) was performed between each of the three DC sessions, and on several occasions after the third DC session. The animal seemed comfortable while DC was delivered, except at the onset of DC when it flexed one of its forelegs for a few seconds, after which it resumed its normal stance posture and continued eating treats. Surprisingly, in light of the results obtained when the same cat was anesthetized, the mean twitch force showed only slight decreases in the first week following the first and second DC deliveries, but had recovered to baseline values

by the second week. No changes in mean twitch force were observed after the third DC delivery.

4.2.2.2 Rabbits

In rabbit 1 which had only one implanted nerve cuff, DC and pulsatile stimulation could not be delivered simultaneously. Acceleration was measured during brief periods of anesthesia before and after DC was delivered to the awake animal as described in section 4.1.3.3. The peak acceleration measured during the brief period of anesthesia after DC was delivered was not significantly different than that measured during the first period of anesthesia prior to DC delivery. Peak twitch force measurements (procedure 1) were conducted in the weeks before and after DC delivery. There was no obvious reduction in force after DC (Figure 4.10).

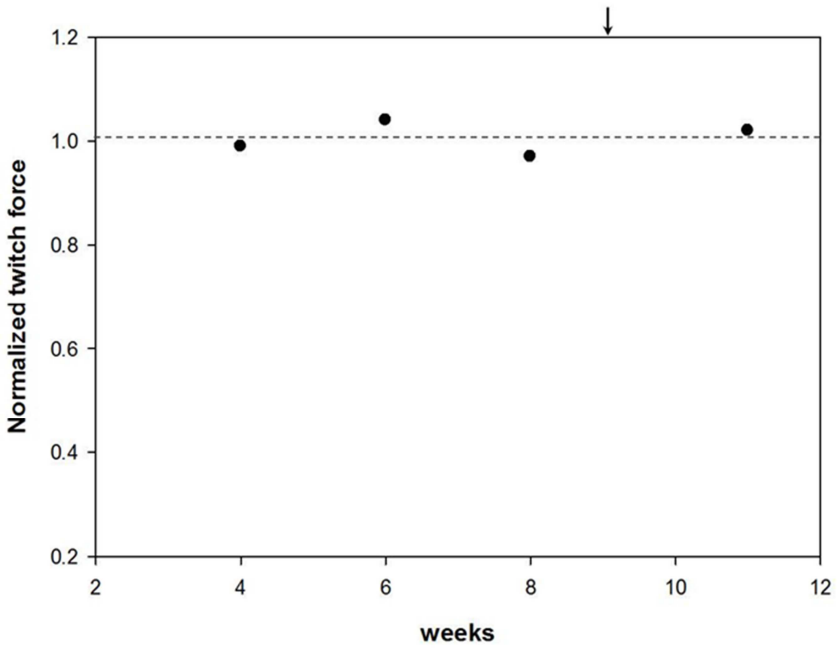


Figure 4.10: Mean peak twitch force measured in the weeks before and after application of DC in rabbit 1. Each dot represents the mean value of five 20 s data segments as described in the methods section. Each arrow represents an occasion when DC was applied to the nerve (procedure 2). No reduction in force was evident in the week following application of DC to the nerve (5ma, 3 min).

Rabbit 2 was implanted with two electrodes (proximal –stainless steel, distal – Pt-Ir) which remained viable throughout. After the animal was awake, DC was applied for 5-6 s as described in section 4.1.3.3 to confirm nerve blockade (Figure 4.11). Once blockade was established DC was applied for longer durations with the aim of ablating the nerve.

On the first occasion 1.5mA was delivered for 6 min 10 s. This resulted in ~20% attenuation of force which recovered over 4-5 weeks (Figure 4.14). On the second occasion, 3mA DC was delivered for 80 s, 15.5 min after anesthesia was discontinued. On the third occasion, 3mA DC was delivered for 67 s, 8.5 min after anesthesia was discontinued. On the fourth occasion, DC was applied three times (Figure 4.12): 3.5mA for 20 s at 8.5 min post-anesthesia, 3mA for 30 s at 11.5 min post-anesthesia and finally, starting at 20 min post-anesthesia, 4mA for the first 7 s then ramping up to 5mA for another 58 s. The raw signals from this third application of DC are shown in Figure 4.13.

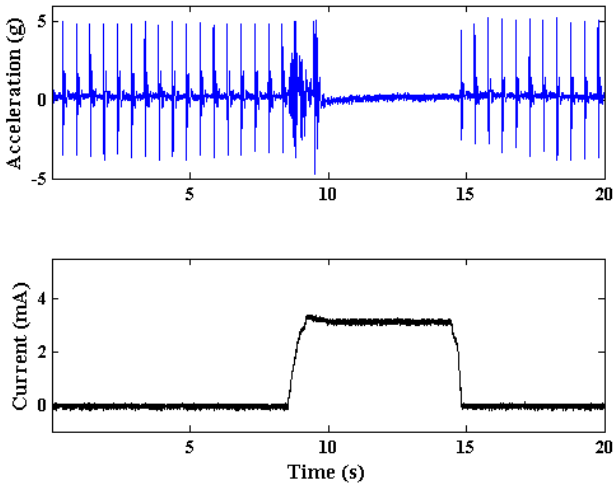


Figure 4.11: Test twitches abolished by 3mA DC applied to the nerve in an awake rabbit. Top: Twitch accelerations elicited by test stimuli applied to the common peroneal nerve via the proximal electrode. Bottom: DC delivered to the nerve via the distal electrode.

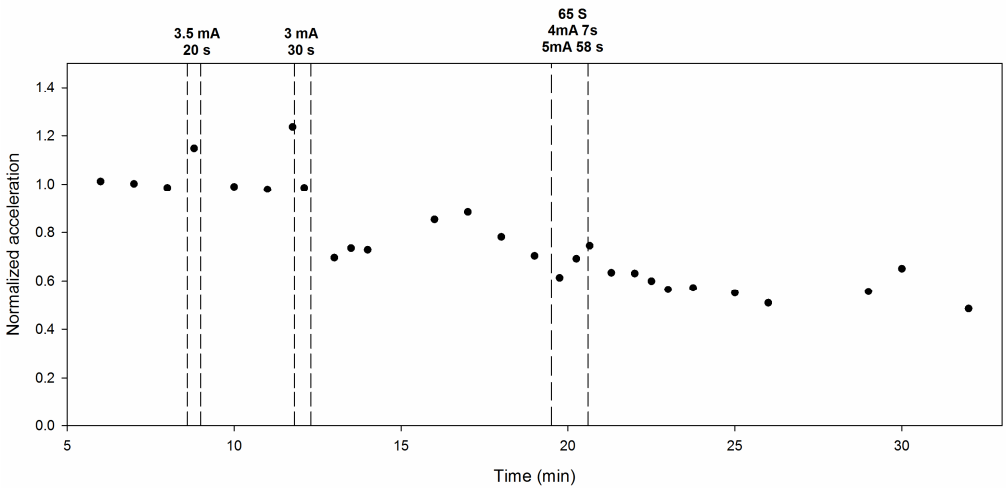


Figure 4.12: Twitch amplitudes before and after three applications of DC in rabbit 2 when it had recovered from a brief period of anesthesia and was fully awake. Each dot represents the mean peak-to-peak acceleration measured in a 20 s data segment. The x-axis shows the time elapsed from the cessation of anesthesia. DC was applied between the vertical dashed lines for 20 s, 30 s and 65 s respectively. Attenuation of acceleration occurred after the second DC application. A further decline in acceleration occurred after the third DC application.

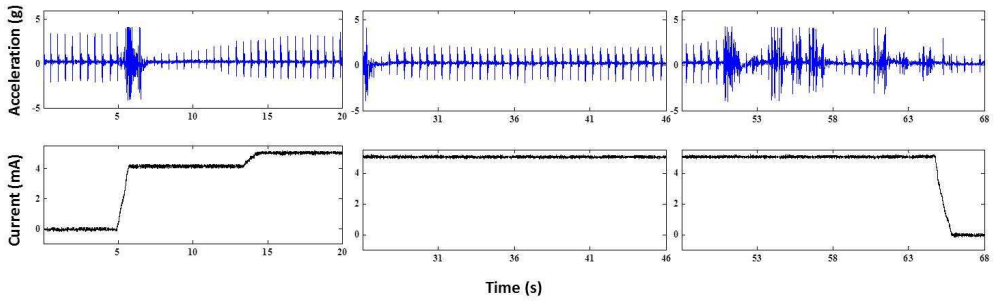


Figure 4.13: Accelerometer and DC signals just before, during and after the third DC application in Figure 4.12. There were two 6 s breaks for data transfer between the three sections of recording. Top: Accelerometer signals. Bottom: DC. A) Accelerations before DC and during two ramp-and-hold increments (to 4mA and 5mA respectively). Twitches were abolished initially, but gradually increased. B) The next 20 s segment recording starts with spontaneous accelerations followed by reduced twitch accelerations compared to segment A. C) The final 20 s segment. Spontaneous bursts of acceleration corresponded to the animal becoming restless. After each such burst, accelerations showed the same pattern of decline and recovery as in segment A.

In rabbit 3 two electrodes were implanted (identical to rabbit 2). Force measurements were conducted under brief anesthesia in the weeks before and after DC was delivered to the awake animal. On the first occasion 1.5mA DC was delivered for 6 min 10 s through the distal cuff (Pt-Ir), no reduction was noted when force was measured under anesthesia in the following weeks (Figure 4.14). On the second occasion DC was delivered continuously through the proximal cuff (stainless steel) after the distal cuff failed. DC was delivered for a total of 5 min (2 mA for 1 min, 2.5 mA for 3 min and 3mA for 1 min). At the onset of DC, as in the case of the awake cat, the rabbit flexed its leg for a few seconds, indicating some discomfort. This occurred again when the current was increased from 2 mA to 2.5 mA, but not from 2.5mA to 3 mA. Mean twitch force measured a week later had dropped by about 25% and remained at this level for another 2 weeks (Figure 4.14). At 3 weeks post-DC, the mean twitch force had returned to baseline levels. The second attempt at delivering DC was made through a stainless steel cuff (not Pt-Ir as originally intended) which might have contributed to the reduction in force. Stainless steel cathodes were shown to undergo very slight changes in weight as opposed to Pt-Ir when under some DC stimulation conditions (Stevenson *et al.*, 2010).

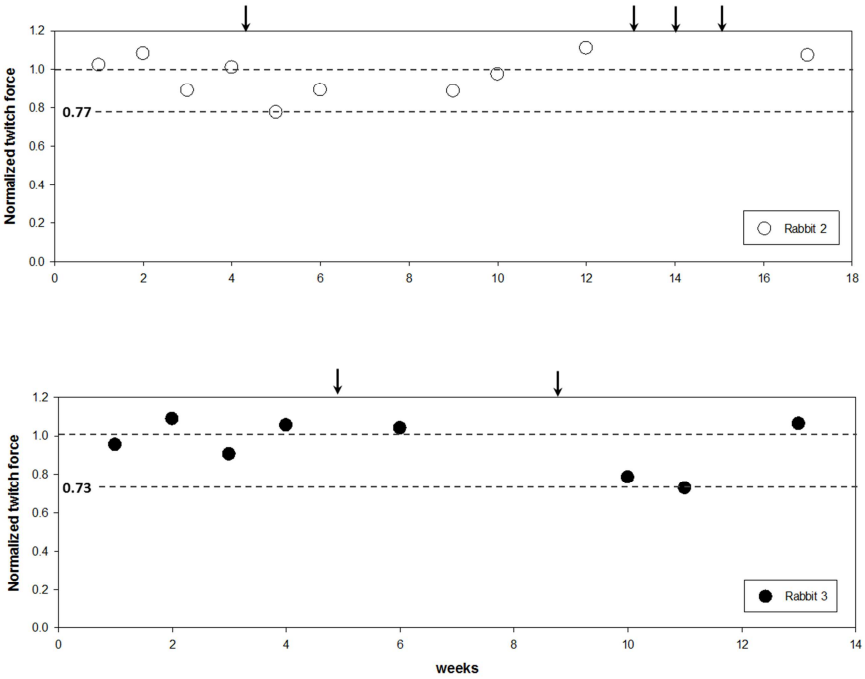


Figure 4.14: Mean peak twitch force measured in the weeks before and after application of DC in rabbits 2 and 3. Each dot represents the mean value of five 20 s data segments as described in the methods section. Each arrow represents an occasion when DC was applied to the nerve (procedure 2). Rabbit 2: a decline in force was observed one week after the first application of DC. Force recovered to baseline values over the next 5 weeks. Three subsequent DC applications (the timing of the first being shown in the right-hand part of the Figure), did not result in force attenuation. Rabbit 3: the first application of DC (delivered through the Pt-Ir electrode) did not result in force attenuation. A second DC application (delivered through the stainless steel electrode) did result in a decline in twitch force, which lasted for at least 3 weeks.

4.3 Discussion

This chapter concerns DC nerve ablation in chronically implanted animals under anesthesia and in the conscious state. In the two anesthetized cats, DC caused graded reductions in muscle force (Figure 4.9), confirming the preliminary findings in a publication (Ravid *et al.*, 2011). The present experiments also showed that nerve ablation using DC is repeatable: DC was applied to the nerve of one cat on two occasions 17 weeks apart, each time resulting in complete conduction block followed by full recovery of the twitch force to baseline values (Figure 4.8).

Nerve tissue damage was previously shown to result from DC application to nerves (Whitwam & Kidd, 1975; Hughes *et al.*, 1981). Following such damage, the distal part of

the nerve undergoes Wallerian degeneration and the proximal nerve portion undergoes "die-back" to the first node of Ranvier (Fenrich & Gordon, 2004). Furthermore, nerve regeneration occurs at 1-3 mm/day. It was also previously shown that after a crush or cut injury of a muscle nerve 40-50 mm from the muscle, motor responses first returned 38-40 days after the crush injury and 54-70 days after the nerve cut and suture procedure (Gutmann *et al.*, 1942).

In the present study, the first signs of recovery of mean twitch force amplitude after DC ablation in Cat 3 occurred at 4 weeks and 7 weeks after DC delivery (Figure 4.8). This is comparable to the 38-40 days quoted by Gutmann et al. for nerve crush (but interestingly, not nerve cut). Some methodological differences should be noted. First, in Gutmann's experiments, the rabbits were tested every day. Because we had to anesthetize the animals in order to conduct force measurement, we only tested the cats every 1-3 weeks, so the very first sign of recovery was most likely missed. The outcome measure in the Gutmann et al. study was reflexive toe-spreading elicited by ground contact. The authors mentioned that the return of dorsiflexion took on average 5 days longer. In the present study, muscle activation was tested by stimulating the nerve proximal to the site of DC delivery, which may be a more sensitive way of detecting first recovery.

Another possible reason for the difference in observations could be the nature of the damage. Gutmann demonstrated that recovery after nerve crush (~3mm/day) was faster than recovery following nerve cut and suture (~2mm/day) (Gutmann *et al.*, 1942). In the experiments presented in this chapter, the nerve was damaged by DC which may have a different recovery time than either cut or crush injuries. The difference in species experimented on (rabbits versus cats) could be a factor as well.

In the studies presented in this chapter, complete recovery of twitch force was observed following nerve ablation and attenuation of force with DC. Studies have shown that after recovery from tibial nerve branch section and suture in cats, the normal proportions of motor unit types were re-established (Foehring *et al.*, 1986; Gordon *et al.*, 2004). In another study, evidence was adduced that 3 years after cutting the nerve to the triceps surae muscles, their ability to generate force had returned to pre-lesioned values, yet their reflex responses to stretching were greatly attenuated (Cope *et al.*, 1994). It was further noted in that paper that the incomplete recovery of stretch response was observed after nerve cut, but not crush. In an earlier study, it had been found that after a nerve transection and subsequent regeneration, the number of functionally identifiable muscle-spindle and tendon-organ afferents was reduced to 25% and 45% of normal, respectively (Banks *et al.*, 1985). This could explain the Cope et al. results. In a clinical application, the effect of nerve transection could be to reduce hypertonus, even after muscle fibers are fully reinnervated. Since one of the characteristics of spasticity is over-active stretch reflexes (Gracies, 2005), the resulting attenuation of the reflex response without loss of motor control or strength following recovery may be beneficial clinically. Further investigations should be carried out in order to test whether this combination of preserved force capability but reduced stretch reflexes applies to DC nerve ablation as well.

In Cat 3 (Figure 4.8), in week 13 post-implantation, just before DC was first applied, the peak twitch force was unexpectedly higher than the forces measured in weeks 9 and 11, which had been assumed to have reached a stable baseline level, consistent with a complete recovery from the effects of surgery. However, more recovery evidently occurred between weeks 11 and 13. DC then produced the anticipated abolition of muscle force, followed several weeks later by full recovery back to the week 13 level, and this sequence was repeated after the second DC application. In Cat 2, 9 weeks after DC application, the force recovered to 130% of the baseline value measured just prior to DC application. This indicates that full recovery had also not occurred at the time the baseline measurement was made. This increase in force was not due to measurement variability since the force transducer was calibrated after each experiment.

In the conscious animal, some brief force responses to DC, particularly at the onset, were observed. These responses did not occur consistently and seemed to depend on the duration of DC ramp-up. In Figure 4.11, the duration of DC ramp-up was less than 1 s and an onset response was clearly visible. In another trial in the same animal (not illustrated), the duration of DC ramp-up was 2.5 s and no onset response was observed. This indicates that there may be a relationship between the occurrence of an onset response and the duration of ramp-up. In a previous experiment on a decerebrate cat (Chapter 3), an onset response occurred even when a five second ramp-up was applied. Therefore a direct relationship between DC ramp-up time and onset response is difficult to establish. In the experiments conducted here, ramp-up times of DC were between 1 and 5 s and were done manually. As can be seen from Figure 4.13 an initial ramp-up of 1 s resulted in an onset response, but an additional rise in DC over 2 s did not elicit a further onset response. Were these onset responses aversive reactions to sensory input, or were they the result of direct activation of alpha motoneurons by the DC? Onset responses to DC ramp-ups have recently been studied in pentobarbital-anesthetized rats (Ackermann *et al.*, 2011). The faster the ramps, the larger the force responses, but in some animals, responses occurred even for very slow (4 s) ramp-ups. At the blood concentrations associated with deep anesthesia, pentobarbital normally abolishes aversive responses to nociceptive input, however, deep anesthesia must be carefully maintained, as aversive responses occur at medium depths of surgical anesthesia. Ackerman et al. administered pentobarbital intra-peritoneally to maintain a level at which eye-blink or withdrawal reflexes did not occur. Taken at face value, this suggests that the onset responses were not evoked reflexly by sensory input. However, the variability of the onset responses in our experiments in the conscious animals, the associated orienting movements, and the lack of onset-responses in most of our experiments performed on anesthetized animals lead us to believe that the onset responses may be partly aversive.

DC delivery in conscious animals did not produce the amount of attenuation in muscle force expected from experiments in which DC was applied during anesthesia or in a decerebrate cat (Chapter 3). For example, in rabbit 1 (Figure 4.10), there was no obvious reduction in force after DC had been applied in the awake state. In rabbit 2, on the first occasion this was done, the force was reduced to 77% of pre-DC levels and this lasted for a few weeks. But on the next three occasions, there were no lasting reductions in force, even though twitches had been abolished by a brief application of DC (Fig 4.11) and reduced after DC was applied in the awake state (Figure 4.12). A week

after this, twitch forces were at pre-DC levels. This is in contrast to the long-lasting attenuation of force caused by applying DC during anesthesia and allowing the animal to recover (Chapter 2 and Figure 4.8 above).

Could the negative results in the conscious animals have been due to inadequate current delivery through the electrodes? The voltage generated by the DC source during DC delivery (in awake and anesthetized animals) was monitored on an oscilloscope. High voltages or large voltage fluctuations that occurred in pilot experiments were associated with electrode failure or pull-out from the subcutaneous ports. However in all three rabbits studied, wires inserted percutaneously into the ports were secured externally with adhesive tape to the animal's body in order to minimize the chance of wire pull-out due to movements during stimulation in the awake animal. Large changes in voltage were not observed during DC delivery in any of the results reported here.

One possibility which might explain the small or absent reduction of force after DC application in the awake animals is that the wires partially or completely pulled out of the subcutaneous ports but remained under the skin. In such an event a large change in voltage would not have occurred, yet little or no DC would have been delivered through the cuff to the nerve. Given that currents up to 5mA were delivered in this way (Figure 4.13), local onset contractions might have been expected in the vicinity of the ports but they may have been obscured by the animals' voluntary movements.

In the awake cat experiment, DC was delivered through a headpiece. The connection to the nerve cuff electrodes in this case was arguably more reliable, yet no significant reduction of force was observed after DC application in the awake state. However, in this case a much lower current was applied (<=0.75mA) and because the cat did not remain still for long enough the current could not be delivered for long enough (6 minutes) to cause lasting nerve ablation.

What could account for a greater resistance to nerve ablation in the awake, compared to the anesthetized animals?

It is possible that anesthesia may suppress mechanisms that in the conscious state protect nerves from DC-induced damage. Nerve damaging processes associated with electrical stimulation have been attributed to reactions at the electrode-tissue interface. These include local changes in pH, metal dissolution and electrode corrosion (Merrill *et al.*, 2005; Stevenson *et al.*, 2010).

For many years it was believed that the neuronal cell membrane was the binding site of anesthetics and therefore the site of their action. It was assumed that anesthetics dissolved in the lipid layer and took effect when a certain concentration had been reached (Evers & Crowder, 2009). Breakthrough experiments conducted on purified luciferase, a protein involved in light production in fireflies, demonstrated that this process can be inhibited by various anesthetics, which bind to the protein (Franks & Lieb, 1984). The authors suggested that the anesthetics compete for the light-activating binding site on the protein. Proteins such as human serum albumin, tested in vitro, have been shown to act as acid-base buffers, attenuating shifts in pH resulting from hydrolysis at electrical stimulation electrodes (Huang *et al.*, 2001). Furthermore, it was

demonstrated that the presence of such proteins resulted in a lower dissolution rate of platinum during electrical stimulation with platinum electrodes (Robblee *et al.*, 1980). Although it is unclear exactly which proteins serve as a buffer in vivo and whether anesthetics affect these proteins at clinically active levels, it may be possible that the protective action of such proteins is inhibited under anesthesia.

Other protective mechanisms that may be affected by anesthetics are the three main physiological buffer systems (bicarbonate, phosphate and proteins). The local blood supply of nerves may be reduced by anesthetics, which would reduce the available supply of these buffers.

The supposition that general anesthesia might have facilitated nerve damage by partially disabling protective mechanisms is not supported by results obtained from the decerebrate cat in which complete ablation was reached (chapter 3: 3mA, 4 min) 1-2h after anesthesia was withdrawn. However, one cannot rule out the possibility that the duration the animal was on the table and the decerebration itself (which might influence breathing and therefore pH levels in the body) may have resulted in conditions which accommodated a more rapid nerve damage.

One of the difficulties encountered with delivering DC to the conscious animal was the limited amount of time for which the animals remained in one place and tolerated the DC application. According to the protocol, DC was discontinued if and when the animal showed signs of discomfort. Another barrier was the measurement of twitch responses to the test stimuli. In the anesthetized animal, twitch forces were easily monitored, providing reliable measures of nerve ablation. In the conscious animal force monitoring was not feasible.

In light of the challenges described in the previous paragraph, one could question to what extent would it be acceptable by humans? Reports of other neuroablative techniques (Simopoulos *et al.*, 2008) and the ability of the rabbits to tolerate a few minutes of DC application imply that DC delivery to the nerve may be acceptable. Continuous radiofrequency (CRF) and pulsed radiofrequency (PRF) are neurolytic techniques used to treat pain by causing a controlled heat lesion (Racz & Ruiz-Lopez, 2006) and were shown to cause nerve tissue damage (Erdine *et al.*, 2009). In a pilot study (Simopoulos *et al.*, 2008) both CRF and PRF were applied to dorsal root ganglia (DRG) and segmental nerves without injection of anesthetics onto the nerves despite the temperatures used for treatment (54^{o} during CRF for 1 min and 42^{o} PRF applied for 2 min). In view of these findings it is reasonable to expect that individuals that would be treated with DC would not require the use of anesthetics on the nerves as in the CRF and PRF applications. Furthermore, it is anticipated that individuals would be able to withstand potential discomfort that could arise during DC application.

Neuropathic pain is known to occur in some cases following peripheral nerve injury (Woolf & Mannion, 1999) and is defined as “pain caused by somatosensory system disease or damage” (Baron *et al.*, 2010). Its symptoms may include pain that is independent of stimuli, allodynia, pain resulting from non-painful stimuli and hyperalgesia, the increased pain response to painful stimuli (Woolf & Mannion, 1999). None of the animals implanted in our experiments exhibited any signs of discomfort or

alterations in behavior indicative of neuropathic pain (Zimmermann, 2001) following ablation of the nerve with DC. Specific testing for allodynia and hyperalgesia were not conducted in this study and therefore cannot be ruled out.

Subcutaneous ports: After several iterations of development, the subcutaneous ports used in the experiments on the conscious animals (described in the methods section) were effective in percutaneously delivering DC and electrical pulses to nerves on repeated occasions. The incorporation of conductive silicone improved the reliability of needle insertion, whether the purpose was to insert a wire or to use the needle as a conductor. Furthermore, conductive silicone held the needles or wires more securely in place than the conductive rubber.

The results of this study provided proof-of-principle that the repeated percutaneous application of DC to nerves can be carried out with subcutaneous ports. Short durations of DC produced controlled nerve ablation, when applied during brief periods of anesthesia. Conclusive evidence of long term force reduction was not observed when DC was applied in the awake state, although short-term conduction block was observed and there was a decline of force during and immediately after DC application. There were no obvious signs of significant or lasting pain during or after the application of DC. Further testing is needed to determine the DC amplitudes and durations required to achieve the controlled amounts of nerve ablation, and therefore reductions in hypertonus, in the conscious state.

4.4 References

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Chapter 5
General discussion and future directions

Spastic hypertonus is a condition that is not easily treatable and several approaches are used today in an attempt to address its debilitating effects. In this body of work, experiments designed to explore a novel way to treat spastic hypertonus were presented. The proposed method is to apply direct current (DC) to nerves innervating over-active muscles via implanted electrodes, for the purpose of partially ablating the nerves and thus reducing the neural drive to the muscles. Chapter 2 describes the initial experiments which involved delivery of DC to a muscle nerve, in order to establish amplitudes and durations required to achieve nerve ablation. These experiments were exploratory in nature. In Chapter 3 a more extensive investigation of the DC parameters (amplitude and duration) required to produce a desired amount of nerve ablation was described. Experiments in which an animal model of spastic hypertonus, namely decerebrate rigidity, are depicted along with an exploration of the possible mechanisms by which DC stimulation induces nerve damage. In Chapter 4, the use of DC in chronically implanted animals, in both the anesthetized and conscious states, is explored and a prototype of an implantable port for the repeated percutaneous delivery of DC is presented.

5.1 Treatment of spasticity with DC nerve stimulation by means of implantable electrodes

Current treatments of spastic hypertonus can help control the condition to various degrees, by reducing hyper-reflexia for example, but surprisingly, little or no improvement in the performance of ADLs has been shown, even when these treatments are effective in reducing muscle tone (Gracies *et al.*, 1997; van Kuijk *et al.*, 2002; Bovend'Eerdt *et al.*, 2008; Esquenazi *et al.*, 2009). There is clearly a need for a treatment for spasticity that will also improve motor function. The use of implantable electrodes to deliver DC may enable clinicians to alleviate spasticity. The subsequent use of these electrodes to deliver FES to the partially ablated nerve may provide a means by which functional improvement may be achieved. Implantable electrodes have long been used in the field of neuroprosthetics (NP) to help restore function after nerve injury (Waters

et al., 1975; Prochazka *et al.*, 2001) and may serve to help rehabilitation after spasticity is reduced, by means of delivery of FES via the same implantable electrodes.

Although further experiments are required before any clinical trials should be conducted, the experiments described in this thesis indicate that implanted electrodes accessed percutaneously may provide a feasible means of delivering DC to overactive nerves to ablate them in a controlled manner for the treatment of spasticity. These experiments addressed solely the issue of alleviation of spastic hypertonus and they did not involve any comparisons of motor function before and after ablation.

Aside from the benefit of having a single system that can both alleviates spastic hypertonus and subsequently deliver FES to produce controlled functional movement, this novel approach may have other advantages over current treatments, as well as some shared disadvantages. These will be described below with reference to the studies' findings. The use of DC for nerve ablation, as compared with other neurolytic agents, is summarized in table 5.1.

5.1.1 Localization of treatment

The use of DC to partially ablate a peripheral nerve does not result in systemic effects such as sedation and dizziness that accompany the use of anti-spastic drugs (Gracies *et al.*, 1997; Kita & Goodkin, 2000; Montane *et al.*, 2004). Furthermore there is no risk of vascular damage due to a misplaced needle during injection, that can occur with phenol or alcohol (Frangiamore & O`Brien, 2004). It has become apparent in the last few years that the action of BtA, a popular neurolytic treatment for spasticity, is not localized to the site of injection as it has been shown to migrate in the body (Gracies, 2004; Fortuna *et al.*, 2011). NFH staining of nerve sections following DC nerve ablation, described in Chapter 2, showed axonal damage localized under the cuff only. No axonal damage was observed distally or proximally to the cuff. However, one local effect of DC that could in principle affect surrounding tissues is a decrease in pH, as we observed in a saline solution in vitro. This may be counteracted in vivo by the body's buffering mechanisms (Huang *et al.*, 2001). The lack of axonal damage in the vicinity of the cuff, as was seen from the NFH staining, implies that pH shifts or other electrode reactions are most likely localized under the cuff as well.

5.1.2 Ease and duration of administration

As opposed to neurolytic injections, such as phenol and alcohol, where significant expertise is required of a physician in order to achieve favorable outcomes (Elovic *et al.*, 2009), application of DC to the nerve once a cuff has been implanted is relatively simple. Administration of phenol or alcohol requires exploration with needles and electrical stimulators to determine the correct site of injection. This process is time consuming (up to 2 hours) and can involve multiple needle insertions prior to the injection itself (Frangiamore & O`Brien, 2004; Horn *et al.*, 2010). Administration of BtA is also recommended to be conducted with electrical stimulation or ultrasound in order to ascertain needle placement (Esquenazi, 2006). In the studies described in this thesis,

connections to the subcutaneous ports, as in Chapter 4, were made under brief anesthesia. This might not be needed in humans. Furthermore, we found that making percutaneous connections to subcutaneous ports required very little time and often, a single needle insertion, which would be a desirable clinical feature.

It was shown in Chapter 2 that low current amplitudes (0.1-0.5mA) require a very long duration (30 to 45 minutes) to achieve the desired attenuation and therefore are less attractive clinically. Brief applications (min) of higher levels of DC (1-3 mA), as described in Chapters 3 and 4, can lead to substantial reductions in force that can last for a long time (weeks) before nerve regeneration occurs (see table B.1 in Appendix B for recommended amplitudes and durations). Results from DC stimulation in the conscious animal indicated that anesthesia might have contributed to the reduction being achieved in such a short time, since similar DC currents and durations did not result in long-lasting force attenuation in the conscious animal as described in Chapter 4. The reduction in twitch amplitudes observed in the conscious animal during application of 4-5mA DC for one minute, though not long-lasting, indicates that a few minutes should suffice to achieve the desired force attenuation. Though further exploration is required to determine a more exact time range for application in the absence of anesthesia, we estimate that the entire process from insertion of the electrode into the subcutaneous port to achieving the desired reduction in force should require no more than 30 minutes.

5.1.3 Frequency of administration

As is the case for phenol and alcohol (Horn *et al.*, 2010), there is no foreseeable restriction on the number of times DC can be applied to the same nerve. BtA, systemic anti-spastic medications and Intrathecal Baclofen (ITB) are all dose-restricted (Penn & Kroin, 1987; Gracies *et al.*, 1997; Horn *et al.*, 2010). During the exploration of DC amplitudes and durations described in Chapters 2 and 3, it was evident that when repeated applications of DC were carried out, nerve conduction block was reached faster and lasted longer with every additional application (Figures 3.8-3.10). This was previously observed by other investigators (Whitwam & Kidd, 1975).

Experiments carried out in chronically implanted cats, described in Chapter 4, showed that muscle force responses to electrical stimuli applied proximal to the lesion recovered to pre-lesion values over some weeks following DC nerve ablation. In a person with underlying hyper-reflexia, this may be accompanied by a return of muscle over-activity as well, at which time the process of delivery of DC can be repeated, much like other neurolytic injections. We assume that the recovery was due to regeneration of motor axons from the site of injury in the DC cuff to the muscle. However, DC presumably ablated sensory axons too. The question then arises, did the sensory axons regenerate at the same rate and to the same extent? It was previously found that following recovery from nerve cut, but not crush, responses to stretch remained impaired (Cope *et al.*, 1994). In an earlier study, it was found that after a nerve transection and subsequent regeneration, the number of functionally identifiable muscle-spindle and tendon-organ afferents was reduced to 25% and 45% of normal, respectively (Banks *et al.*, 1985). This could explain the Cope results. The effect could be

to reduce hypertonus, even when muscle fibers are fully reinnervated. Further studies should be conducted in order to determine if this is the case when a nerve is ablated with the use of DC. Such an outcome might be favorable in reducing spasticity and may result in fewer repeated applications.

5.1.4 Time duration from administration to effect

In the acute and chronic setting, when DC amplitudes above 0.5mA were applied, only a few minutes were required to result in force declines which lasted for the remaining hours of the acute experiments and for weeks after successful ablations in chronic experiments. The rapid onsets and extended durations of force attenuation are similar to those seen with phenol or alcohol injections (Frangiamore & O`Brien, 2004) whereas force reductions after BtA injections can take 2-4 weeks to develop (Kirazli *et al.*, 1998). In some cases, force was completely abolished during DC application and then returned to lower levels when DC ceased (e.g. Figure 3.10 and 3.11), whereas in other cases a gradual reduction in force during DC application was observed (e.g. Figure 4.8). In the former cases, to produce a desired amount of attenuation, it would be necessary to apply DC for several successive short periods until the desired force is reached. The proposed percutaneous delivery system, would allow DC amplitudes and durations to be adjusted in this way.

5.1.5 Risks and side effects

Spasticity treatments that target central nervous mechanisms, such as systemic anti-spastic drugs, are often accompanied by undesirable side effects such as sedation, dizziness, reduced cognition, weakness and liver damage (Gracies *et al.*, 1997; Kita & Goodkin, 2000; Elovic, 2001). Treatments such as BtA and phenol injections are applied to specific nerves and muscles and thus avoid major systemic side effects, though it should be remembered that BtA has recently been shown to produce muscle weakness in distant muscles in the body (Fortuna *et al.*, 2011). Nerve lesioning with DC acts the same way therefore no systemic effects are expected.

There are two main types of risk associated with the application of DC via implanted electrodes: risks associated with surgery and the risk of developing neuropathic pain as a result of nerve ablation. The main risks associated with the surgery are infections and pain following the operation (Gan, 2009). Neuropathic pain may occurs following peripheral nerve injuries (Woolf & Mannion, 1999) and its prevalence is estimated at 1-3% in the general population (Hayes *et al.*, 2002). It is hard to predict what the incidence of neuropathic pain would be following a DC nerve lesion. One would expect that treatments such as phenol and alcohol injections, which lead to neuronal degeneration (Zafonte & Munin, 2001), may also result in neuropathic pain, though the incidence of such events is unclear.

The chronically implanted animals in our study showed no signs of pain over the weeks and months after DC nerve ablation. They did not show an increased tendency to flex the lesioned limb, as they might if the limb were constantly painful. However, because the incidence of pain after nerve injury is 1-3% (Hayes *et al.*, 2002), it is reasonable to expect a similar incidence after DC nerve ablation. To evaluate this issue properly would therefore require a large number of animals. This was outside the scope of the present studies, but it is an important point that needs to be cleared up before human clinical trials could be contemplated.

5.1.6 Cost-effectiveness

Cost-effectiveness of a treatment can be very hard to estimate since in addition to the direct costs of the treatment itself, physicians, nurses and physiotherapists' time, the impact of side effects on quality of life and most importantly, attaining of pre-treatment targets should all be included (Ward *et al.*, 2005). Treatment of spasticity with DC delivered via implantable electrodes would require surgery, which would involve significant costs at the outset, particularly when hospitalization and recovery costs were included. However, it has been suggested that 64% of failed treatments with BtA and orally administered drugs result in corrective surgery anyway (Ward *et al.*, 2005). Thus, although BtA and anti-spastic medications are widely used, surgery is still needed in some cases of spasticity.

Clinicians estimate that only 35% of patients treated with anti-spastic drugs see sufficient benefits from the treatment to warrant the cost, as opposed to 73% treated with BtA (Ward *et al.*, 2005). BtA treatment is expensive (Horn *et al.*, 2010). A recent analysis indicated it was more cost-effective than orally administered drugs (Ward *et al.*, 2005), but it was estimated to be twice as expensive as treatment with phenol, even when side effects were considered (Horn *et al.*, 2010).

The treatment of spasticity is a big burden on the healthcare system (Rothwell, 2001; Roger *et al.*, 2012). An implantable system will have a high cost initially, due to the costs of the device and surgery. However, unlike BtA, the cost of repeated treatments with DC, when applied through implanted ports, would be low, as the treatments could be performed by medical technicians. Furthermore, the same implanted leads could in principle be used to apply FES to improve activities of daily life, further reducing the incurred physiotherapists cost and potential assisted care cost. To achieve this, the implanted ports would have to be modified so that they could deliver either DC via the percutaneous wires described in this thesis, or FES pulses through another device such as an implanted radio frequency pulse generator. All things considered, an implantable system that addresses spasticity and reduced function might in fact turn out to be a more cost-effective treatment for spastic hypertonus than present-day treatments.

	DC	Phenol and alcohol	BtA
Localization	Very localized Under the cuff only	Localized to site of injection	Migrate in the body
Ease and duration of administration	Approximately 30 minutes	Up to 2 hours	30 minutes to an hour
Frequency of administration	No restriction	No restriction	Every 3 month, due to restricted dosage
Time duration from administration to effect	Minutes	2-60 minutes	2-4 weeks
Side Effects and risks	Risks associated with surgery Discomfort during DC application Neuropathic pain	Pain during application Multiple needle insertions Vascular injections Neuropathic pain	Pain during application Flu like symptoms Migration in the body Muscle loss
Cost effectiveness	Initial expense – surgery Cost effective - address spasticity and reduced function	Low cost	Costly

Table 5.1: Comparison of DC nerve ablation for the treatment of spasticity and current neurolytic treatments (phenol , alcohol and BtA).

5.2 Future directions

The work presented in this dissertation is an early investigation of a novel treatment for spasticity using DC to lesion nerves in a controlled manner; therefore there are several research avenues to pursue. The following sections outline areas of particular interest.

5.2.1 Mechanisms of damage in the conscious and anesthetized animal

As was shown in Chapter 4, DC amplitudes and durations that were effective in ablating nerves in anesthetized animals were less effective when applied in conscious animals. Studies have shown that proteins such as human serum albumin may impede the damaging effects of the electrode reaction for example by buffering pH changes (Robblee *et al.*, 1980; Huang *et al.*, 2001) and anesthesia is known to bind to proteins and have the ability to alter their activation (Franks & Lieb, 1984). Another possibility is that the blood supply to peripheral nerves may be reduced by anesthetics, and this might reduce the availability of the body's three main acid-base buffering systems. Further investigations into the mechanism of damage due to DC stimulation are needed. The emphasis should be on understanding the mechanism of DC ablation using in vitro and in vivo preparations, and the role of anesthesia in making nerves more vulnerable to DC.

5.2.2 Neuropathic pain

The possible emergence of neuropathic pain following DC nerve ablation should be evaluated prior to clinical application. Two main points should be considered before any such testing is carried out. The first issue concerns the animal models used to study neuropathic pain. It was suggested by Costigan et al. (Costigan *et al.*, 2009) that while rodents may be a good model for studying the mechanism of pain they are not accurate predictors of the type of pain that might emerge in humans. The second point to be considered is the finding that the incidence of neuropathic pain in cats and dogs following trauma was higher than in humans (Mathews, 2008). As was previously mentioned, neuropathic pain may result from peripheral nerve damage. In the general population, the prevalence of neuropathic pain is hard to estimate but is thought to be approximately 1.5% (Taylor, 2006). An Acute Pain Services (APS) center in Australia estimated the incidence of neuropathic pain between 1% and 3% (Hayes *et al.*, 2002). A different study, looking at traumatic nerve injuries, suggested that the incidence of neuropathic pain after peripheral nerve trauma is closer to 50% (Ciaramitaro *et al.*, 2010). The incidence of neuropathic pain may in fact depend on the size of the nerve involved: a recent extensive review (Costigan *et al.*, 2009) reported that sectioning of large nerves was associated with a 30%-60% incidence of sustained pain, whereas damage of a small nerve during hernia repair only resulted in a ~5% incidence. The

same review cites several epidemiological studies that report an overall prevalence of neuropathic pain of ~5% (Costigan *et al.*, 2009).

A new study suggests that the nerve response to a mild preconditioning nerve lesion leads to neuroprotection from neuropathic pain that would otherwise be expected to result from a subsequent lesion (Moalem-Taylor *et al.*, 2011). This can be easily tested with an implantable electrode system such as the one described in this dissertation work and could be easily clinically implemented if proven to work.

5.2.3 Restoration of function following reduction of spasticity

Alleviation of spastic hypertonus does not in general improve the voluntary control of muscles paralyzed as a result of central nervous system disorder. The same implanted leads that are used to deliver DC ablation could subsequently be used to deliver FES to the nerve to help restore voluntary function, provided there was a means of connecting a controllable pulse generator to the leads. The efficacy of implantable FES systems in improving motor function has previously been demonstrated in several studies (Kilgore *et al.*, 2008; Gan *et al.*, 2012).

5.3 Significance of research and concluding remarks

Due to the dependency of the outcome of DC ablation on the number of DC applications in a single session and the parameters used, and in light of the variability of force reduction observed between different animals of the same species, it is recommended that some method of evaluating nerve-evoked muscle force should be carried out during DC application (e.g. applying low-frequency pulsatile stimulation of the nerve and measuring twitch force). Furthermore it is recommended that DC is not applied more than 3 times in a single treatment and that the applications should be spaced by 5-10 min to allow outcome evaluation. From the results in the anesthetized animals it seems that mid-range amplitudes (1-1.5mA) do not result in immediate conduction block but rather in graded attenuation when applied for durations of 2-3 min. Since the results in the conscious animals were inconclusive, it is not feasible at this time to estimate the amplitude and duration of DC that would be required to achieve graded reductions of muscle force in humans. This requires further testing in both conscious animals and humans.

The objective of this work was to test a novel method of alleviating spastic hypertonus, a condition that afflicts millions of people around the world, reducing their quality of life, impeding basic functions of daily life and costing millions of dollars to health care systems. The current treatments for spastic hypertonus address spasticity with little consideration to the side effects of the treatment, or to functional improvement. The system suggested in this dissertation offers an alternative means of

reducing spastic hypertonus. It may prove to be a cost–effective treatment with few side effects and the potential to improve voluntary motor function.

5.4 References

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Appendix A:

Summary of animals used, experimental parameters and results

Rabbit number	DC amplitude (mA)	DC duration	Order of application (amplitude/duration)	Final attenuation In force	chapter
1	0.5, 1	2.5, 5 min	2x(0.5/2.5), 0.5/5, 1/2.5	80%	2
2	0.1, 0.2	2.5, 5, 10 min	0.1/2.5, 0.1/10, 0.2/5, 0.2/10, 0.2/5	60%	2
3	1	2, 5 min		100%	2
4	0.2, 0.3, 0.4, 0.5	5,10, 20 min	0.2/10, 2x(0.3/5), 0.3/10, 0.3/20, 0.4/10, 0.5/20	No permanent reduction	2
5	0.5	10,20 min		100%	2
6	0.1, 0.2, 0.3, 0.4	10, 20, 30, 35, 45 min	0.1/45, 0.2/45, 0.3/45, 0.3/35, 0.4/10, 0.4/20, 0.4/30	100%	2
7	0.3	45, 35, 30 min		65%	2
8	0.75	2 min	6x(0.75/2)	100%	3
9	0.75	2 min	14x(0.75/2)	63%	3
10	2, 2.5	20, 30, 40 s 1, 1.5 min	2/20 5x(2/30), 3x(2/40), 2.5/30, 2x(2/1)	100%	3
11	1, 1.5, 2, 2.5	30 s	3x(1/30), 3x(1.5/30), 3x(2/30), 3x(2.5/30)	90%	3
12	1	45 s, 1, 1.5, 2 min	3x(1/45), 3x(1/1), 3x(1/1.5), 2x(1/2)	100%	3
13	2	30 s, 1, 1.5 min	4x(2/30), 4x(2/1), 3x(2/1.5)	80%	3
14	1.5	30 s, 1min	1.5/30, 7x(1.5/1)	60%	3

Table A.1: Rabbit used in acute experiments - data reported in thesis

Rabbit	DC amplitude	DC duration	Order of application (amplitude/duration)	Technical problems rendering results inconclusive, so not reported in the thesis
1	0.5, 0.75	2 min	13X(0.5/2), 2x(0.75/2)	Muscle puller had to be readjusted
2	0.75	2 min	3x(0.75/2)	Cuff broke, problems with DC stimulator
3	2	30 s, 45 s, 1 min, 1.5 min, 2 min	4x(2/30), 2/45, 3x(2/1), 2/1.5, 2/2	Used rabbit implanted with initial version of subcutaneous ports, had to change the anode in the middle of the experiment
4	2	1	2/1	Used rabbit implanted with initial version of subcutaneous ports. Muscle puller jittered. Length tension curve was performed and muscle puller was set.

Table A.2: Rabbit used in acute experiments – no results

Cat number	Time after implant	DC amplitude	DC duration	Force attenuation	Time to recovery from final attenuation	chapter
1	3 weeks	0.3 mA	37min	90% - temporary	2 month	2
	6 weeks	0.4 mA	35 min	50%		
	11 weeks	0.4 ma	30 min	100%		
2	4 month	0.5mA	10 min	60%	7 weeks	4
3	13 weeks	1mA	10 min	100%	16 weeks	4
	30 weeks	1mA	9 min	100%	20 weeks	

Table A.3: Chronically implanted cats – DC under anesthesia

animal	L-leg	R-leg	DC amplitude	DC duration	Attenuation and recovery	chapter
Rabbit 1	Lidocaine Lidocaine + DC	DC only	3 mA	4 min	100% R+L leg no recovery	3
Rabbit 2	Lidocaine Lidocaine+ DC	DC only	3 mA	4 min	100% R+L leg no recovery	3
Cat	Lidocaine Lidocaine+ DC	Lidocaine +DC	R-leg: 3 mA L-leg: 1mA	R-leg : 4 min L-leg: 10 min	100% R+L leg No recovery	3

Table A.4: Animals used for Lidocaine tests reported in thesis

Animal	L-leg	R-leg	Result	Technical problems rendering results inconclusive, so not reported in the thesis
Rabbit 1	Lidocaine	-	Initial conduction block lasted more than 2 hours	Initial conduction block lasted more than 2 hours (very young animal)
Rabbit 2	Lidocaine	-	Initial conduction block lasted more than 2 hours	ditto

Table A.5: Animal used for experiments with Lidocaine – no results

Animal	# of cuffs	Time after implant	DC amplitude (mA)	DC duration	Attenuation measured under anesthesia	Time to recovery from attenuation	chapter
Cat 2 (same as cat 2 in Table A.3)	2	12 month	0.5 0.5	3.5 min 4 min	Slight attenuation	One week	4
			0.75 0.75 0.75	6 min 3 min 40 s 2 min 40 s	Slight attenuation	One week	
			0.75	5 min 40 s	No attenuation		
Rabbit 1	1	2.5 month	5	3 min	No attenuation		4
Rabbit 2	2	18 weeks	1.5	6 min 10 s	23%	5 weeks	4
		28 weeks	3	80 s	Not measured		
		29 weeks	3	67 s	Not measured		
		30 weeks	3.5 3 4, 5	20s 30s 65 s (7 s 58 s)	No attenuation		
Rabbit 3	2	4 month	1.5	6 min 10 s	No attenuation		4
	1	5.5 month	2, 2.5, 3	1 min, 2min, 1min in one continuous trial	27%	4 weeks	

Table A.6: Chronically implanted animals – DC delivery while awake

Rabbit number	Reason for failure as a chronic	Alternative use	Description of alternative use	Reported in chapter
1	Implanted with initial version of subcutaneous electrodes (right leg). Rabbit chewed stainless steel electrode.	Used acutely	Rabbit 3 in table A.2 (used left leg)	
2	Implanted with initial version of subcutaneous electrodes (right leg). Stainless steel electrode failed.	Used acutely	Rabbit 13 in table A.1 (used left leg)	3
3	Implanted with initial version of subcutaneous electrodes (left leg). Could not stimulate through subcutaneous port.	Used acutely	Rabbit 4 in table A.2	
4	Implanted with a newer version of subcutaneous ports. Electrodes failed after 4 months before sufficient data could be collected.	Used acutely	Tested the latest version of subcutaneous electrodes.	
5	Implanted with final version of subcutaneous electrodes using conductive silicone. A single Pt-Ir electrode was implanted. Wire broke off.			

Table A.7: Chronically implanted rabbits - could not be used for awake experiments

Appendix B:

DC amplitudes and duration that may be effective at reducing spasticity as were concluded from animal experiments

duration / amplitude	30s	60 s	90 s	120 s	180 s
1mA	x	x	x	x	x
1.5 mA	x	x	x	x	
2 mA	x	x	x		
2.5mA	x	x			
3mA					

Table B.1: Recommended combinations of amplitude and duration (marked "x") to produce graded reductions of force as concluded from the results presented in this thesis.