DEVELOPMENT OF AN UPPER LIMB NEUROPROSTHESIS: CONQUERING WEAKNESS AND FATIGUE

By

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Alie Buckmire, titled "Development of an Upper-Limb Neuroprosthetic: Conquering Weakness and Fatigue" and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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Appendix A.

ABSTRACT

Neuroprosthetics are devices that substitute or supplant motor, sensory or cognitive modalities damaged as a result of spinal cord injury or stroke. Functional electrical stimulation (FES) neuroprosthetics utilize artificial stimulation to restore motor function in paralyzed muscles, where control exerted by higher nervous system centers over muscle may be impaired. Although promising, FES has failed to gain widespread acceptance due in part to weak contraction strength and rapid fatigue observed with artificial stimulation. This dissertation documents an attempt to create an upper limb FES neuroprosthetic and subsequently to address the issues of weakness and fatigue. To exploit the capabilities of the musculoskeletal system the neural drive to muscle first must be decoded. Decoding the neural drive for specific movements has been approached using either a deterministic (engineering) or machine learning model. While a deterministic model accounts for all components of a limb, number of joints, degrees of freedom, limb length, muscle length, etc, machine learning characterizes the relationship between select variables, in this case whole muscle electromyographic data (EMG) and limb kinematics. Ultimately, the output of both approaches is used to predict the neural drive required to generate movements. In this study we first attempt to build an upper limb FES neuroprosthetic. Utilizing machine learning, we characterize the relationship between limb kinematics and EMG. Then, predict EMG based solely on limb kinematics. Finally, stimulation pulses were generated and delivered via intramuscular electrodes to produce movement. Additionally, to address force generation we hypothesized that due to the distributed nature of motor axons within a muscle stimulating with multiple spatially distributed electrodes would activate a larger muscle volume thus generating additional force. This in turn would facilitate

load sharing among muscle fibers, and reduce fatigue. To evaluate fatigue we compared interleaved and synchronous patterns of stimulation as well as single electrode vs multiple electrode stimulation. We approached these questions and aims with a combination of strategies and techniques including machine learning, implantation of stimulating electrodes in a non-human primate model and finally human subjects. While machine learning provided EMG predictions with high R values, we were unable to generate substantive movements activating all the muscle in a complete joint system. However, we were able to generate movements stimulating a single muscle in an intact joint system. We found that single electrode force could be augmented with multiple electrodes. Additional results indicate that multiple electrode stimulation was less fatiguing than single electrode stimulation. Interleaved stimulation however, did not result in less fatigue than synchronous stimulation.

CHAPTER 1

INTRODUCTION

SPINAL CORD INJURY

People rarely stop to think about the communication superhighway that is their spinal cord or its role in facilitating communication between the periphery of their body and their brain. Even fewer people are aware of the neuronal computations that take place within the spinal cord. Indeed, communication enabled by the spinal cord is pivotal to life itself. Therefore, any event or condition that compromises signaling between the brain and the body has devastating effects, including loss of sensory or motor function and paralysis. Only when this ability is impaired to some degree is awareness and perhaps an appreciation gained for the spinal cord.

The information and studies discussed in this work focus on the development of an upper-limb neuroprosthetic for the restoration of movement following paralysis. Broadly, the goal of this work was to address three problems facing such neuroprosthetics: (1) determining the patterns of muscle activation needed to elicit complex motor behaviors, (2) increasing the contractile forces generated using artificial stimulation and (3) reducing the high degree of fatigue associated with artificial stimulation.

In order to understand the scope and challenges of treating spinal cord injury with an upper limb neuroprosthesis (as proposed in this dissertation), it is important to consider the complex biological environment that develops following spinal cord injury. The following sections briefly addresses this.

1.2: Mechanism of injury and paralysis:

Spinal cord injury (SCI) results from an insult inflicted on the spinal cord that compromises either completely or incompletely its major functions of communication and processing of motor, sensory, and autonomic signals. Trauma to the spinal cord is secondary to a mechanical injury to the surrounding bony structure. The National Spinal Cord injury Statistical Center (NSCISC, 2018) reports that approximately 17,000 people suffer a SCI of varying magnitude each year. While the average age of injury has increased from 29 in 1970 to 43 between 2015 and 2017, men continue to be the prominent group affected. A bimodal distribution occurs, however, with a second group of individuals with an average age 60 being affected primarily by falls (Dobkin & Havton 2004; Jain et al., 2015; Tator & Fehlings 1991).

Injuries are often a result of trauma caused by some external force exerted on the brain, spinal cord or body. External causes include falls, a subset of which includes falls from standing in the elderly (~60 years) (Jain et al., 2015; Dobkin & Havton 2004), motor vehicle crashes, firearms (acts of violence), and striking against or being struck accidentally by an object or person (NSCISC 2018; Jain et al., 2015). Non-traumatic causes of SCI includes chronic degenerative disorders, spinal cord compression as a result of disc pro-lapse, bone metastasis that results from cancer, as well as multiple sclerosis (Lee & Thumbikat 2015; Lassman 2015).

The mechanism of injury to the spinal cord includes distraction, laceration, transection, shearing forces, and compression (including axial compression while the neck is slightly flexed). Distraction occurs when the bony spinal components are hyper-extended as often occurs in rapid acceleration or deceleration injuries (Schwartz & Fehling 2002; Winter & Pattani, 2011).

Laceration and transection are often due to missiles penetrating the spinal cord (e.g. gunshot wounds). Sharp bony fragments dispersed by the missile impact disrupts the soft tissue components of the spinal cord resulting in varying degrees of damage. It should be noted however that instances of traumatic injury resulting in complete transection of the spinal cord are extremely rare (Tator & Fehlings 1991). Compression, the most common mechanism of injury results when bony fragments generated from the initial impact compresses the cord (Tator 1983; Tator 1996; DeVivo et al., 2002). Indeed, the efficacy and timing related to resolving persistent compression of the spinal cord is highly debated and will be discussed later (Wilson et al., 2017; Dimar et al., 1999; Ramer et al., 2014). Regardless of the mechanism of traumatic injury to the spinal cord, it results in some degree of motor, sensory, and autonomic impairment (Tsintou et al., 2015; Lee & Thumbikat 2015; Ramer et al., 2014), ranging from transient deficits to complete and permanent paralysis (Kwon et al., 2004).

Empirical and experimental evidence suggest that the severity of the mechanical disruption is directly proportional to the amount of energy transferred to the bony structures in the acute insult (Blight & DeCrescito 1986). In turn, transfer of this energy into the soft tissue determines the severity of the lesion and resultant impairments.

Accordingly, Wolman (Wolman 1965), proposed that soft tissue damage is disproportionately greater in gray matter compared to white matter tissue due, in part, to the greater vascularity of gray matter and its softer consistency (Wolman 1965; Dumont et al., 2001). Additionally, because gray matter has a higher metabolic demand, damage typically is irreversible within the first hour following injury, whereas white matter has a 72-hour window before damage is considered permanent (Dumont et al, 2001; Blight & Young 1989). Thus,

injury to neural elements is largely mediated by disruption of blood flow to and within the cord. Hypoxia, driven by disrupted blood flow results in local infarctions and an inflammatory response which leads to the cord swelling and compressing against the rigid spinal canal.

Clinically, the extent of the SCI is assessed as complete of incomplete. Absence of motor and sensory function below the level of the lesion signifies a complete injury. Partial preservation of neurological function below the level of the injury is designated an incomplete injury.

The American Spinal Injury Association (ASIA) impairment scale further stratifies the severity of the SCI between grades A through E as follows; ASIA grade A – Complete, no motor or sensory function present, ASIA grade B – Incomplete, sensory but not motor function is preserved, ASIA grade C - Motor function is preserved below the lesion and half of the key muscle groups have a muscle grade less than 3, ASIA grade D – Incomplete, preserved motor function below the lesion and key muscle below the lesion have a muscle grade greater than 3, ASIA grade E – motor and sensory functions are normal. Muscle grades refer to active movement, where grade 3 is active movement against gravity, while grade 4 and 5 includes movement against some resistance and full resistance respectively.

Other quantitative and qualitative measures are also used to assess the severity of the acute injury. For example, Miyanji et al. (2007) reviewed MRI measures of SCI including: maximum spinal cord compression (MSCC), maximal canal compromise (MCC), and lesion length. Additionally, six qualitative measures used included: intramedullary hemorrhage, edema, cord swelling, soft tissue injuries, canal stenosis, and disk herniation (Miyanji et al., 2007).

Taken together, these measures along with the ASIA impairment scale provide assessment indices characterizing the extent of SCI.

1.3 Secondary Injury

While the acute mechanical insult associated with SCI results in a wide range of outcomes and neurological deficits, SCI also has systemic ramifications affecting all organs systems. More threatening, however, is an array of cellular and biochemical processes triggered by the acute mechanical trauma, known as secondary injury. First proposed by Allen (1911), secondary injury is now known to involve a number of pathophysiological mechanisms including, but not limited to, hemorrhage, edema, neurogenic shock, systemic hypotension, vasospasm, post traumatic ischemia, inflammation, excitotoxicity, calcium mediated injury, demyelination, fluid electrolyte disturbances, neurotransmitter and ionic disturbances, immunological injury, mitochondrial function disturbances, free radical production, lipid peroxidation, axonal and neuronal necrosis, apoptosis, and channel and receptor impairment (Dumont et al, 2001; Tator et al., 1991; Tsintou et al 2015; Silva et al., 2014; Bareyre & Schwab 2003; Springer et al., 1999; Schwartz & Fehlings 2002; Silva et al. 2014). Occurring on a time course of minutes, hours, weeks, months and even years, this biological cascade extends the development of injury leading to further neurological damage and a chronic injury state (Gensel & Zhang 2015).

An aggressive pathophysiology exists following SCI, initiated and exacerbated by the initial mechanical disruption of neural tissue and the vascular system in and around the spinal

cord (Tator et al., 1991). The normal physiological responses following injury fall into three progressive and overlapping phases; (1) an inflammatory phase, (2) a proliferative phase and (3) a remodeling phase (Gensel & Zhang 2015; Novak & Koh 2013; Gurtner et al., 2008). The inflammatory phase is initiated in response to the mechanical damage and loss of tissue homeostasis. Activated inflammatory cells including microglia, macrophages, leukocytes and neutrophils migrate to the injury site with the primary function of removing damaged tissue (Gensel & Zhang 2015; Donnelly & Popovich 2008).

Kwon, (Kwon et al., 2004) along with others assert that the stereotypical inflammatory response should be considered in two phases, an early neurotoxic phase and late phase neuroprotectic properties (Chan 2008; Donnelly & Popovich 2008; Kwon et al., 2004). One side of the inflammatory response contains a cellular component, which includes neutrophils, macrophages and T-cell, while the other is comprised of molecular components containing pro-inflammatory molecules, including cytokines, upregulated chemokines, along with the production of free radicals and oxidative stress (Donnelly & Popovich 2008; Siddiqui et al., 2015). Neutrophils, first on the scene, recruit other inflammatory cell types but also secrete lytic enzymes, which further damage local tissue (Gonzalez et al., 2003; Donnelly & Popovich 2008; Dumont et al., 2001; Fleming et al., 2006). Blood borne monocytes and macrophages, along with resident microglia serve to phagocytose injured tissue. Cytokines including tumor necrosis factor (TNF-α) and interleukins mediate the inflammatory response and contribute to additional tissue damage (Dumont et al., 2001; Gensel & Zhang 2015). Indeed, the role cellular and molecular components serve, either deleterious or advantageous, depend on their targets and

timing of expression. The immediate and continue consequence is cell death at the lesion site and beyond, resulting in the formation of a fluid filled cyst and a glial scar.

Gensel and Zhang proposed that macrophages, important in all phases of repair, are of particular importance in the inflammatory phase due to their ability to regulate the transition through different stages of the healing process (Gensel & Zhang 2014). Indeed, it is proposed that the functional adaptability of macrophages to change phenotypes in response to environmental and tissue specific stimuli results in the SCI environment resembling a chronic non-healing wound state (Gensel & Zhang 2014). Thus, a maladaptive macrophage response to the milieu of environmental cues maintains the inflammatory response. This and other factors result in the creation and maintenance of the inflammatory environment.

1.4: The Inflammatory Environment & Excitotoxicity

Vascular alterations leading to ischemia are cited as critical to the secondary injury cascade (Tator et al., 1991; Kwon et al., 2004). Ischemia, defined as a local deficiency of blood supply secondary to vasoconstriction or obstruction of arterial blood flow, leads to the production of oxygen-derived free radicals and other high energy oxidants (Dumont et al., 2001). The presence of high energy oxidants, reactive oxygen, and nitrogen species contribute to oxidative stress leading to pathogenesis (Gensal & Zhang 2015).

Included in the large number of factors released following SCI, glutamate, the most prevalent excitatory neurotransmitter, has perhaps the most profound and deleterious effect (Kwon et., al 2004; Dumont et al., 2001). The effects of excess glutamate on its receptors and

resultant processes that lead to neuronal cell death are referred to as excitotoxicity (Choi 1992; Dumont et al., 2001). Glutamate receptors include ionotropic, N-methyl-D-aspartate (NMDA) and metabotropic, alpha-amino-3-hydroxy-5-methyl-isoxazolapropionate (AMPA)/kainate receptors. Activation of NMDA receptors by glutamate release allows extracellular calcium and sodium to move via a concentration gradient into cells (Kwon et al., 2004). Elevated cytosolic calcium can trigger alterations in cellular metabolism with lethal results (Kwon et al., 2004, Dumont et al., 2001). Excitotoxicity also contributes to neuronal cell death via inhibition of Na+ -K+ ATPase activity, lipid peroxidation, altered calcium homeostasis; inhibition of mitochondrial respiratory chain enzymes, oxidative modifications to proteins, along with activation of lytic enzymes (Dumont et al., 2001; Kwon et al., 2004; Sekhon & Fehlings 2001; Hulseboch 2002).

2.0: Therapeutic Approaches to Spinal Cord Injury

The primary injury along with the resultant cascade of cellular and biochemical processes creates a therapeutically challenging environment. Due to the dynamics of the environment and the large number of potential targets, a single therapeutic solution to SCI is unlikely. Instead, complex multifaceted approaches must be undertaken.

Therapeutic strategies proposed can be categorized into general areas aimed at restoring function to the injured spinal cord; rescue, rewire, and reactivate ((Ramer et al., 2014; Dell'Anno & Strittmatter 2017; Ribotta et al., 2002; Hulsebosch 2002; Ramer et al., 2005).

Preventing the spread of damage from the initial site of the mechanical injury is the focus of strategies aimed at rescuing the spinal cord (Ramer et al., 2014). These early interventions include surgical decompression and stabilization, cooling and hypothermia, neuroprotection and strategies aimed at specific biological processes that are a direct result of the primary injury, such as inflammation and ischemia (Ramer et al., 2014; Wilson et al., 2017; Siddiqui et al., 2015; Collis 2017; Cappuccino et al., 2017; Alkabie & Boileau 2015; Dietrich et al, 2011; Levi et al., 2010; Dimar et al., 1999; Batchelor et al., 2013; Ramer et al., 2005; Schwartz & Fehlings 2002).

Rewiring the injured spinal cord includes refers to strategies aimed at regrowth of injured axons or repurposing spared axons (Dell'Anno & Strittmatter 2017; Ramer et al., 2014; McDonald & Howard 2002; Ribotta et al., 2002). These strategies seek to stimulate the intrinsic growth/regenerative capacity of neurons and reduce inhibitor of growth present in the SCI environment (Ohtake & Li 2015; Schwab 2002; Gimpe & Silver 2002).

Reactivation of neural elements involves strategies that seek to use spared systems through rehabilitation, pharmacological intervention, and electrical stimulation (Ramer et al., 2017; Stein et al., 2002). A major component of this strategy includes artificial activation of neural elements that can no longer be engaged by the injured spinal cord.

2.1 Rescuing the spinal cord

Rescuing the spinal cord is the initial step in any therapy strategy seeking to restrict the deleterious effects of secondary damage. Techniques such as surgical decompression, therapeutic hypothermia, and drug treatments seek to targeted inflammation and excitotoxicity. The

requirement for, and timing of, surgical decompression, however has been debated (Dimar et al., 1999, Wilson et al, 2017). In the earliest recorded treatment of spinal cord injury, contained in the Edwin Smith surgical papyrus, SCI are referred to as, "an ailment not to be treated (Donovan 2007). A shift from this early conservatism has occurred, perhaps driven by research data, improvement in technology, techniques and the availability of hardware (Dimar et al., 1999; Fehlings et al., 2012). As a result, surgical intervention is used to decompress the spinal cord, remove obstructive bone, and stabilize the fractured vertebrae. A longitudinal study by Perkins and Deane showed complete recovery in 3 of 6 patients following surgical decompression (Perkins and Deane 1988). Additional evidence supporting early versus late decompression was provided in a meta- analysis completed by Liu et al. (2016) along with a multicenter cohort study by Fehling et al. (2012). Liu et al. (2016) reported that surgery within 24 hours of the acute spinal cord injury improves neurologic outcomes (Liu et al., 2016). Fehlings et al. (2012) reported a minimum of 2 grade AIS improvement at 6 months in SCI patients who underwent decompression within 24 hours of SCI (Fehlings et. al., 2012).

2.2: Therapeutic Hypothermia

First introduced in 1940 by the pioneering work of Dr. Temple Fay, therapeutic hypothermia (TH), or refrigeration can be used as a therapeutic and neuroprotective strategy following acute SCI. Fay (1940) reported on an anesthetized patient undergoing refrigeration using surface cooling for 18 hours recovering without knowledge of the procedure or any discomfort (Smith & Fay 1940). More recently, a comparative study by Levi et al. (2010) between hypothermia treated and control patients, showed an encouraging trend for improvement in function compared to control. The findings were encouraging enough to warrant a phase 2 and phase 3 multicenter trial (Levi et al. 2010).

3.0: Rewiring the Spinal Cord

The adult CNS does not easily regenerate, and as a result, individuals who experience SCI show only marginal amounts of spontaneous recovery. However, a highly variable amount of recovery can be experienced between the first 3 to 6 month post injury (Fawcett et al., 2007; Kirschblum et al., 2004). Steeves et al. (2011) indicated that among ASIA complete SCI patients, only 10% regain some degree of motor function and only 10% regain sensory function in the absence of motor recovery (Steves et al, 2011).

Historically, research on axonal regeneration has focused on two approaches; remove or reduce inhibitors of axonal growth in the SCI environment or increase the limited intrinsic regenerative capacity of neurons (Ramer et al., 2005). The intent is to regrow neurons, repurpose neurons

spared in the initial trauma and secondary destruction, or reduce inhibitor to growth present due to the interaction of local cells with molecular participants.

Cellular properties including the potential to form myelin, promote and guide axonal growth, as well as the ability to bridge the injury site are considerations for cell types and cell based therapies aimed at restoring function. The cell types of most interest include: Schwann cells, embryonic stem cells, induced pluripotent stem cells, neural stem cells, mesenchymal stem cells, and olfactory ensheating cells (Tetzlaff et al., 2011; Ruff et al., 2012; Assinck et al., 2017).

Schwann cells, the myelinating glia of the peripheral nervous system (PNS), form the basis of many research programs, among them the Miami project to cure paralysis (Bunge & Wood 2012). Schwann cells are multifunctional, able to support and encourage growth of new axons, guide regeneration following injury, as well as secrete trophic factors and growth promoting matrix (Guest et al., 2013; Bunge & Wood 2012; Hulseboch 2002). As a result, Schwann cells have been the focus of rewiring research more than any other cell-based therapy (Bunge & Wood 2012).

Stem cells (SC) have been proposed as the solution to many medical problems including SCI (Ramer et al., 2014). Stem cells show potential to bridge the lesion, replace lost neurons, glia, and other cells (Ramer et al., 2014). Such enthusiasm has surrounded SC that in 2009, a phase 1 clinical trial was undertaken by the Geron company (Menlo Park California). A cohort of thoracic SCI patients were transplanted with oligodendrocyte precursor cells derived from human embryonic SC (Ramer et al., 2014). Unfortunately, in 2011 the study was halted (Ramer et al., 2014; Pollack 2011). Geron cited a change in research focus to cancer therapies as the reason for halting the trial (Pollack 2011). SC therapies remains one of the most highly

investigated therapies for SCI (Hulseboch 2002; Kanno et al., 2015; Deng et al., 2013; Guest et al., 2013).

Induced pluripotent stem cells (IPSC) provide a way to obtain stem cells directly from adult tissue for autologous transplantation. This reduces the ethical dilemma associated with embryonic stem cells (Cohen & Melton 2011). Pioneering work by Yamanuka in 2006 demonstrated that using 4 transcription factors, differentiated adult cells could be made to revert to pluripotent cells (Takahashi & Yamanaka 2006). IPSC could offer an unlimited supply of autologous cells because they can be derived directly from adult tissue (Cohen & Melton 2011) and would allow cell transplantation back into the host without risk of immune rejection. Indeed studies have shown that transplanted progenitor cells derived from IPSC survive and differentiate into neurons, oligodendrocytes, and astrocytes (Kobayashi et al., 2012, Nori et al., 2011).

Other cell-based treatments include olfactory ensheating cells (OEC), a glia cell found in the olfactory system (Richter & Roskams 2008; Ramer et al., 2014; Assinck et al., 2017). OEC are extremely plastic and are able to retarget across the PNS (Richter & Roskams 2008). OEC are easily accessible in many model systems, and because of properties like remyelination of axons, and the ability to enhance growth in intact and lesioned axons, they hold promise as therapeutic tools (Richter & Roskams 2009). Results of a phase 1 clinical trial in 2013 demonstrated that human OECs can be isolated, purified, and safely transplanted into the human spinal cord (Tabakow et al. 2013).

In 2014, Tabakow and colleagues published a case report demonstrating the result of transplanting bulbar olfactory ensheathing cells in a 38 year old patient who had sustained a traumatic transection of the thoracic spine resulting in paralysis (ASIA-A). Cultured OEC were

transplanted into the lesion site following resection of the glial scar (Tabakow et al., 2014). Tabakow et al. (2014) reported that the patient improved from ASIA-A to ASIA-C. Improved trunk stability, along with partial recovery of voluntary movements was reported (Tabakow et al. 2014).

Yet, in a letter to the editor of the Journal of Neurotrauma, Guest and Dietrich (2014) suggested, claims made by Tabakow et al. were potentially overstated and merited cautious examination of the evidence presented (Guest & Dietrich 2014). Others have asserted that the research on OEC often contains claims that are unable to be independently confirmed Tetzlaff et al., 2011). The reasons for these discrepancies are not often clear but certainly suggest some experimental bias.

3.1: Bio-materials

Biomaterials offer an alternative approach to rewiring the injured spinal cord by providing a medium in which endogenous or transplanted cells can grow (Assuncao-Silva et al., 2015). Biomaterial must meet specific criteria to be clinical applicable outside of simply being biocompatible. This includes mechanical and physiochemical properties, attachment and growth properties, and degradation (Assuncao-Silva et al.; 2014; Slaughter et al., 2009; Foyt et al., 2018). Indeed, among biomaterials, hydrogels meet these criteria while mimicking the soft tissue of the CNS (Foyt et al., 2018). Slaughter et al. (2009) defines hydrogels as three-dimensional networks formed from hydrophilic homopolymers, copolymers, or macromers cross-linked to form insoluble polymer matrices. Derived from natural polymers for biocompatibility, hydrogels

mimic macromolecular compounds found in the body (Slaughter et al., 2009; Assuncao-Silva et al., 2014).

Classes of hydrogels include; natural based hydrogels, synthetic biodegradable hydrogels, and non-biodegradable methacrylate based hydrogels. Natural based hydrogels are based on substances that occur naturally in the extracellular matrix with properties that are recognized by cells (Pego et al., 2012; Assuncao-Silva et al., 2014). Natural based hydrogels include: alginate, agarose, collagen, fibronectin, fibrin, matrigel, chitosan and hyaluronic acid, to name a few (Foyt et al., 2018).

In a rat subacute spinal cord injury model, Johnson et al., (2010), using a fibrin scaffold implantation, showed that fibrin is conducive to regeneration and cellular migration (Johnson et al., 2010). While this study did not show any functional recovery, the authors postulate that a fibrin scaffold could enhance host cell proliferation and axonal regeneration.

In contrast to natural based hydrogels, synthetic biodegradable hydrogels offer the advantage of being tailored for specific applications. For example, Patist et al. (2004) tested the effects of poly (D, L - Lactic acid) macroporous guidance scaffold impregnated with brain-derived neurotrophic factor (BDNF) in a model of transected rat spinal cord. Patist et al. (2004) reported that cells and axons more rapidly invaded the BDNF foam when compared to control, demonstrated increased vascularization, and that the foam was tolerated well within the cord (Patist et al., 2004). In a recent non-human primate study of acute spinal cord injury Slotkin et al. (2017) reported that SCI monkeys exhibited significantly improved recovery and locomotion in response to implantation of a biodegradable scaffold (Slotkin et al., 2017). Additionally, the

authors stated that even though their results were modest, support was offered for the efficacy and safety of polymer scaffolds as a potential therapy in spinal cord injury (Slotkin et al., 2017).

Finally, non-biodegradable methacrylate based hydrogels remain stable upon implantation but suffer the disadvantage of being non-biodegradable (Assuncao-Silva et al., 2014). Additionally these hydrogels retain large amounts of water without dissolving. These hydrogel are of particular interest because they are moldable into tubular shapes that might facilitate axonal growth (Dalton et. al., 2002). Indeed, hydrogels hold promise but require additional testing to validate their efficacy.

4.0: Reactivating the spinal cord and peripheral nerves

The chronic SCI environment presents a myriad of challenges that, to date, are not easily addressed by any one solution. Another potential therapeutic approach utilizes neural elements beyond the lesion site to restore function. These strategies include artificial stimulation of circuits and/or peripheral nerves to restore function.

4.1: Functional electrical stimulation

Functional electrical stimulation (FES) is defined as the artificial activation of paralyzed muscle to perform a functional task. FES has been used in patients who have sustained a SCI, where control from higher nervous system centers is impaired (Doucet et al., 2012; Ducko 2011; Rupp & Gerner 2004). FES has been used successfully for the restoration of some degree of

control over bladder and bowel, diaphragm pacing, and to restore limited function to paralyzed limbs, trunk, and legs (Ragnarsson 2008; Peckham & Knutson 2005; Ducko 2011, Handa et al., 1989; Keith et al., 1987). As a rehabilitative technology, FES has been used in FES cycling and body weight supported treadmill walking (BWST) to ameliorate muscle atrophy and build muscle endurance and strength (Kralj & Bajd 1989; Nataraj et al., 2017; Dutta et al., 2011; Popovich et al., 2003).

To restore function to paralyzed muscles, artificial stimulation is delivered via surface, percutaneous, intramuscular, or nerve cuff electrodes placed in the periphery to activate nerves supplying muscles. An electric field centered at the electrode tip activates nerves that reach threshold due to the stimulating current. Action potentials generated as a result of the artificial stimulation propagate via afferent and efferent (sensory and motor) axons (Collins 2007; Popovic 2014). Activation of efferent axons results in muscle activation while activation of afferent pathways have the potential to generate reflexes and deliver ascending sensory information to the brain, but only if ascending sensory pathways are intact.

Electrical stimulation can be delivered anywhere along the length of a nerve, proximally near (or even within) the spinal cord to its dispersed distal innervations within the muscle. Motor axons, rather than muscle fibers are activated by the current field due to a markedly lower activation threshold of axons compared to muscle fibers (Mortimer 1981; Jacobs & Nash 2004; Peckham 1995; Peckham et al., 2005).

Electrode types are often chosen based on their functional objectives, the effort required to implant or use them and duration of use. While nerve cuff electrodes enable high contraction forces to be developed using relatively low stimulus intensities, they require surgery to implant

and typically lack single muscle selectivity. Surface electrodes, on the other hand, are easy to use but are prone to movement with the skin and do not have access to deep muscles. Intramuscular electrodes offer a compromise, allowing access to deeper muscles, selectivity of individual muscles and when used percutaneously are easily removed.

The components of an FES system are relatively simple and typically include a power source, a controller or processing unit, stimulator, wire leads, and electrodes (Ragnarsson 2008; Bajd & Munih 2010). Three stimulation parameters determine the strength of the evoked contraction; pulse duration, pulse amplitude, and pulse frequency (Bajd & Munih 2010; Atrens et al., 1979, Peckham & Knutson 2005, Kesar et al., 2007). Modulation of these three parameters control contraction strength. Pulse amplitude and duration govern the recruitment of motor axons (and thereby their innervated muscle fibers) while pulse frequency modulates force by varying the rate at which muscle fibers are activated. Stimulus pulses are typically rectangular in shape and monophasic or biphasic. Biphasic pulses provide charge balance, helping to reverse the electrochemical processes that lead to electrode deterioration and tissue damage (Mortimer 1981; Gorman & Mortimer 1983; Badj & Munih 2010).

Control schemes for FES systems vary. Handa et al. (1989) and Kameyama et al. (1999) used intact respiratory function (puff & sip sensor) to activate pre-programmed stimulus patterns to excite muscles in quadriplegic patients (Handa et al., 1989; Kameyama et al., 1999). Keith et al. (1989) used an external movement sensor placed on the contralateral shoulder (over which the patients had voluntary control) to select and activate preprogrammed stimulus patterns in tetraplegic patients (Keith et al., 1989). Nathan (1989) used voice control to deliver stimulation to surface electrodes placed over muscles of the arm (Nathan 1989). Myoelectric control and

brain machine interfaces (BMI) have also been explored as control methods for FES (Hart eat al., 2001; Ajiboye et al., 2017). Myoelectric control uses electromyographic (EMG) signals produced by voluntary muscle contraction (Hart et al., 2001; Ambrosini et al., 2013) to trigger stimulation. Alternatively, BMIs interpret signals directly from the brain and use these signal to activate FES systems (Moritz et al. 2007; Ethier et al., 2012; Bouton et al., 2016; Ajiboye et al., 2017). Additional control schemes could include eye tracking, head, or tongue movements. All the control methods described function by selecting and activating pre-preprogrammed and stored patterns of muscle stimulation and as a result, offer a limited repertoire of movements that can be evoked.

4.2: Example of FES upper limb motor prosthesis

The following are examples of FES systems that have been developed to restore motor behavior in paralyzed individuals. There are, however, no commercially available implantable upper-limb FES systems. The Freehand system was developed at Case Western Reserve University, first implanted in 1986 and approved by the Food and Drug Administration in 1997 (Keith et al., 1989; Cornwall & Hausman 2004). Prior to being withdrawn from commercial availability in 2001, 250 patients with C5 and C6 tetraplegia were implanted (Ragnarsson 2008). Eight intramuscular electrodes were implanted into the muscles of the hand and forearm to provide lateral and palmar grasp (Keith et al., 1989; Taylor et al., 2002). A radio frequency transmitter was attached to the skin directly above a stimulator implanted in the chest wall. The control unit was activated by voluntary contraction of the contralateral shoulder. This selected

preprogrammed patterns that allowed the user to open and close the hand (Keith et al, 1989, Taylor et al., 2002).

In reporting on the functional impact of the Freehand System, Taylor et al. (2002) indicated that subjects were able to produce reasonably function grips. These subjects lacked grip strength prior to the implantation of the neuroprosthetic. Additionally, the Freehand system improved the ability of C5 and C6 tetraplegics to complete activities of daily living (Taylor et al., 2002). Additional support for increased in grip strength and improved activities of daily living were reported by Malcahey et al. (2004), Memberg et al. (2003), Smith et al. (2001).

Memberg et al. (2014) described a study involving 2 high-level tetraplegia patients who were implanted with 24 intramuscular and nerve-cuff electrodes. Stimulators were implanted into the abdomen were used to activate the upper extremity electrodes. With this system, subjects were able to produce different motor behaviors including hand opening/closing, shoulder abduction–adduction, and internal rotation of the shoulder (Memberg et al., 2014). While one subject demonstrated a significant improvement in activities of daily living (ADL), the second subject was only able to partially complete two ADL task (Memberg et al., 2014). This FES system required multiple surgeries to complete the implantation of all the required hardware yet it was only able to demonstrate functional improvements in one subject.

Ajiboye and colleagues described an upper limb motor prosthesis implanted in a high level tetraplegia that allowed the subject to control the motor prosthesis using signals from an intracortical brain computer interface (iBCI) (Ajiboye et al, 2017). Two micro-electrode arrays were implanted into the hand and arm areas of the subject's motor cortex, while 31 percutaneous electrodes were implanted into muscles of the upper and lower arm (Ajiboye et al., 2017). The

subject was able to command single joint and coordinated multi-joint movements with this system (Ajiboye et al., 2017).

This system, combining iBCI and chronically implanted muscle electrodes is perhaps the current state of the art in FES neuroprosthetics. While very impressive, it is important to be circumspect as to what was accomplished with respect to the control of FES with that system. The iBCI in that study served primarily as a switch box to select one of a few fixed sequences of stimulation to be played out. Furthermore, the subject could only activate one sequence at a time resulting in sequential (rather than concurrent) movements at each involved joint.

4.3: Problems with FES

Despite significant advances in technology, materials, and surgical techniques, upper limb FES systems continue to experience low acceptance and translation into clinical settings. A major reason for low acceptance is that FES systems can only produce a few simple movements. Indeed the difficulty in generating motor behaviors lies in identifying the patterns of stimulation needed to generate complex movements. Indeed, even simple movements often involve many muscles working across multiple joints and with complex timing of their activations.

Several strategies have been deployed to resolve this challenge. Hoshimiya et al. (1989) used EMG signals recorded from heathy subjects to create stimulus templates needed to active muscles in SCI patients. This approach, while important, was only able to produce a few movements because EMG activities were only recorded for a few simple tasks.

The Freehand system developed by Case Western Reserve University, used an alternative approach by defining the functional outcomes and tuning that activity pattern for each muscle to produce the results. This approach involved trial and error until the desired motor behavior was produced and then the associated stimulation pattern was stored. Again, only a limited number of simple movements could be generated using this labor-intensive method (Keith et al., 1989; Taylor et al., 2002).

Another approach was to develop a deterministic musculoskeletal model of the limb in order to predict activation patterns for each muscle using inverse dynamics (based on Newtonian mechanics) and the biophysical properties of each muscle (Blana et al., 2008). When tested in healthy subjects, this highly sophisticated model, however, predicted EMG patterns that poorly fit actual EMG (Blana et al., 2008).

Therefore, a primary goal of this dissertation was to use machine-learning methods to predict patterns of stimulation need to produce a wide range of complex, multi-joint movements. This issue is addressed in Chapter 3 of this dissertation.

Another obstacle limiting the utility of FES is weak contraction strength elicited with artificial stimulation. Such weak contraction strength is not simply the result of muscle atrophy or fiber type conversion following paralysis. Indeed, the maximum force that can be evoked in healthy subjects using artificial stimulation is markedly less than that produced with voluntary contractions (Enoka & Fuglevand 1991; Kramer et al, 1984; Maffiuletti et al., 2014). One reason for this weakness may be related to the widespread distribution of motor nerve branches within muscle. As such, a single stimulating electrode (as conventionally used) may be incapable of

activating the entire array of motor axons supplying a muscle. This possibility is addressed in Chapter 4 of this dissertation.

Finally, the rapid onset of muscle fatigue is also identified as a major obstacle to the effectiveness of FES systems (Doucet et al. 2012; Nguyen et al., 2011; Sayenko et al. 2014; Wise et al. 2001; Yoshida & Horsch, 1993). A reason often cited is that the normal size principle of motor unit recruitment (Henneman 1957, Henneman et al., 1965) is reversed such that large more fatiguable motor units are recruited prior to small, less fatiguable units (Bickel et al. 2011; Yoshida & Horch, 1993; Bajd & Munih, 2010; Peckham & Knutson 2005). We believe, however, that excessive fatigue comes about primarily because of an inherent inability of conventional electrode systems to access the entire pool of motor units within a muscle. This issue is addressed in Chapter 5 of this dissertation

CHAPTER 2

EFFORTS TOWARDS DEVELOPMENT OF AN UPPERLIMB NEUROPROSTHETIC

1.0: INTRODUCTION

The work presented in this dissertation was aimed at the development of an upper-limb neuroprosthetic for the restoration of movement in individuals paralyzed as a result of spinal cord injury or stroke. While functional electrical stimulation (FES) systems have been developed and deployed to restore motor function, the number of patients that utilize these systems is limited. This is due in part to the difficulty associated with identifying the patterns of muscle activity needed to produce desired motor behaviors. Indeed, even the simplest movements often require coordination of many muscles across multiple joints, involving multiple degrees of freedom (Schieber 1995; Valero-Cuevas et al. 2000; Fuglevand 2011).

Previous studies have used sophisticated biomechanical models in an attempt to predict muscle activity patterns (e.g Blana et al. 2008). Such approaches use inverse dynamics based on classical mechanics (e.g. Winter 1990) to predict the net torques generated at each joint needed to produce some desired trajectory of the limb. Because multiple muscles typically cross any joint, the net torques need to be resolved into individual muscle forces using various optimization strategies (Crowninshield 1978; Pedotti et al. 1978; Zajac & Gordon 1989; Anderson & Pandy 2001). Once individual muscle forces are predicted, the patterns of activation of those muscles can be estimated taking into account a variety of biophysical properties of skeletal muscle contraction (e.g. force-length, force-velocity relations)(Hatze 1978; Delp et al. 2007; Kosterina et al. 2013). The resulting patterns of muscle activation then can serve as templates for time-varying electrical stimulation delivered to the muscles in order to evoke the desired movement (Yamaguchi & Zajac 1990). Unfortunately, such approaches have

performed relatively poorly in predicting muscle activity patterns associated with even simple arm movements (Blana et al., 2008). This may be because such complex systems do not lend themselves to structured analytic solutions.

Work in our laboratory, however, has demonstrated that non-deterministic machine learning methods can be used to predict muscle activity with good fidelity associated with complex movements (Seifert & Fuglevand 2002; Anderson & Fuglevand 2008; Johnson & Fuglevand 2009; Tibold & Fuglevand 2015). These studies, however, involved predictions of activities of only a small number of muscles, for example, those needed to control movements of a single finger (Siefert & Fuglevand 2002). In order to control movements of an entire limb, electrical stimulation needs to be delivered through intramuscular electrodes to several muscles. Such a requirement precludes testing in human subjects at this time. Therefore, we sought to determine whether machine-learning based control of functional electrical stimulation could be used to evoke complex movement of the upper limb in temporarily paralyzed rhesus monkeys, an animal with an upper-limb anatomy similar to that of humans.

2.0: METHODS

Two separate experimental phases were undertaken as part of this study (figure 1). In phase one, we sought to determine whether patterns of muscle activity could be predicted from 30 upper limb muscles associated with complex free movements of the upper limb using machine-learning algorithms. Predicted muscle activity is compared to the actual recorded EMG to evaluate the machine learning algorithm. In the second phase, figure 1(B), stimulus pulses

were generated from predicted muscle activity and delivered to paralyzed muscles to evoke kinematics. Likewise, evoked kinematics is compared to desired kinematics to determine if evoked kinematics compared to desired kinematics.

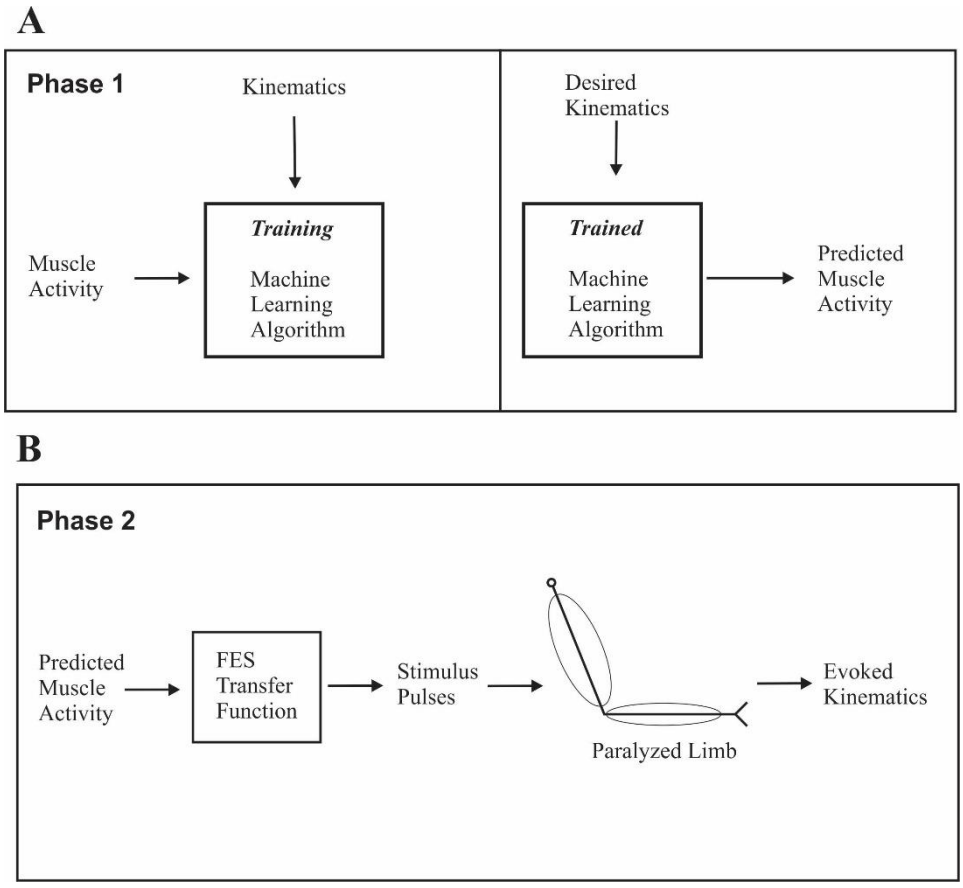

Figure 1. *(A) Phase 1 – Kinematics & EMG are used to train a machine learning algorithm (B) Kinematics are evoked in a paralyzed limb using artificial stimulation.*

Subjects: Three adult male rhesus monkeys (Macaca mulatta) ages 11, 13, 14 were included in this study in accordance with IACUC guidelines and approved by the University of Arizona institutional review board.

Training: Monkeys were trained to sit in a primate chair with their left arm restrained and their right hand positioned in a start box in front of them. The correct start position was signaled by a low-frequency audible tone when the monkey's hand interrupted the beam of a photodiode switch inside the start box. At the start of each trial, a food morsel was positioned at different locations within the monkey's reach space. To increase the complexity of the movements completed, the experimenter moved the food morsel through complex trajectories, changing direction and speed of the morsel as the monkey tracked the morsel with his hand. The monkey grasped the food morsel, placing it into his mouth and then returning his hand to the start position. The monkeys typically performed this task for 15 to 30 minutes until satiated. To ensure that the reach space was fully sampled, these procedures were repeated over several training sessions.

Electrodes: In the first monkey tested, implanted muscle electrodes were Teflon coated, multi-stranded, low corrosion, biocompatible, stainless steel wires (Crooner wire AS633 – 36 gauge) (Fig. 2A).

Because there was some evidence suggesting that some of the electrodes migrated out of their muscle targets, in the second and third monkeys implanted, the simple stainless steel electrodes were replaced by electrodes designed for chronic implantation in human, (Ardiem Medical Inc.)(Fig. 2B). These electrodes had the following characteristics to improve their resilience: 1. polypropylene barbs that served to anchor the detection/stimulating surface within

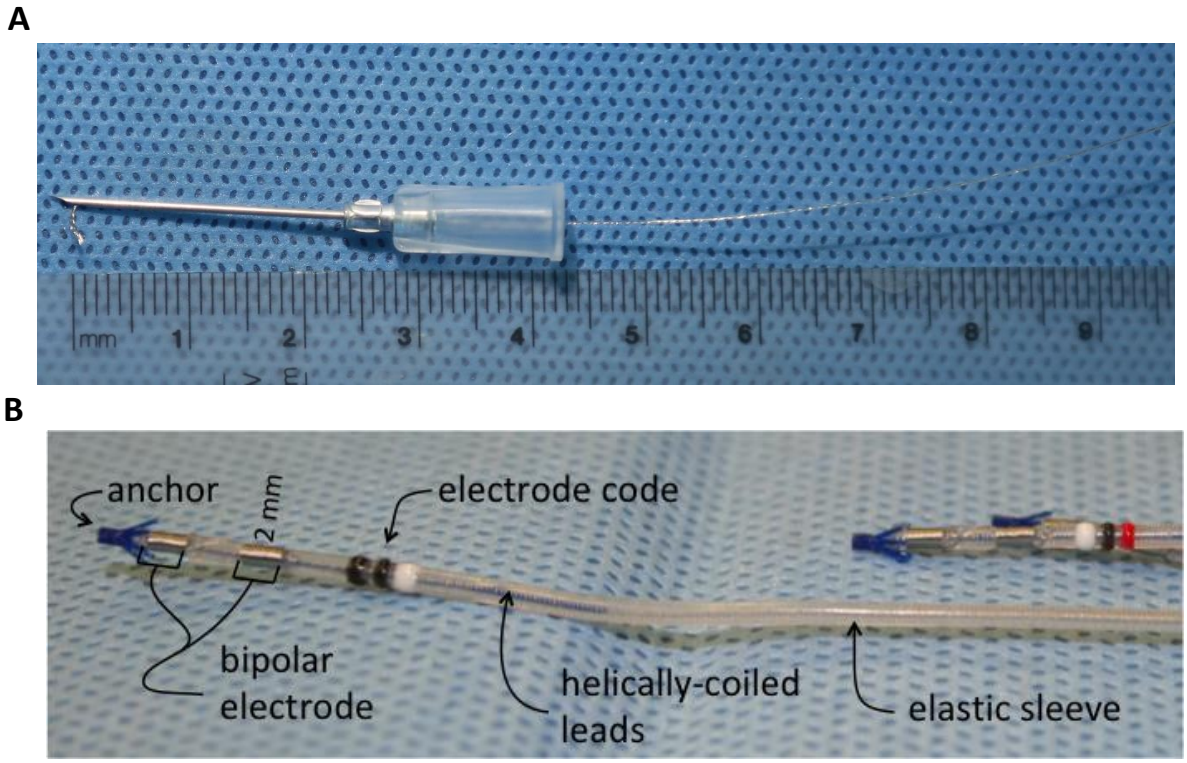

Figure 2. *(A) Crooner wire electrode inserted into a hypodermic needle, insulation has been removed from the distal end, which is folded back against the needle shaft. (B) Ardiem bipolar electrode, 2mm uninsulated spiral segments are used to stimulating and recording electromyographic signals . Polypropylene barbs serve as anchor to maintain position within the muscle. Individualized electrode code corresponds to specific muscles.*

the muscle. 2. the electrode leads were helically twisted along their length and housed within an elastic sleeve, allowing the electrodes to stretch, relieving potential strain on the leads. 3. the electrodes were custom-designed to have two independent uninsulated spiral areas that were used for bipolar recording and stimulating surfaces.

In all cases, electrode leads were soldered to a small board (Neuralynx pcb board ADPT-HS-36PSR). The board included connectors for cables that interfaced with differential

amplifiers (for EMG recording associated with stage 1 of the experiment) or to multichannel stimulators (for delivering current pulses for stage 2). The board and connectors were protected within a Delrin plastic encasement that was mounted on the monkey's skull (see below).

Muscles: To generate complex three-dimensional arm movements requires simultaneous activation of muscles controlling the scapula, shoulder, elbow, and wrist. We implanted 30 muscles, including 3 scapular muscles (rhomboids, trapezius, serratus anterior), 9 shoulder muscles (supraspinatus, infraspinatus, subscapularis, teres major, anterior deltoid, middle deltoid, posterior deltoid, latissimus dorsi, pectoralis major), 8 elbow muscles (long head of the triceps, lateral triceps, medial triceps, short head of the biceps, long head of the biceps, brachialis, brachioradialis, corocobrachialis medius), 7 wrist and forearm muscles (flexor carpi ulnaris, extensor carpi ulnaris, flexor carpi radialis, extensor carpi radialis, palmaris longus, pronator teres, supinator) and 3 extrinsic finger muscles, capable of generating significant wrist torque were also implanted (flexor digitorum profundus, extensor digitorum communis, and flexor digitorum superficialis).

Surgery: The surgery to implant electrodes was completed in two phases, mounting of the electrode encasement on the skull, followed by implantation of 30 intramuscular electrodes. Using sterile surgical conditions and under isoflurane gas anesthesia, a coronal incision was made in the skin on the dorsal surface of the monkey's skull. Periosteal tissue was removed once the skin was retracted. The electrode encasement was affixed to the skull surface using acrylic cement anchored to approximately 12 bone screws (Fig. 3).

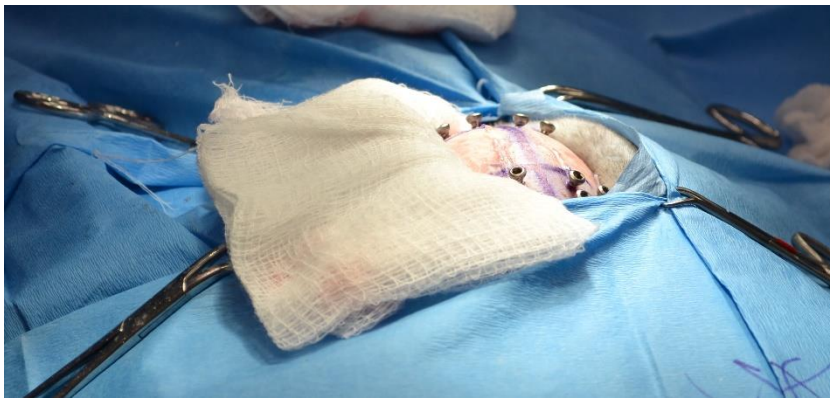

Figure 3. *Dorsal surface of the skull exposed, bone screws will serve as the anchor for the electrode encasement.*

Electrodes were then tunneled subcutaneously from the encasement on the skull to a mid-line incision between the scapulae and externalized. The electrodes were then separated into dorsal and ventral bundles. Electrodes were passed subcutaneously from the midline incision to specific incisions overlying the target muscles. Unique color codes identified each electrode to their target muscle (Fig. 4). An optimal insertion site was determined for each muscle using a small probe that delivered brief trains of stimulus pulses of fixed current amplitude into the target muscle. The probe was placed in different locations in the muscle and the evoked contractions were observed visually. The location that appeared to evoke the strongest contraction was then targeted for implantation with the electrode.

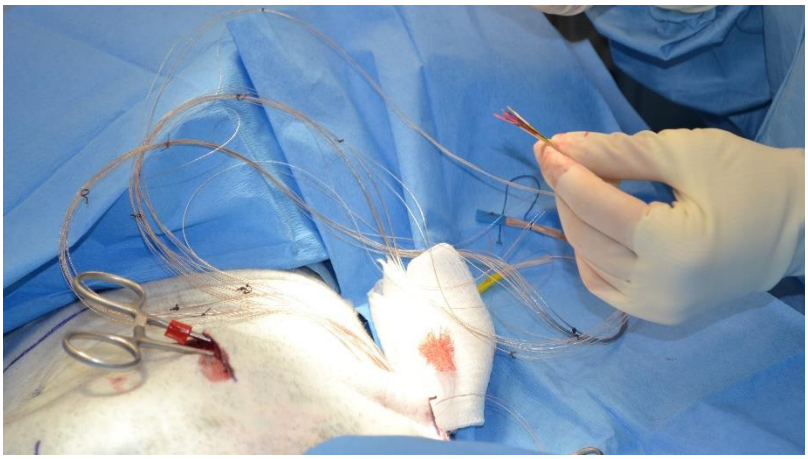

Figure 4. *Color-coded crooner wire electrodes externalized. Electrode bundles are separated into dorsal and ventral bundles.*

Two insertion methods were used for the two types of electrodes. For the simple stainless-steel electrodes, the wire was passed through the eye of a curved needle and pulled through the targeted region of the muscle. Double knots were tied near the distal end of the lead and approximately 3 mm of insulation was removed from the end of the wire using a thermal wire stripper. The double knots and the denuded end were then pulled back into the muscle with the knots serving to help anchor the electrode in the muscle.

For the Ardiem electrodes, a custom-built insertion tool was fabricated from 14-gauge needle to accommodate insertion of the electrodes. The insertion tool was built to hold the polypropylene barbs over the edge of the needle opening. The insertion tool holding the Ardiem electrode was inserted into the muscle and retracted, leaving the electrode embedded in the muscle. A slot within the insertion tool allowed the lead to be separated from the tool. Once the electrode was placed, brief trains of stimuli were delivered through each lead of the Ardiem bipolar electrode and the evoked responses/movements observed. If stimulation failed to evoke a

robust contraction, the electrode was removed and reinserted. Large surface area ground electrodes were placed under the skin near the midline incision.

All incisions were closed with sutures, treated with antimicrobial cream, and covered with gauze. The arm was immobilized before the monkey recovered from anesthesia. Immobilization remained in place for 5 days following surgery to encourage electrode anchorage within the muscle (Bhadra & Mortimer 2006).

Figure 5 shows X-ray images of the implanted electrode system. Figure 5A shows the electrode connector and bone screws on the skull and the bundle of electrodes extending down from the encasement under the skin of the dorsal neck region. Figure 5B depicts Cooner wire electrodes with knotted terminations in muscles near the elbow in one monkey. Figure 5C shows bipolar Ardiem electrodes in muscles near the elbow in a different monkey.

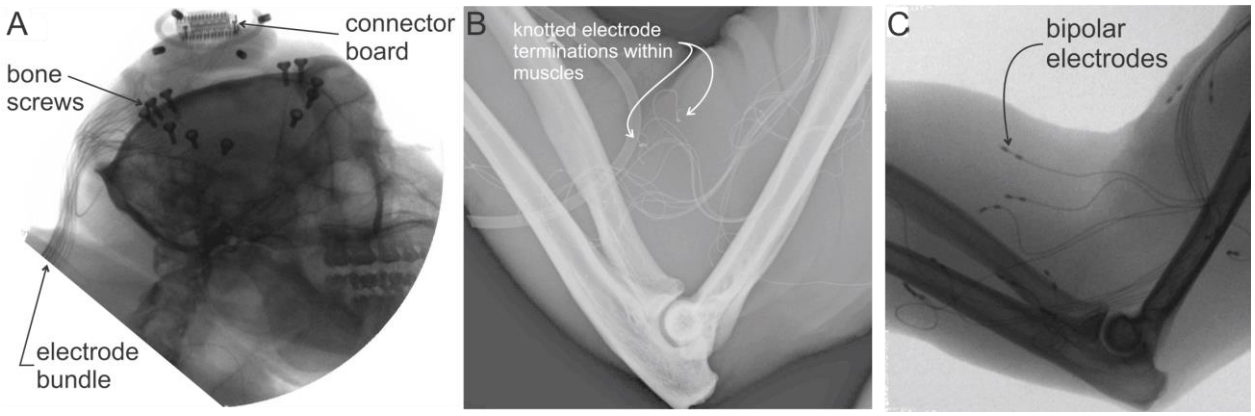

Figure 5. *Panel A. Skull mounted encasement and Neuralynx board. Panel B. Crooner wire electrodes. Panel C. Ardiem bipolar electrodes.*

Phase 1 - Predicting Muscle Activity Patterns

EMG & Kinematics: Approximately two weeks post surgery, kinematics and EMG signals were recorded while monkeys made complex reaching movements (Fig. 6). Electromagnetic tracking (120 Hz/channel, Liberty Systems, Polhemus, Inc.) was used to record six degrees-of-freedom (X, Y, Z positions, as well as pitch, roll and yaw orientations) motion of the hand. Small sensors (0.7 cm x 0.5 cm) were attached to the hand and shoulder using elastic wrap and medical tape. Shoulder position was used to represent the origin of a reference frame for measuring hand position.

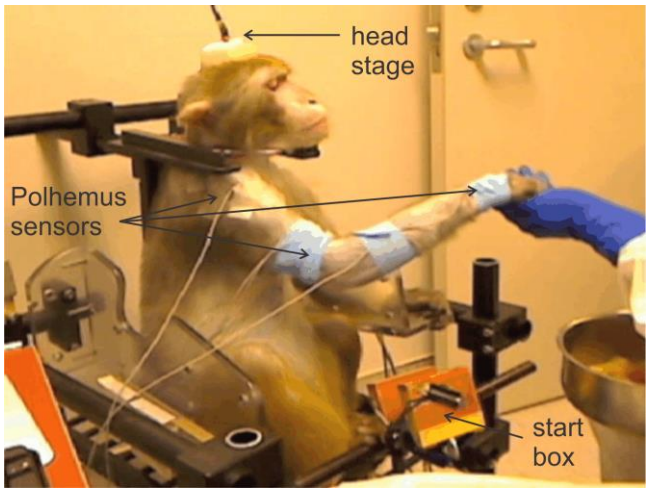

Figure 6. *Training session to record EMG and Kinematic data used to train the machine learning algorithm. Electromagnetic Sensor were placed at the shoulder, elbow and back of the hand. EMG data is recorded via the head stage.*

To record EMG activity, two lightweight cables with local head stages were attached to the connectors inside the encasement on the skull (see Fig. 6). The EMG signals detected with

the intramuscular electrodes were routed by the cables to four, 8-channel differential amplifiers (Lynx 8, Neuralynx Inc.). EMG signals were amplified (gain = 1000), band-pass filtered (100 – 475 Hz), and digitally sampled (2500 Hz/channel) using a computerized data acquisition system (Power 1401, Spike 7, Cambridge Electronics Design, UK). To synchronize the kinematic and EMG data, a TTL pulse generated by the Polhemus system was recorded in the data acquisition system. Additionally, a signal generated by the photodiode switch mounted in the start box indicated when the limb was in the starting position.

Signal Processing: All data were processed offline using custom-designed Matlab scripts (Mathwork, Natick, MA). Hand position (x -anterior/posterior; y - medial/lateral; z - vertical) data were expressed relative to the shoulder position and normalized to the maximal displacement of the hand recorded during each experimental session. Additionally, pitch, roll and yaw orientations of the hand were expressed relative to an earth-based reference frame. Kinematic data were then low-pass filtered (6Hz cut-off, six order Butterworth, zero phase). EMG signals were full-wave rectified and low pass filtered at 2 Hz (sixth order, Butterworth, zero phase). EMG signals were then down-sampled to 120 Hz/signal and synchronized with the position data. EMG magnitude was normalized to a percentage of the peak EMG recorded over all training sessions.

Training Data: Data used to train the machine-learning algorithm and data used to test the algorithm were either from the same monkey (within-subject analysis) or from different monkeys (across-subject analysis). Within-subject analysis represents the best-case scenario for predictions because subject differences in the relationship between EMG and kinematics do not contaminate predictions (Johnson & Fuglevand 2009). Across-subject analysis (where data

collected from one monkey was used to train the algorithm, while data from a different monkey was used to test the predictive ability of the algorithm) represents a more realistic evaluation of machine learning as a means to develop an FES controller (Johnson & Fuglevand 2009). When applied to actual patients, able-bodied subjects would be needed to provide the data to train the algorithm to be deployed in paralyzed individuals.

Machine Learning: Machine learning refers to a host of computational methods (artificial neural networks, Bayesian probability, support vector machines, etc.) that use existing data (both input and outputs from some system of interest) to learn associations among the variables (in a process called "training"). The training in turn generates computer algorithms that can be use make predictions based on a new set of inputs. Previously, our lab has demonstrated the utility of using machine learning to predict EMG from desired kinematics (Siefert & Fuglevand 2002, Anderson & Fuglevand 2008, Johnson & Fuglevand 2009, Tibold & Fuglevand 2015). Here we use an artificial neural network (ANN) as our machine-learning algorithm based on its slightly better performance and computational efficiency compared to other algorithms for this type of data (Johnson & Fuglevand 2009).

For each time point, six kinematic parameters were used as inputs to the ANN (X, Y, Z position of the hand relative to the shoulder, pitch, roll and yaw orientations of the hand). In addition, the kinematic values from the two immediately preceding time points were also included as inputs. In previous work it was demonstrated that additional kinematic features such as velocities, accelerations, and other joints positions (e.g. the elbow), had only a limited effect on the predictions of EMG patterns, as a result we did not include these features (Anderson &

Fuglevand 2008). The ANN was a multilayer perceptron involving a feed-forward network created in the Neural Networks Toolbox of Matlab (Mathworks, Natick, MA).

After the network was trained, new predictions were made from data not included in the training. These testing data were processed in the same way as the training data. Predictions were low-pass filtered with a 10-point moving average filter to remove high-frequency deflections.

Analysis of predicted EMG signals: Once the ANN was trained, EMG signals were then predicted from the kinematics recorded for a set of 16 test movements, each ~ 10 s in duration. The EMG and kinematic data recorded from the test movements, which involved a variety of movements including simple and complex trajectories, were not used to train the ANN. EMG signals here refer to the rectified, low-pass filtered, and amplitude normalized EMG recorded from any muscle. The main metric used to measure the quality of predictions was the coefficient of determination (R^2). The coefficient of determination is based on the correlation between the actual and predicted EMG signals for each muscle. It is an indication of the amount of variance in the recorded EMG signals explained by the predicted EMG signals and indicates how closely the predicated and actual activity patterns are matched.

Phase 2 - Evoking Desired Movements With Multi-Muscle Stimulation

Conversion of EMG to Stimulus Pulses: Predicted EMG signals associated with a set of test movement trajectories were converted off-line into trains of current-regulated stimulus pulses (0.5 ms duration) to be delivered to the 30 implanted muscles in temporarily paralyzed monkeys (see Stage 2, Figure 1). To do this, we used a 'transfer function' described by Johnson and Fuglevand (2011) that converted EMG signals into amplitude and frequency modulated

stimulus pulse trains. Increasing pulse amplitude increases the number of motor axons recruited to the contraction. Increasing pulse frequency increases the contraction intensity by temporal summation of contractile impulses (i.e. rate coding). The transfer function modulated pulse amplitude and pulse frequency both as sigmoid functions of EMG amplitude. As such, as predicted (or actual) EMG increased, both pulse amplitude and pulse frequency increased monotonically and in parallel with one another. Such co-modulation of amplitude and frequency approximates natural activation of muscle wherein motor unit recruitment and rate coding operate in parallel over much of the force range of typical muscles (Johnson & Fuglevand 2011). For each muscle, the pulse amplitudes were normalized from the minimum current needed to evoke a detectable response to the pulse amplitude above which no further increase in contraction strength could be discerned. Stimulus frequencies were normalized between 10 and 60 Hz. These frequencies represent the typical range of firing rates recorded in human motor units. Little such data exists for firing rate ranges in monkeys.

Stimulation Experiments: Once stimulus patterns had been generated for each test movement using the transfer function, we then played out those patterns to the 30 muscles in experimental sessions during which the monkeys were anesthetized. Monkeys were first sedated in their home cage with Ketamine HCL (10 – 15 mg/kg IM) and transported to the procedure room. Atropine (0.04 mg/kg IM) was given to reduce hyper-salivation common with Ketamine sedation. Carprofen (2.2 mg/kg SQ) was also given to reduce inflammation associated with endotracheal intubation. A 22-gauge intravenous catheter was placed in the saphenous or cephalic vein to deliver lactated Ringers (5-10 ml/kg/hr) to maintain hydration. Anesthesia was induced with isoflurane (1.5 – 2% in 100% oxygen, ~ 1 L/min) via mask insufflation. Following

induction, an endotracheal tube was inserted to maintain airway patency and deliver anesthesia (1 – 2% isoflurane in 100% oxygen, ~1 L/min).

Monkeys were then placed into a modified infant car seat in a seated position. A neonatal cervical collar was used to maintain the head in an upright position. The cervical collar was fixed in place to the car seat with cable ties passed through holes drilled in the car seat and through slots in the back of the collar. Straps situated midway between the neck and shoulder and across the torso secured the animal to the chair. The right (test) arm was placed in a position that approximated placement of the hand was in the start box during the training phase.

Monkeys were instrumented with an esophageal thermometer to measure core temperature and a SpO2 monitor placed on one of the digits of the left hand. Heart rate, respiratory rate, electrocardiogram, end-tidal CO2, and non-invasive blood pressure (cuff over radial artery) signals were monitored throughout the experiment. Core temperature of ~ 98 º F was maintained via a forced warm air blanket and bubble wrap placed over the torso. Physiologic parameters were noted every 5 – 10 minutes.

With the monkey anesthetized and positioned in the modified car seat, the four 8-channel, STG-4008 stimulators were attached by two cables to the electrode connectors mounted on the skull. Muscle thresholds, (minimum current to evoke a detectable response) established previously was verified with 0.1 mA steps and compared to previous measures. The Polhemus liberty system was used to record motions of the hand relative to the shoulder position, using sensor placed on the hand. Six degrees of freedom (X,Y,Z positions, as well as pitch roll and yaw orientation) were recorded. Trains of stimulus pulses associated with each of the test movements were delivered to all muscles in separate trials with about 1 minute of rest between

trials. The evoked movements were recorded and compared to the desired test movements using R^2 measures.

3.0: RESULTS

Examples of hand trajectory tracked during a single training session are shown in Figure 7. Figure 7A shows a single reach to a food morsel and then to the mouth, while figure 7B shows all trajectories recorded during a single training session. It can be seen from Figure 7B that a relatively wide expanse of the reach space was sampled during this session. Likewise, Figure 8 shows a brief sample of the rectified low-pass filtered EMG signals recorded for a number of example muscles during a training session. The example data shown in Figures 7 and 8 represent the type of data used to train the ANN.

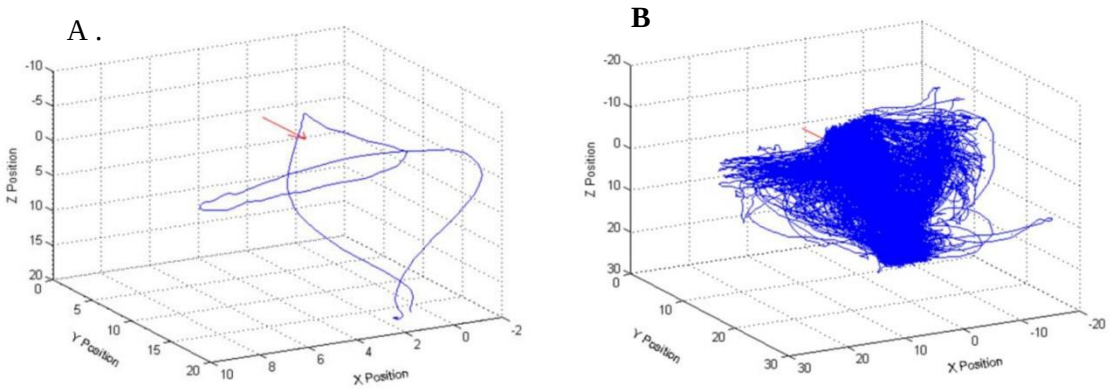

Figure 7. *(A). Trajectory of the arm during a single reach. (B). Trajectories recorded during a single training session. Red arrow indicates the direction the monkey is facing. Monkey's mouth in 7(A) is approximately at X-2, Y-2, Z-1.*

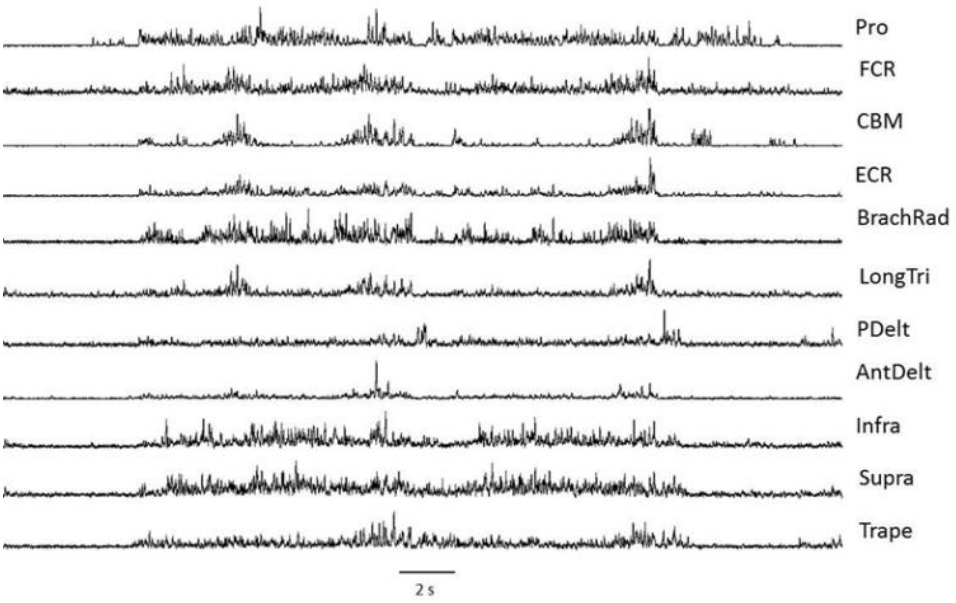

Figure 8. *Example Electromyographic (EMG) recorded during a single training session. EMG is rectified and low pass filtered.*

Figure 9 shows representative examples of EMG signals predicted by the trained ANN. The inputs to the trained network were hand kinematics from movement trials that were not used to train the network. The black traces indicate the actual EMG recorded while the red trace indicates the predicted EMG signals. The predictions match the actual EMG signals quite well in both amplitude and temporal specificity.

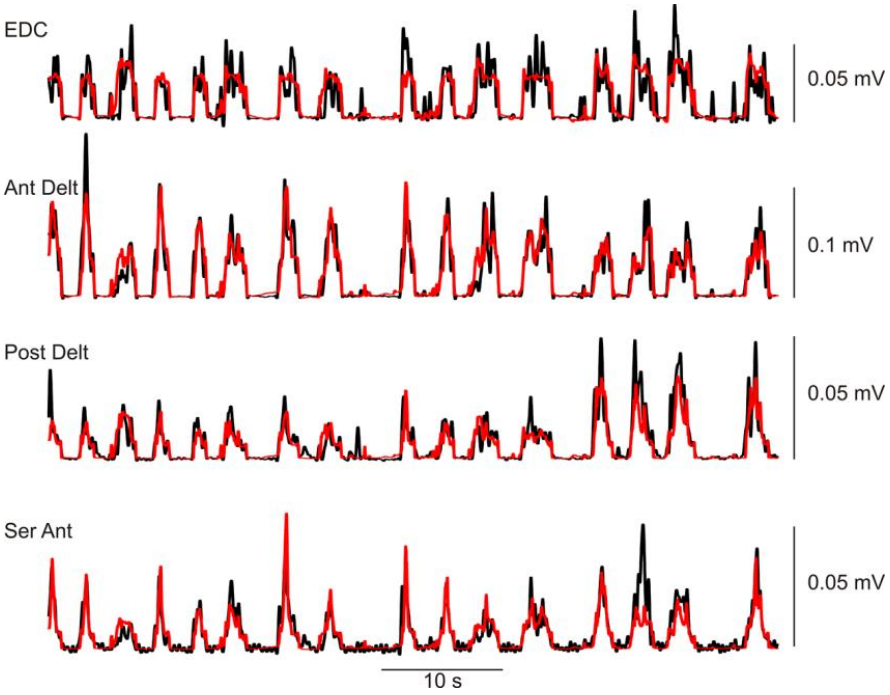

Figure 9. *Predicted EMG (Black) overlaid on actual EMG (Red).*

In one monkey (the only monkey in whom we were able to record sufficient data for analyses), the coefficient of determination (R^2) between actual and predicted EMG averaged across a set of 64 test-movement trials and across all muscles was 0.56. The predictions, however, varied across muscles. For example, large prime movers like the anterior deltoid (0.74), Triceps (0.73), and posterior deltoid (0.52) were fit best. Muscles of the forearm, however, such as brachiradialis (0.15), flexor carpi radialis (0.18) and flexor digitorum profundus (0.12) showed poorer fits. These differences likely reflect the degree to which individual muscles contributed to the actual movement. Nevertheless, given these promising results for the prime movers of the arm, we were encouraged to use these predicted signals as templates for electrical stimulation in an attempt to evoke targeted movement trajectories.

Stimulation. We delivered the stimulus-pulse patterns (Fig. 10) derived from our FES transfer function (Johnson & Fuglevand 2011, see Fig. 1, Stage 2) and based on the ANN predictions of EMG activity to the muscles of anesthetized monkeys in an attempt to generate test movements. Disappointingly, stimulation during these experiments failed to generate all but minor displacements of the limb. Stimulation was unable to elevate the arm from the starting position; instead the arm typically flexed slightly at the elbow and was associated with oscillations of the wrist and fingers. Even the simplest trajectory sought with artificial stimulation was not attained. It should be noted that in a single trial in one monkey, the arm did elevate modestly. However, we were unable to duplicate those results during this experiment or any other.

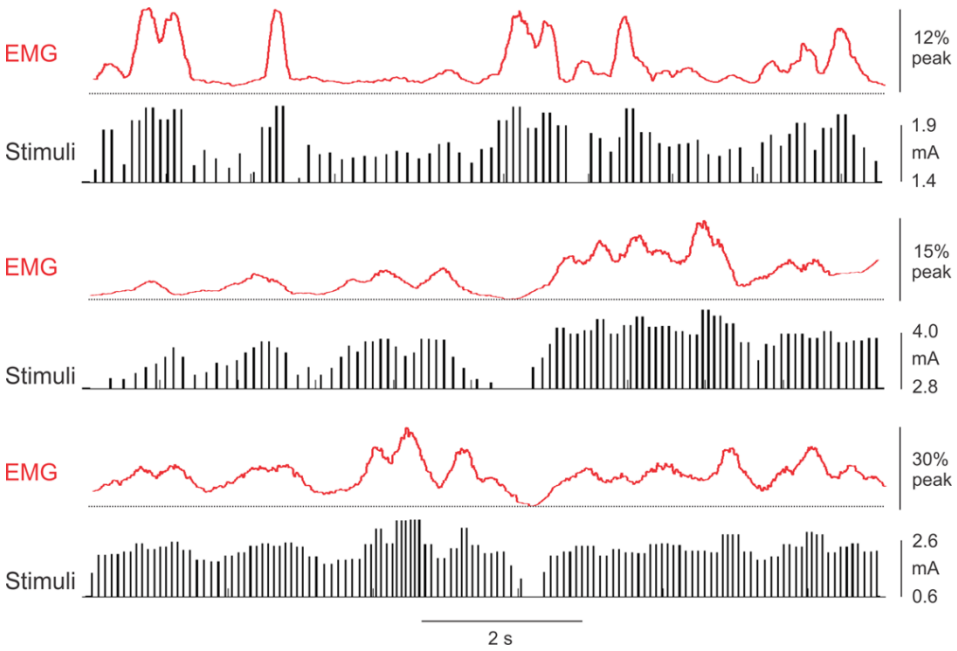

Figure 10. *Example filtered and smoothed EMG. Lower part of the figure shows amplitude and frequency modulated stimulus patterns derived from predicted EMG.*

4.0: DISCUSSION

At present, upper-limb neuroprosthetic systems can evoke only a small number of motor behaviors in paralyzed individuals (Kilgore et al., 1989; Memberg et al. 2014; Ajiboye et al. 2017). A key factor that limits the production of a broader repertoire of movements with FES is the difficulty in identifying the patterns of muscle stimulation needed to evoke desired movements. Previously, we have shown that EMG signals can be predicted from desired kinematics with good fidelity for relatively simple movements (Siefert & Fuglevand 2002; Anderson & Fuglevand 2008). Here, we sought to markedly increase the types of movements available through FES systems. We showed that EMG patterns associated with complex, multi-joint, three-dimensional movements can be predicted with good accuracy using machine learning.

Unfortunately, while the machine-learning algorithm performed well, we were unable to generate robust movements of the limb using artificial stimulation. Our inability to generate clear movements could have been due to a number of problems. For example, electrode migration (confirmed in post-mortem evaluation for some electrodes) may have partially contributed to deficits in the efficacy of stimulation in the first monkey who was implanted with simple hook-wire electrodes. However, electrode migration was unlikely to have accounted for the inability to evoke movements in the other two monkeys implanted with Ardiem electrodes. The Ardiem electrodes possess a barb-like collar specifically designed to prevent electrode migration when implanted in human patients. It should be noted, however, that the Ardiem electrodes inexplicably led to breakdown of the skin overlying the path of the electrodes and had

to be removed. Once the electrodes were removed the lesions healed. However, we were not able to actually confirm that the electrodes had remained in the target muscles.

After ensuring that the failure to evoke robust movements was not due to other technical problems, we speculated that the most likely cause was associated with an intrinsic inability of electrical stimulation to evoke strong muscle contractions. Indeed weak force generation along with rapid fatigue had been cited previously as primary problems of FES systems (Enoka and Fuglevand 1991; Maffiuletti et a. 2014; Bhadra & Peckham 1997; Mizrahi 1997; Kesar et al. 2008; Doucet et al. 2012; Guiraud et al. 2014; Ibitoye et al. 2016; Barss et al. 2018; Naess & Storm-Mathisen 1955; Binder-Macleod & Snyder-Mackler 1993; Nyugen et al. 2010). In particular, it has been shown in a few studies that maximum electrical stimulation evoked only modest force compared to that produced voluntarily in able-bodied subjects (Vanderthommem & Duchateau 2007; Milner et al. 1969; Kramer et al. 1984).

To evaluate this possibility, we initiated pilot experiments to compare force generated as a result of voluntary contractions to that evoked with artificial stimulation in human subjects (N =2). We first tested flexion of the interphalangeal joint of the thumb because this is an action that is controlled by a single muscle (flexor pollicus longus, FPL).

The force generated by intense percutaneous intramuscular stimulation of the FPL produced values that were only about 60% of that which could be produced during a maximum voluntary contraction. It was noted, however, that in the one subject on whom this was tested, substantial involuntary co-activation of other muscles of the arm and torso occurred during the electrical stimulation. This seemed to be largely associated with the pain caused by the strong electrical stimulation. Such co-contraction of antagonist muscles will reduce the external force

detected by a transducer. These finding were replicated when testing another muscle, the anterior deltoid. Therefore, because of the substantial pain and inadvertent co-contraction of other muscles, these studies were stopped.

Nevertheless, these pilot studies led us to develop experimental models where; (1) the effects of the discomfort and associated co-contraction caused by electrical stimulation could be removed and (2) the target muscle could be isolated for study. The studies described in the following two chapters of this dissertation used these experimental models to help understand the causes of weak contraction and fatigue that severely undermine the utility of functional electrical stimulation to restore movement in paralyzed individuals.

CHAPTER 3

DISTRIBUTED STIMULATION INCREASES FORCE ELICITED WITH FUNCTIONAL ELECTRICAL STIMULATION

The work contained within this chapter has been published in the Journal of Neural Engineering. It is now copyrighted material, the paper has been included in this dissertation as **Appendix A**. A summary of the work and a contribution summary has been included within this chapter.

Study summary

The work contained in this chapter is presented as a published article in Appendix A. Here, we compared the maximum isometric force that could be evoked in the anterior deltoid using one or two intramuscular electrodes. We also examined whether temporarily interleaving stimulation between two electrodes might reduce fatigue compared to simultaneously stimulating through two electrode.

To test this idea we selected a non-human primate as our model animal, due to comparable musculoskeletal systems and upper limb anatomy. The two primates participated in a total of 29 trials. We compared the maximum isometric forces that could be evoked with two intramuscular electrodes. In separate trials we evaluated whether temporally interleave stimulation might reduce fatigue as opposed to synchronized stimulation.

We found that two electrode stimulation produced greater force than maximal stimulation with only one electrode. In our evaluation of fatigue using interleaved or synchronous stimulation we found no reduction in fatigue with interleaved stimulation.

Contribution Summary: This work was completed in conjunction with the authors listed on the title page of the publication manuscript in **Appendix A**. As first author, I completed all the experiments and was responsible for the analyzing the data, compiling figures and writing the manuscript. Dr. Andrew Fuglevand provided additional analysis of the experimental results. I received undergraduate assistance from Danielle Lockwood in completing the experiments.

CHAPTER 4

MITIGATION OF EXCESSIVE FATIGUE ASSOCIATED WITH FUNCTIONAL ELECTRICAL STIMULTATION

The work contained within this chapter has been published in the Journal of Neural Engineering. It is now copyrighted material, the paper has been included in this dissertation as **Appendix B**. A summary of the work and a contribution summary has been included within this chapter.

Study summary

The work contained in this chapter is presented as a published article in Appendix B. In this portion of the study, we examined how multi-electrode stimulation could affect the fatigue due to functional electrical stimulation. We compared endurance time in three scenarios including multi-electrode stimulation and peri/intra-neural stimulation, compared to voluntary contraction. The impetus for this study arose from our desire to address the second major problem effecting Functional electrical stimulation, that of fatigue. Rapid fatigue is a hallmark of artificial stimulation and is well documented in the research literature. Traditionally, a single electrode is used to activate muscles with FES. We hypothesized that due to the highly distributed branching of intramuscular motor axons, a single electrode may be insufficient to activate the entire nerve array supplying a muscle. Stimulating with multiple spatially distributed electrodes however, should enable access to a larger volume of muscle fibers. This in turn, facilitates contractile load-sharing among muscle fibers, reducing fatigue.

Additionally, FES indirectly evokes reflex contractions, confounding measurements of the directly activated muscle response. Removing the reflex component of the evoke contraction would provide a more accurate measure of fatigue resulting from artificial stimulation. The aim of this study therefore, is to compare endurance time, in the absence of reflex contractions, using a single percutaneous electrode, multiple percutaneous electrodes, or a single intra-neural electrode.

Reflex contribution to the contractile force measured was removed, using a localized nerve block, applied to the peroneal nerve, proximal to the knee. Tungsten microelectrodes were inserted into the Tibialis Anterior (TA) of human subjects (n=4). Using one voluntary and three

artificial stimulation tasks, we compared endurance time while subjects dorsiflexed their foot against a force transducer. A target force of 20% of the subject's maximum voluntary contraction (MVC) was used for all trials. Baseline endurance time was measured with a voluntary contraction of the TA. In artificial stimulation trials, continuous stimulation was delivered at 25 Hz until the evoked force could no longer be maintained within 5% of the target force. The stimulus intensity was automatically adjusted using a feedback control algorithm to maintain the target force at 20% of each subject's MVC. Only one fatigue trial was tested per experimental sessions with 48 hours rest between sessions.

Overall, our findings show that endurance times were significantly increased in all scenarios. Specifically, multiple electrode endurance time was 139% greater than single electrode time. Additionally, nerve stimulation was 952% and 341% greater than single and multiple electrode stimulation respectively. Thus, it appears that endurance time can be increased using multiple electrodes and an intra-neural electrode.

Contribution Summary: This work was completed in conjunction with the authors listed on the title page of the publication manuscript in Appendix B. As first author, I completed all the experiments and was responsible for the analyzing the data, compiling figures and writing the manuscript. Dr. Andrew Fuglevand provided additional analysis of the experimental results. I received assistance from Tapas Arakeri in completing the experiments. He programmed the control algorithm used to maintain the target force and would control the stimulator as we probed the muscle for electrode placement.

CHAPTER 5

DISCUSSION

Functional electrical stimulation offers the potential to restore movement in paretic limbs by artificially activating skeletal muscle. However, this potential has not been realized due in part to the difficulty associated with predicting the patterns of muscle stimulation needed to evoke all but the simplest motor behaviors. Additionally, weak contraction strength and rapid fatigue are major problems limiting the development and deployment of FES systems.

Therefore, the primary goal of this work was to advance the development of an upper limb neuroprosthetic capable of generating an unrestricted range of complex motor behaviors. Three challenges associated with this goal were addressed in this dissertation: 1) accurate prediction of the patterns of muscle stimulation required to generate complex motor behaviors, 2) increasing the force evoked by electrical stimulation, and 3) increasing endurance time (reducing fatigue) of electrically-evoked contractions.

To determine whether we could estimate the muscle activation patterns associated with complex multi-joint behaviors, we employed a machine learning algorithm involving an artificial neural network. Inputs used to train the algorithm included arm kinematics and EMG signals from ~ 30 muscles that were recorded while monkeys made complex reaching movements. Once trained, the neural network was able to predict the activation patterns associated a new set (i.e. not used to train the algorithm) of arm movements. The predicted patterns were temporally and spatially similar to the actual EMG signals recorded during these new movements. Therefore, in principle, one could use these predicted patterns of muscle activity as templates for electrical stimulation needed to evoke a wide range of movements in paralyzed individuals. However, when we attempted to demonstrate this in temporarily paralyzed monkeys by

converting those patterns into amplitude and frequency modulated stimulus pulses, our attempts were unsuccessful because of weak contractions and rapid fatigue.

As is the convention in FES, we delivered stimulus current to single electrodes in each muscle. In attempting to understand why evoked muscle contractions were so weak, we recognized (based on relatively new findings in the literature; Won et al. 2011; 2012; 2015) that innervation of skeletal muscle involves a highly diffuse network of nerve fibers distributed throughout a muscle rather than a singular and centrally-located motor point, as has been largely presumed. On this basis, we hypothesized that a single electrode may be insufficient to activate all nerve branches innervating a muscle even when using high currents. We carried out experiments to test this hypothesis in anesthetized non-human primates by comparing the maximum muscle force exerted in a test muscle in response to stimulation with one electrode to that evoked with multiple electrodes. In all cases, greater forces were elicited when using multiple electrodes, indicating that a greater volume of muscle fibers was activated when using multiple electrodes. This finding suggests that FES can be made more efficacious in terms of force generation by using more than one site of stimulation, particularly for large muscles.

It also seemed to follow that weakness associated with conventional FES was itself a primary cause of rapid fatigue with FES. Indeed, if only a fraction of muscle fibers within a muscle can be enlisted with a single electrode, then a smaller reserve of fresh muscle fibers will be available to be activated to compensate for force loss in those muscle fibers initially activated during a sustained contraction to a given target level. This would mean that the target force should not be maintained as long as compared to a situation where more muscle fibers can be called upon as in the case of multiple electrodes or direct stimulation to the muscle nerve.

To test this idea, we compared endurance times using a single intramuscular electrode, multiple intramuscular electrodes, and direct nerve stimulation. Additionally we measured the voluntary contraction time for comparison. We demonstrated that endurance time was greater when using multiple electrodes compared to single electrodes. Furthermore, endurance time using nerve stimulation was substantially longer than that using intramuscular stimulation and could even exceed voluntary endurance time. Therefore, future implementations of FES systems to restore movement in paralyzed limbs should attempt to include multiple intramuscular electrode or nerve-based electrodes to help mitigate fatigue.

While the work presented in this dissertation may help advance the utility of FES systems, there remain a number of other challenges that should be addressed in the future. For example, actual deployment of an upper limb neuroprosthetic would require real-time predictions (rather than off-line, as done in this dissertation) from artificial neural networks to generate patterns of muscle stimulation needed to produce desired movements. Importantly, there will also need to be some efficient means by which the user provides the desired limb trajectory as the input to the trained artificial neural network. This might be accomplished with detection of gaze direction when fixating on target objects, verbal commands, or even brain-machine interfaces that decipher desired trajectories from neural activity recorded in the cerebral cortex.

Additionally, future upper limb neuroprosthetics systems must be intelligent and adaptable enough to account for interactions with objects in the environment (not just free movement as done here). Systems must include provision for on-line feedback adjustments to stimulus parameters, accommodating for unexpected perturbations of the limbs, muscle fatigue or even errors in the algorithm. Longevity of such implanted systems necessitates that subjects

be untethered from external cables, utilizing instead wireless technology, and that implanted components including electrodes not illicit an immune response, are robust but reduced in size and most importantly remain at their optimal implantation site.

Indeed, such advances would move upper limb neuroprosthetic and the restoration of paralysis out of the laboratory and into daily lives, allowing paralyzed individuals to regain an increased degree of freedom and autonomy, even normalcy.

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APPENDIX A

DISTRIBUTED STIMULATION INCREASES FORCE ELICITED WITH FUNCTIONAL ELECTRICAL STIMULATION

Distributed Stimulation increases force elicited with functional electrical stimulation

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ABSTRACT

Objective: The maximum muscle forces that can be evoked using functional electrical stimulation (FES) are relatively modest. The reason for this weakness is not fully understood but could be partly related to the widespread distribution of motor nerve branches within muscle. As such, a single stimulating electrode (as is conventionally used) may be incapable of activating the entire array of motor axons supplying a muscle. Therefore, the objective of this study was to determine whether stimulating a muscle with more than one source of current could boost force above that achievable with a single source.

Approach: We compared the maximum isometric forces that could be evoked in anterior deltoid of anesthetized monkeys using one or two intramuscular electrodes. We also evaluated whether temporally interleaved stimulation between two electrodes might reduce fatigue during prolonged activity compared to synchronized stimulation through two electrodes.

Main Results: We found that dual electrode stimulation consistently produced greater force (~50% greater on average) than maximal stimulation with single electrodes. No differences, however, were found in the fatigue responses using interleaved versus synchronized stimulation.

Significance: It seems reasonable to consider using multi-electrode stimulation to augment the force-generating capacity of muscles and thereby increase the utility of FES systems.

INTRODUCTION

Functional electrical stimulation (FES) involves artificial activation of muscles to restore some measure of motor function in paralyzed individuals. One vexing problem undermining the utility of FES is that the maximum forces exerted with stimulation are relatively weak. Indeed, even in healthy subjects, the largest forces that can be evoked with electrical stimulation are inexorably less than that produced during maximum voluntary contraction (Milner et al. 1969; Marsh et al. 1981, Kramer et al. 1984; Enoka and Fuglevand 1991; Koh and Herzog 1995; Vanderthommen and Duchateau 2007; Maffiuletti et a. 2014). Many factors likely contribute to this weakness in individuals with spinal cord injuries, including muscle atrophy and muscle denervation. As a consequence, most FES systems applied to high-level tetraplegics, for example, require an external frame to support the arm because muscle stimulation alone is insufficient to elevate the limb against gravity (Hoshimiya et al. 1989; Nathan 1989; Schill et al. 2011; Memberg et al 2014).

In addition, it seems possible that single site stimulation (as typically used in implanted FES applications) may simply be insufficient to fully activate most muscles, particularly larger muscles controlling more proximal joints like the shoulder. A widely held view is that if isometric force saturates with escalating stimulating current delivered by a single electrode, then the maximal force capacity of the muscle has likely been achieved (e.g. Merton 1954; Bigland-Ritchie et al. 1979; Rutherford et al. 1986). However, the highly distributed branching of motor nerves in human muscle (Sunderland and Hughes 1946; Amarali et al. 2007; Mu and Sanders 2010; Won et al. 2011; 2012; 2015) combined with the steep decay in the electric field strength with distance from a stimulating electrode (McIntyre and Grill 2002; Rattay 2004) may preclude

activation of the entire array of motor axons within a muscle with a single intramuscular electrode (Memberg et al. 2014).

Therefore, the aim of this study was to determine whether stimulating a muscle using intramuscular electrodes but with more than one source of current could boost force above that achievable with a single source. An additional potential benefit of such distributed stimulation is that some degree of load sharing among the muscle fibers activated by different electrodes might help minimize fatigue during prolonged activity (Mortimer 1981; Yoshida and Horch 1993; Wise et al. 2001; McDonnall et al. 2004; Nguyen et al. 2011; Sayenko et al. 2014; Lou et al. 2017; Wiest et al. 2017). Because of the relatively prolonged force response to each stimulus pulse, it seems feasible that a target force could be maintained by alternating stimuli among the electrodes using lower rates than would be needed by a single electrode. In turn, lower rates of muscle fiber activation might lessen the overall degree of muscle fatigue. Therefore, a secondary aim of this study was to determine whether temporally interleaved stimulation between stimulation sites might reduce fatigue compared to synchronous stimulation.

METHODS

Subjects. Two adult male rhesus monkeys (Macaca mulatta), ages 11 and 13 years, were included in this study in accordance with IACUC guidelines and approved by the University of Arizona institutional review board. Subjects were anesthetized during the experiments (see details below). We initially attempted these experiments in awake healthy human subjects but the discomfort (Merton 1954; Edwards 1984, Vanderthommen and Duchateau 2007; Maffiuletti et al. 2014) and inadvertent co-contraction (Marsden et al. 1983) associated with intense stimulation precluded valid assessments. Because macaques have relatively large skeletal

muscles and an upper limb anatomy similar to that of humans (Cheng & Scott 2000), they provide a reasonable experimental model for testing FES. Furthermore, because we were interested in the practical implementation of FES operating within intact joint systems, we opted not to surgically isolate the test muscle, the anterior deltoid. The anterior deltoid was selected for study because it is a key contributor to arm elevation particularly for reaching movements (Soechting & Flanders 1997; Gronley et al. 2000). In macaques, the proportion of the anterior deltoid composed of slow twitch oxidative fibers is about 33% (Singh et al. 2002) whereas it is about 50% in humans (Tesch and Karlsson 1985). As such, the anterior deltoid of the macaque may be slightly faster and somewhat less resistant to fatigue than in humans. To minimize the number of animals tested, we carried out repeated testing of the two subjects across multiple sessions. The data reported here were obtained from 28 sessions, 15 for monkey S and 13 for monkey R. A minimum of seven days recovery were allowed between testing sessions for both monkeys.

Subject preparation. Prior to each experimental session, monkeys were sedated in their home cage with Ketamine HCL (10 – 15 mg/kg IM) and transported to the procedure room. Atropine (0.04 mg/kg IM) was given to reduce hyper-salivation common with Ketamine sedation. Carprofen (2.2 mg/kg SQ) was also given to reduce inflammation associated with endotracheal intubation. A 22-gauge intravenous catheter was placed in the saphenous or cephalic vein to deliver lactated Ringers (5-10 ml/kg/hr) to maintain hydration. Anesthesia was induced with isoflurane (1.5 – 2% in 100% oxygen, ~ 1 L/min) via mask insufflation. Following induction, an endotracheal tube was inserted to maintain airway patency and deliver anesthesia (1 – 2% isoflurane in 100% oxygen, ~1 L/min).

Monkeys were then placed into a modified infant car seat in a seated position (Fig. 1). A neonatal cervical collar was used to maintain the head in an upright position. The cervical collar was fixed in place to the car seat with cable ties passed through holes drilled in the car seat and through slots in the back of the collar. Straps situated midway between the neck and shoulder and across the torso secured the animal to the chair. The right (test) arm hung in a pendant position, free of obstruction.

Monkeys were instrumented with an esophageal thermometer to measure core temperature and a SpO2 monitor placed on one of the digits of the left hand. Heart rate, respiratory rate, electrocardiogram, end-tidal CO2, and non-invasive blood pressure (cuff over radial artery) signals were monitored throughout the experiment. Core temperature of ~ 36.7 º C was maintained via a forced warm air blanket and bubble wrap placed over the torso. Physiologic parameters were noted every 5 – 10 minutes.

Force measurements. Once the animal was positioned in the chair, a custom-built force transducer was attached to the pendant arm by a strap that encircled the wrist and was used to measure shoulder flexion force. The transducer position was adjusted to pull the limb slightly in extension and thereby maintain a resting tension of ~ 2 N. The force transducer signal was amplified (x 1000, Transbridge, World Precision Instruments, Sarasota FL, USA) and digitally sampled (1000 Hz, Spike2, Cambridge Electronics Design, Cambridge England).

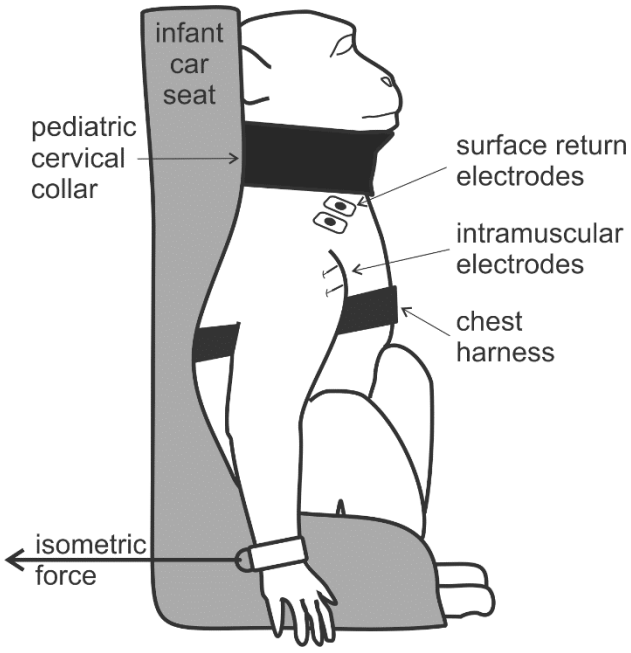

Figure 1. *Experimental set up. Anesthetized monkeys were positioned and secured upright in a modified infant car seat. Two intramuscular electrodes were inserted percutaneously into the right anterior deltoid muscle. Current-regulated pulses were delivered independently through each electrode and the evoked isometric shoulder-flexion forces were recorded with a transducer attached to the wrist. Surface electrodes placed over the clavicle served as return electrodes for each stimulation channel.*

Electrical stimulation. Hair was removed from the skin overlying the right deltoid and surrounding areas. The skin surface was then cleaned with sterile alcohol and gauze. Intramuscular cathode electrodes (tungsten, 250 μm shaft diameter, 1 – 5 μm tip diameter, 2 – 4 mm of insulation removed from the tip) were then inserted through the skin and into the anterior deltoid along the proximal-distal axis of the muscle. Individual electrodes were placed ~ 2 cm apart. In preliminary experiments using up to four intramuscular electrodes, the preponderance of force summation was achieved with two electrodes. As such, two independent intramuscular

electrodes were used for the experiments reported here. Surface electrodes (1.5 cm diameter) placed over the clavicle and acromion process served as the return electrodes.

A programmable multi-channel stimulator (STG4008 MultiChannel Systems, Reutlinger, Germany) generated current-regulated rectangular pulses (0.5 ms duration). Given a compliance voltage of 160 V, and an approximate electrode/skin impedance (at 1000 Hz) of 8 kΩ, we estimated the maximal controlled current available for these experiments to be ~ 20 mA.

Initially, 1 Hz pulses (2 mA amplitude) were delivered to each electrode while the depth position of the electrode was manually adjusted in small increments until the largest twitch forces were elicited. The electrode was then left in that position for the remainder of the experiment. A more comprehensive mapping of the entire anterior deltoid to determine the optimal sites for stimulation was not undertaken primarily for the sake of expediency, as we tried to minimize the time monkeys were under anesthesia. The threshold current needed to elicit a detectable force response was then determined for each electrode. One-second trains of pulses (35 Hz, 4 s inter-train intervals) were delivered in 0.1 mA steps up until a force response was detected on the transducer. The magnitude of the current associated with the first detected response was deemed the threshold current.

Force-frequency relationship. In some of the sessions (10 for monkey R, 12 for monkey S), we characterized the relation between stimulus frequency and isometric force for the anterior deltoid. One-second trains of stimuli (0.5 ms, 6 mA) were delivered at 10, 20, 30, 40, 50, 60, 80, and 100 Hz with 4 s between trains. This was done using one electrode only. As far as we are aware, no previous studies have characterized the force-frequency relationship in limb muscles

of non-human primates. Therefore, this procedure helped us to determine stimulation frequencies needed to evoke maximum force. Furthermore, because there is growing use of non-human primates as model systems for FES and motor neuroprosthetics (e.g. Moritz et al. 2008; Pohlmeyer et al. 2009; Ethier et al. 2013; Capogrosso et al. 2016; Ethier et al. 2017), such force-frequency data could be useful for identifying suitable stimulation frequencies for specific applications.

Force–current relationship. The relationship between current-pulse amplitude and evoked force was assessed using 1-s trains of pulses delivered at 35 Hz first to one electrode then to the other. Trains were delivered repeatedly with pulse amplitude incremented in 1 mA steps from 1 mA to 10 – 20 mA with 4-s delay between trains. During this procedure, we observed the evoked actions of the limb for any signs (e.g. shoulder adduction, abduction) indicating that neighboring muscles were being recruited at higher stimulus intensities. If activation of other muscles was detected, stimulation was halted. On the few occasions when this did occur, the most common added action was shoulder abduction, suggesting activation of the middle head of the deltoid. In these cases, the position of the electrode was changed which usually involved removing the electrode and reinserting at different location farther away from the muscle that was unintentionally recruited. After a few minutes of rest, the procedure for evaluating the relationship between current-pulse amplitude and force was repeated for the new electrode position. The entire procedure was then repeated for the second electrode. For each electrode, the minimum current above which no clear increment in force was observed over the entire range of currents tested was identified and referred to as the ‘maximum’ current.

Force Summation. We then sought to compare the force exerted during ’maximal‘ stimulation delivered by each electrode individually to that generated by simultaneous stimulation through both electrodes. For this procedure, we stimulated using the maximum current identified for each electrode. Stimulus trains were 2-s in duration. Because we were also interested to estimate the overall maximum force that could be evoked in anterior deltoid, for these procedures we stimulated at 60 Hz. In preliminary experiments, 60 Hz was the most common frequency associated with the initial plateau in the force-frequency relation. ‘Maximal’ stimulation was delivered first to one electrode, then to the other, followed by two trials in which maximal current was delivered simultaneously to both electrodes. This was then followed by stimulation to the second electrode by itself and finally to the first electrode by itself. The duration between stimulus trains for this procedure was 10 s.

Fatigue. As mentioned in the Introduction, a possible advantage of using multiple electrodes is that force might be better maintained during prolonged activity by interleaving stimulation to each of the electrodes. In this way, the stimulus frequency delivered to any one electrode (and the muscle fibers it activates) can be lessened, while the net frequency delivered to the muscle is maintained at a higher level enabling larger target forces to be achieved. This in turn might help minimize fatigue that is associated with high-frequency stimulation of muscle fibers (Naess and Storm-Mathisen 1955; Jones et al. 1979; Metzger and Fitts 1986). To test this idea, we recorded the force evoked by repetitive stimulation of anterior deltoid using 2-s trains, with a 50% duty cycle, for 4 minutes. Stimuli were delivered to both electrodes using one of three distinct patterns outlined below. Only one pattern was tested during a given session.

In some sessions, stimuli were delivered at 35 Hz synchronously to both electrodes. Thirty-five Hz was selected because at this rate, force was relatively well fused yet did not produce full tetanic tension. In other sessions, stimuli were delivered at 17.5 Hz but with stimuli interleaved between the electrodes (i.e., 180 degrees out of phase with one another) such that the net stimulus frequency was 35 Hz. And to partially evaluate the effect of interleaved versus synchronous stimulation but using the same stimulus frequency, in a third set of sessions stimuli were delivered in an interleaved fashion at 35 Hz per electrode. Prior to each fatigue run, stimulus intensity (current amplitude) was adjusted individually on each electrode such that the forces produced by each electrode were similar to one another and that the total initial force (due to the combined actions of both electrodes) detected at the wrist was approximately 2 N above the resting level and was same across all sessions. Finally, to assess recovery following the fatigue protocols, we recorded force responses to 2-s stimulus trains (using the same stimulus patterns for that session) at 1, 2, 4, 8 and 16 minutes following the end of the fatigue protocol.

Data analysis – For each train of stimuli, force was averaged over two 0.5 s windows registered to the beginning and end of the peak force. The larger of the two averages was taken as the force measurement. For force summation experiments, in each session the mean force for the 2 trials involving dual-electrode stimulation was normalized as a percentage of the mean force evoked during the 4 trials of single-electrode stimulation. A one-sample t-test was performed on normalized dual-electrode forces (using a hypothesized mean of 100%) to determine whether force exerted with dual-electrode stimulation was greater than that evoked by single-electrode stimulation.

For each fatigue trial, we made three measurements: endurance index (ratio of force of last train to that of the first train), normalized force area (summation of all force responses during the trial divided by the sum of all force responses had no fatigue occurred, i.e. all force response the same as the first), and slowing index (ratio of the half-relaxation times of last train to that of the first train). Half-relaxation time (1/2 RT) was calculated as the time required for force to decay from the peak value measured immediately following the last stimulus pulse in a train to 50% of that value. A one-way ANOVA was performed on each these fatigue measures with stimulation pattern (35-Hz synchronous, 17.5 interleaved, and 35-Hz interleaved) as the factor. Data are reported as means ± standard deviation (SD) with $P < 0.05$ considered significant.

RESULTS

Both animals underwent several sessions of general anesthesia, each lasting about 2 hours. No adverse events occurred in any of the sessions. The animals recovered within ~ 45 minutes following cessation of anesthesia, exhibiting fully coordinated movements, eating and drinking. The animals remained healthy throughout the entire testing period (~ 4 months) and showed no long-term effects of repeated anesthesia. As such, this method can be used to repeatedly and safely evaluate aspects of FES delivered with percutaneous or chronically implanted electrodes in macaque monkeys.

Examples of anterior deltoid force responses to varying stimulus frequencies delivered through a single electrode are shown in Figure 2A. Force increased progressively with increased frequencies up to about 60 Hz beyond which there was little additional force increase. Unfused

ripple can be observed at frequencies up through ~ 30 Hz with the contraction becoming almost completely fused at 40 Hz.

A similar profile was observed for all force-frequency trials carried out on the two monkeys across 22 sessions (10 for monkey R, 12 for monkey S). Figure 2B shows mean (SD) force versus frequency relationships for the two monkeys separately. The responses were very similar across the two subjects with force increasing steeply from 10 – 30 Hz, increasing modestly from 40 – 60 Hz, and not further increasing at frequencies greater than 60 Hz.

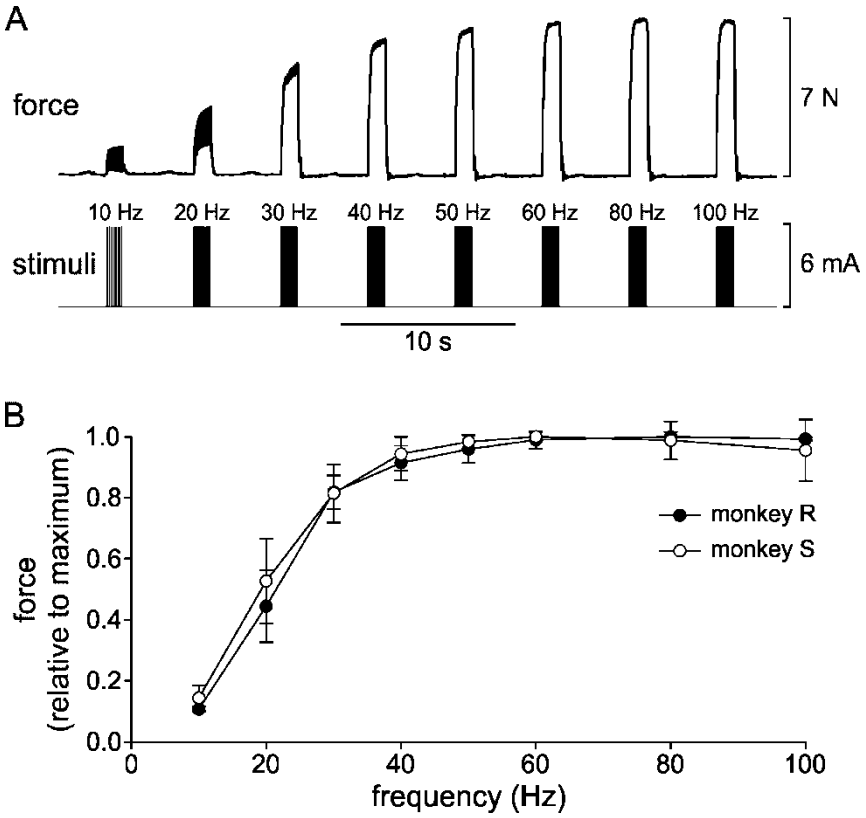

Figure 2. *(A) Example set of isometric force responses to 1-s trains of stimulus pulses (0.5 ms, 6 mA) at increasing frequencies from 10 – 100 Hz delivered through one electrode. (B) Mean (SD) force as a function of stimulus frequency for monkey R (10 sessions) and monkey S (12) sessions.*

Force– current relationship. Figure 3A shows an example trial of isometric force responses to progressively increasing pulse amplitudes using 35 Hz trains delivered through one of two electrodes. Note that for stimulus currents from 2 to 5 mA, force appeared to saturate (highlighted by red line above force traces). However, above 6 mA, force increased markedly for increments in current up to 15 mA when force again appeared to saturate. Because we did not stimulate above 16 mA in this experiment, we could not be certain whether another escalation in force would have occurred at higher stimulus intensities. There was no detectable activation of neighboring muscles even for the highest currents delivered. For purposes of these experiments, the minimal current associated with 'maximal' force (dashed horizontal line) was deemed to be 15 mA for this trial.

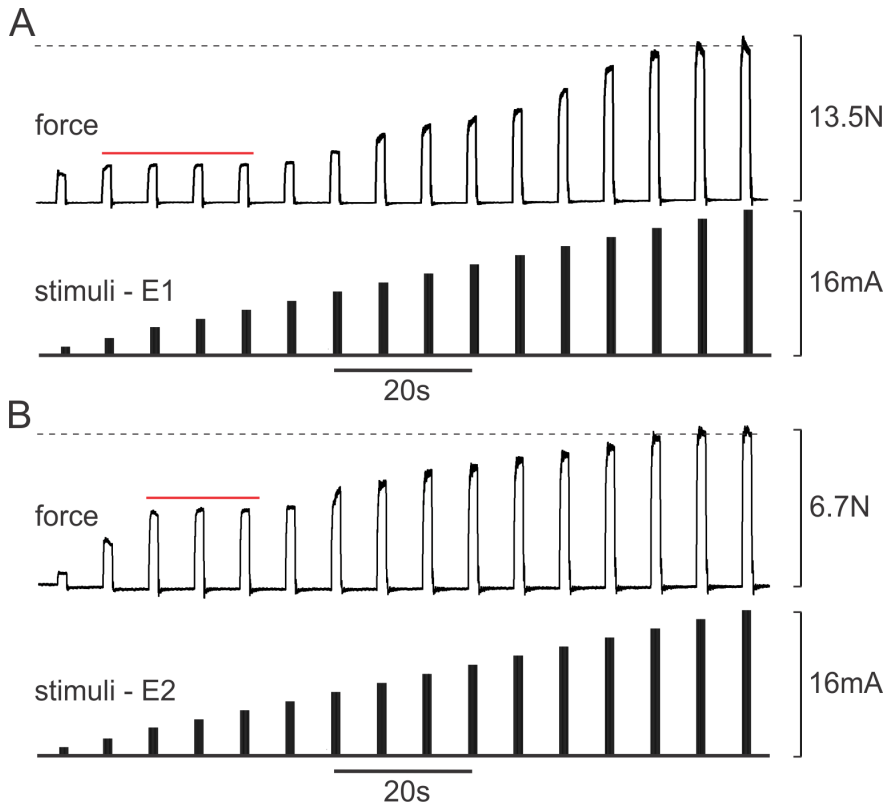

Figure 3. *Examples of force response to 1-s trains of stimulus pulses (0.5 ms, 35 Hz) with increasing current amplitudes from 1 – 16 mA delivered to (A) electrode 1 (E1) and (B) to electrode 2 (E2). Red horizontal lines indicate intermediate plateaus in force responses to increasing current. The dashed horizontal lines indicate force saturation for highest current levels tested.*

Figure 3B shows the force responses to incrementing current pulses obtained in the same experiment as for Figure 3A but for the other electrode (electrode 2) that was placed about 2 cm distal to the one used in Figure 3A (electrode 1). The pattern of force increases to incrementing current delivered through electrode 2 was roughly similar to that for electrode 1. An

intermediate plateau was observed for currents from 3 to 5 mA followed by a ~ linear increase in force with current up to about 14 – 15 mA at which point force appeared to saturate. The stimulus current needed to elicit 50% of 'maximal' force was only about 3 mA for electrode 2 (Figure 3B) whereas it was about 10 mA for electrode 1 (Figure 3A). The most striking difference, however, between the responses elicited with the two electrodes was the absolute magnitude of the forces. The 'maximal' force evoked by electrode 1 (13.5 N) was twice as large as that for electrode 2 (6.7 N). That disparity, in itself, indicates that the force evoked by electrode 2 was not maximal despite the presence of the plateau in force at the highest currents tested with that electrode.

False plateaus. The average current at which force responses were first detected was 0.9 ± 0.4 mA. The average current associated with maximal force was 11.8 ± 3.4 mA. It should be noted, however, that in about half of the sessions (mainly the initial sessions), we did not always allow current to reach the maximum capacity of the stimulator (~20 mA). Often we halted the current steps once we observed what appeared to be a plateau in the force responses. We later noticed the presence of intermediate plateaus, wherein force would appear to saturate for two or more 1-mA increments in current (see Figs. 3A, 3B). Consequently, it is possible that when we halted current steps before reaching the maximum current capacity, we had actually encountered an intermediate or 'false' plateau.

To quantify the incidence of such false plateaus, we calculated the change in force associated with each increment in current for all of the current-force sequences. Figure 4A shows an example of the relation between force and current for one sequence while Figure 4B shows the associated change in force for each current step. Based on plots like that shown in Figure 4B, we then determined the number of cases for which the change in force fell below 5% for two or more steps in current and which was then followed by increases in force above 5%. Figure 4B shows one such case of a false plateau (highlighted in red) for which little change in force was detected for increments in current from 2 – 5 mA but was then followed by clear increases in force at higher current levels. In total, 42% of the current-force sequences recorded had one or more such false plateaus. We believe that this percentage would have been even

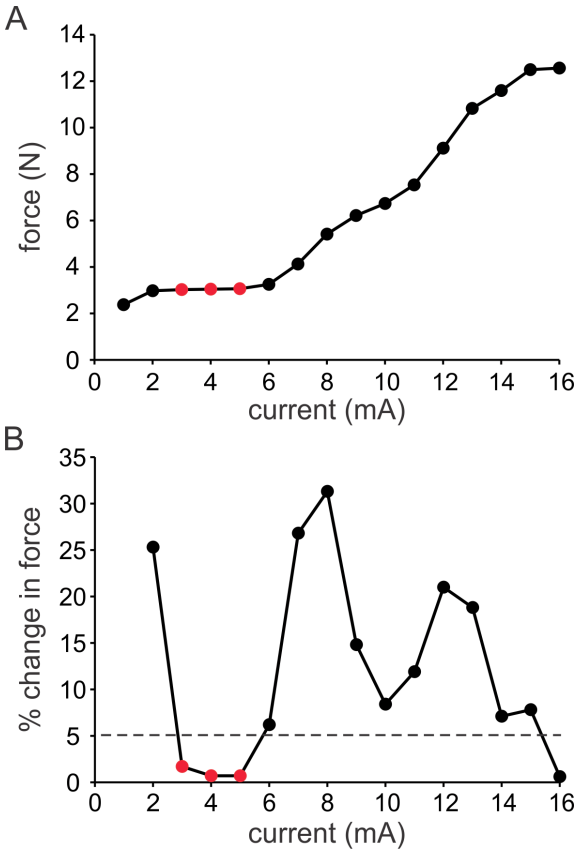

Figure 4. *(A) Example force – stimulus current relationship for one electrode showing little increase in force for three, 1-mA step increments in current above 2 mA (red circles). (B) Percentage change in force for each increment in current depicted for the force – current relation shown in in panel A. Three consecutive increments in current (to 3, 4, and 5 mA – red circles) evoked less than a 5% increase in force (dashed horizontal line), and which were then followed by a greater than 5% increase in force (at 6 mA). Such a sequence was deemed a 'false' plateau.*

higher had we tested up through the maximum current capacity in all trials. The physiological and practical significance of such false plateaus will be addressed in the Discussion.

Force Summation. Figure 5A shows example force responses to 60-Hz trains of stimuli using 'maximal' current pulses delivered first to one electrode, then to the other electrode, and then to both electrodes together (first three trials). The order of stimulation was then reversed for the last three trials. Sixty-Hz stimulation was used in these experiments in an attempt to elicit the overall maximum force capacity of the anterior deltoid. The recordings shown in Figure 5A were taken from the same experimental session as that used for Figure 3. For the first trial, 'maximal' stimulation to electrode 1 (E1) produced a force of 12.8 N while 'maximal' stimulation to electrode 2 (E2) produced only 7.0 N of force. Simultaneous 'maximal' stimulation to both electrodes, however, produced a force of 16.5 N. A reduction in the evoked forces for the last three trials suggests some development of fatigue.

The average force for the two trials using dual-electrode stimulation in the experiment shown in Figure 5A was 15.2 N whereas the average force across the four trials using single electrode stimulation was 8.6 N. In this case, dual-electrode stimulation produced 177% of the force generated by single electrode stimulation.

Figure 5B shows the mean (SD) and individual values of isometric force resulting from dual-electrode stimulation, measured as a percentage of single electrode 'maximal' force, across all 28 sessions for the two subjects. In every case, dual-electrode stimulation produced greater force than single-electrode stimulation. On average, dual-electrode force produced 147.0 ± 22.3 % of single-electrode force ($P < 0.001$ compared to 100%). These results strongly indicate that

using two (or more) sites of intramuscular stimulation enables activation of a greater volume of muscle than that which can be readily achieved with single site stimulation.

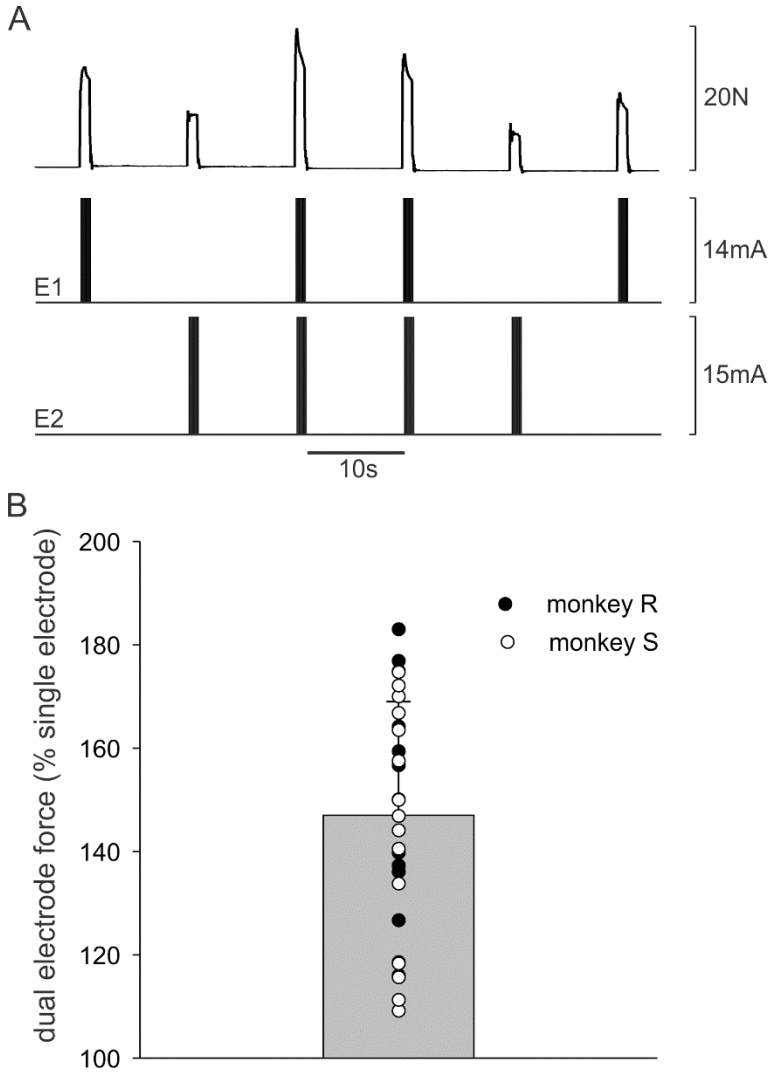

Figure 5. *(A) Example force responses (top trace) to 'maximal' stimulation delivered at 60 Hz to electrode 1 (E1), electrode 2 (E2), or to both simultaneously. Forces produced in response to stimulation through single electrodes were exceeded with simultaneous stimulation through both electrodes. (B) Mean (SD) isometric force resulting from dual electrode stimulation expressed as a percentage of single electrode "maximal" force across all 28 sessions for the two monkeys. Individual values are shown separately for each session and for each monkey (open and closed symbols).*

Fatigue. Another possible advantage of multiple-site stimulation is that interleaving stimulation between electrodes might enable a given target force to be maintained for longer durations by using lower stimulus frequencies at each electrode than if stimulation were delivered synchronously to all the electrodes. Figure 6 shows example recordings made in three separate sessions from one monkey in response to repetitive 2-s trains of pulses using: A) synchronous stimulation at 35 Hz/electrode, B) interleaved stimulation at 17.5 Hz/electrode, and C) interleaved stimulation at 35 Hz/electrode. The insets show one-second segments of force and stimulus pulses at the outset of the tenth train from the end of each fatigue protocol to help illustrate differences in the stimulus patterns across sessions.

In all three sessions, the initial force was slightly greater than 2 N. For synchronous 35-Hz stimulation (Fig. 6A), after an initial relatively stable period of force production, force declined progressively over the remaining stimulus trains. The force produced by the last train was 36% of that produced by the first train. The force profile associated with interleaved 17.5 Hz stimulation (Fig. 6B) was very similar to that produced with synchronous stimulation at 35 Hz (Fig. 6A) with the last train producing 34% of the force of the initial train. Stimulation using interleaved stimulation at 35 Hz (Fig. 6C) had a slightly different profile with force appearing to drop a little more quickly at the outset of stimulation and with the last train producing 29% of the force of the initial train.

Figures 7A and 7B show the averaged force profiles for the three fatigue tasks for monkey R and monkey S, respectively. The averages in Figure 7A included 4 trials of

synchronous 35-Hz stimulation, 4 trials of interleaved 17.5-Hz stimulation, and 5 trials of 35-Hz interleaved stimulation. The averages in Fig. 7B included 5 trials for each pattern of stimulation. The overall average current amplitudes used for the three tasks were: 2.0 ± 1.1 mA, 4.8 ± 2.6 mA, and 2.9 ± 1.2 mA for the 35-Hz synchronous, 17.5-Hz interleaved, and 35-Hz interleaved

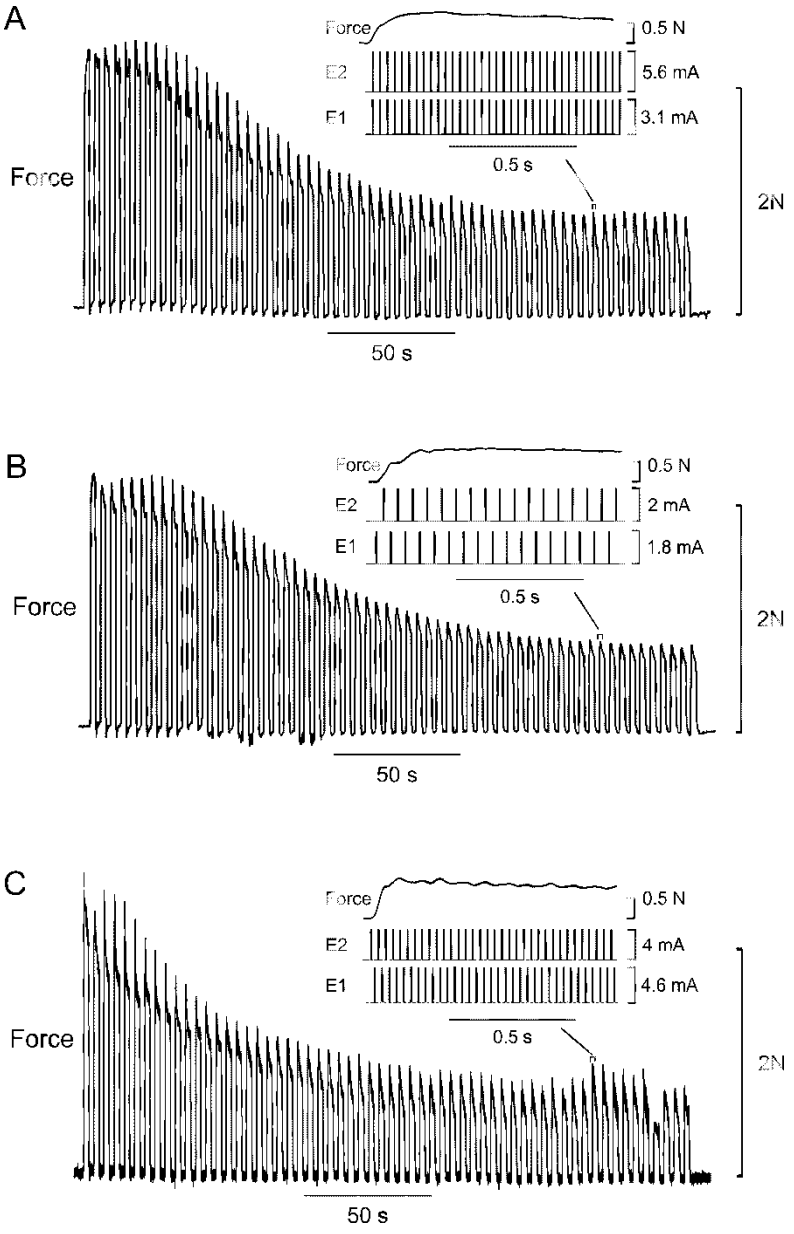

Figure 6. *Examples of isometric force responses generated by anterior deltoid of one monkey during four minutes of repetitive stimulation using two electrodes across three sessions involving: (A) synchronous stimulation at 35 Hz, (B) interleaved stimulation at 17.5 Hz, and (C) interleaved stimulation at 35 Hz. In all cases, stimulus trains were 2-s in duration with a 2-s inter-train interval. Insets show stimulus pulses and the initial portion of the force response for the 51st train in each sequence.*

protocols, respectively. The higher currents for the 17.5 Hz stimulation were needed to offset the weaker forces produced with lower stimulus rates in order to achieve the target force.

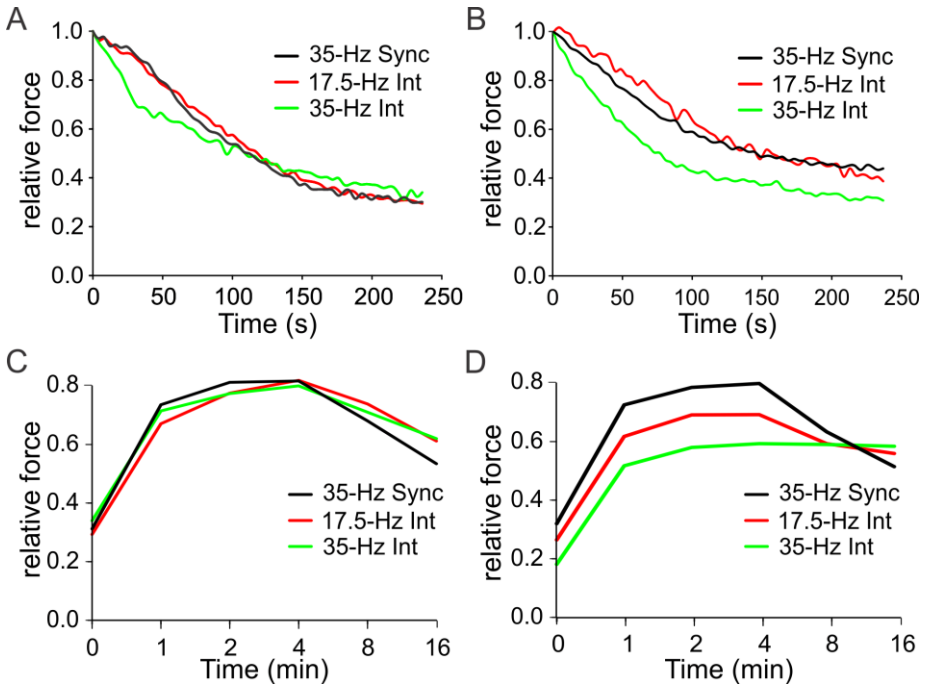

Figure 7. *Averaged force as a function of time for three fatigue tasks involving two electrodes: 35-Hz synchronized, 17.5 Hz interleaved, and 35-Hz interleaved in monkey R (A) and monkey S (B). Averaged force responses to 2-s trains of stimuli delivered at 1, 2, 4, 8, and 16 minutes after the three fatigue tasks in monkey R (C) and monkey S (D).*

For monkey R (Fig. 7A), the pattern of force loss over time was very similar across the three fatigue protocols. The main difference was a slightly steeper force loss at the outset of the 35-Hz interleaved stimulation protocol, similar to that shown for the example recording in Figure 5C. Likewise, for monkey S (Fig. 7B), the patterns of force loss were similar across stimulation

protocols and were also similar to that of monkey R (Fig. 7A). However, for monkey S, the initial steep force loss with 35-Hz interleaved stimulation was sustained throughout the entire protocol.

One-way ANOVAs run on endurance index and normalized force area for monkey R yielded no significant effects (Table 1) of stimulation pattern. For monkey S, while there was no significant effect of stimulation pattern on endurance index, there was a significant effect ($p = 0.04$) on normalized force area. Post-hoc analysis indicated that normalized force area for 35-Hz interleaved stimulation was significantly less than that of either 35-Hz synchronous or 17.5-Hz interleaved stimulation. Such a tendency for greater fatigue with interleaved 35-Hz stimulation might be due to the higher *net* frequency delivered (70 Hz) to muscle fibers that happened to be

	Monkey R				Monkey S				Both			
	35-Hz Synch.	17.5-Hz Interleaved	35-Hz Interleaved	p value	35-Hz Synch.	17.5-Hz Interleaved	35-Hz Interleaved	p value	35-Hz Synch.	17.5-Hz Interleaved	35-Hz Interleaved	p value
n	5	4	4		5	5	5		10	9	9	
Endurance Index	0.30 ± 0.11	0.30 ± 0.07	0.34 ± 0.10	0.78	0.35 ± 0.03	0.29 ± 0.06	0.20 ± 0.14	0.07	0.33 ± 0.08	0.29 ± 0.06	0.26 ± 0.14	0.15
Normalized Area	0.55 ± 0.13	0.56 ± 0.10	0.53 ± 0.11	0.93	0.55 ± 0.08	0.58 ± 0.07	0.40 ± 0.14	0.04*	0.55 ± 0.10	0.57 ± 0.08	0.46 ± 0.14	0.08
Normalized 1/2 relax. time	2.14 ± 0.14	2.07 ± 0.25	1.96 ± 0.13	0.37	2.10 ± 0.23	2.32 ± 0.42	1.95 ± 0.24	0.2	2.12 ± 0.18	2.21 ± 0.36	1.95 ± 0.19	0.12

Table 1. *Mean (SD) values of endurance index, normalized force area, and slowing index (change in ½ relaxation time) in response to three types of fatigue protocols involving dual electrode stimulation.*

activated by both electrodes. Such high-frequency stimulation can promote more rapid fatigue than stimulation at lower (and more natural) frequencies (Naess and Storm-Mathisen 1955; Jones et al. 1979; Metzger and Fitts 1986; McDonnall et al. 2004).

In addition to force loss, sustained activity can lead to slowing in muscle contraction and relaxation. For all stimulus patterns and for both monkeys, one-half relaxation time (½ RT) doubled over the course of the fatigue trials (Table 1). There was no significant effect, however, of stimulus pattern on ½ RT for either monkey individually or with data from both monkeys combined.

Following the fatiguing contractions, single brief (2 s) trains of stimuli were delivered at various intervals to evaluate recovery from fatigue. Figures 7C and 7D show the mean force (normalized to that of the first train at the outset of each fatigue protocol) during the recovery period for each of the three fatigue protocols for monkeys R and S, respectively. For both animals, force recovered steeply with one minute of rest, then continued to recover more slowly out to 4 minutes of rest up to 60 – 80% of the pre-fatigue force. Interestingly, for both animals, force then progressively diminished beyond 4 minutes out to 16 minutes (longest duration tested). Such delayed fatigue has been observed previously in fast twitch motor units in cat (Jami et al. 1983; Bevan et al. 1993), in rat motor units (Lannergren et al. 1989), and in whole human muscle (Edwards et al. 1977; Fuglevand et al. 1993). A two-way ANOVA using the combined data from the two monkeys with stimulus pattern and recovery time as factors yielded a significant effect of recovery time ($P < 0.001$) but no effect of stimulus pattern ($P = 0.07$) and no significant interactions. Post-hoc analysis indicated that force was significantly ($P < 0.001$) greater at all recovery times compared to that immediately after the fatigue test (i.e. at time 0), and that the force at 16 min recovery was significantly ($P <0.001$) less than that at 2 and 4 minutes of recovery.

Evoked Movements with Multi-electrode Stimulation. As a simple test of the efficacy of multi-electrode stimulation to evoke movements, in one monkey we delivered stimuli synchronously to two intramuscular electrodes inserted into the anterior deltoid and recorded the evoked displacement of the hand using electromagnetic position sensors (Liberty, Polhemus) placed on the hand and shoulder (Figure 8A). Stimuli consisted of constant amplitude pulse trains (4 mA, 0.5 ms pulse duration, 3-s trains, 30 Hz). The limb hung pendant at the side of the monkey before each trial. Dual electrode stimulation evoked strong flexion at the shoulder causing the hand to briskly rise (peak speed ~ 250 cm/s) above the shoulder before settling and holding for ~ 2-s just below shoulder height (Fig. 8B). After each trial, the hand was repositioned to approximately the same starting position. The time between successive trials was about 3 s. The evoked trajectory was quite similar across 10 repeated trials (Fig. 8C) and was almost entirely in the sagittal plane. We then strapped a 100 g load in the hand of the monkey and delivered the same stimulus sequence 3 times (Fig 8D). While the extent of arm movement was not as great as for the unloaded case (as expected for uncompensated stimulus strength), the arm reliably lifted and held the load against gravity. On numerous occasions (not shown), single electrode stimulation using high currents evoked only modest movements, often advancing the hand only a few centimeters forward of the starting position.

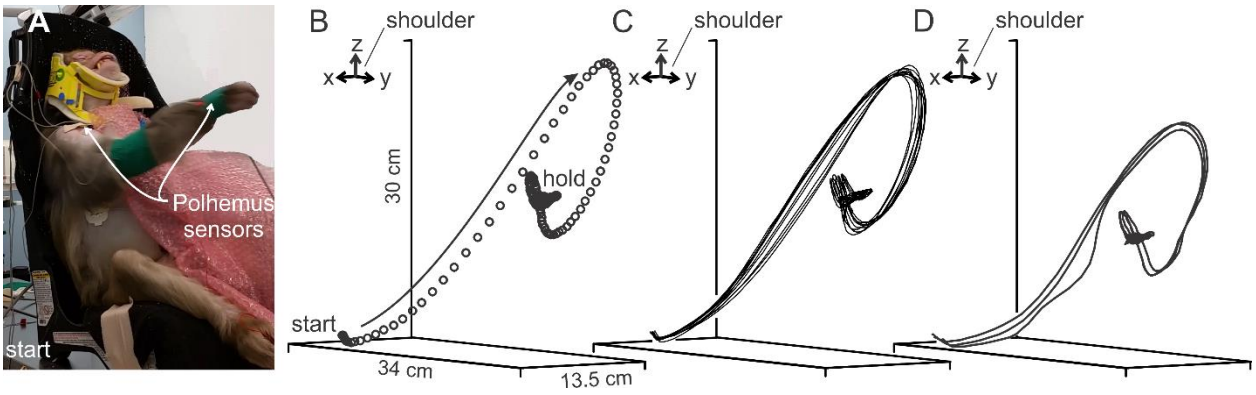

Figure 8. *Movements evoked by stimulation of anterior deltoid in anesthetized monkey using two intramuscular electrodes. (A) Image captured from video depicting arm being elevated in anesthetized monkey in response to stimulation (4 mA, 0.5 ms pulse duration, 3-s trains, 30 Hz) of anterior deltoid with two electrodes synchronously. The start position of the hand is indicated. Polhemus sensors were placed on the hand and shoulder to record the evoked movements. Image was retouched to remove extraneous physiological-monitoring equipment in the background. B) Displacement of hand during a single trial. Each dot indicates sampled position of hand relative to shoulder (sampling interval ~ 8 ms) C) evoked hand trajectory for 10 trials superimposed, D) evoked trajectory for 3 trials with 100 g load in strapped in the hand.*

DISCUSSION

Here we have shown that stimulation of muscle with more than one intramuscular electrode clearly produces greater forces than can be readily produced with single electrodes. This occurred even when force responses to single electrodes appeared to saturate – traditionally considered a sign that the maximum force capacity of the muscle was achieved by the stimulation. It seems reasonable, therefore, to consider using multi-electrode stimulation to augment the force-generating capacity of muscles (particularly large proximal muscles) to increase the utility of chronically implanted FES systems. Indeed, we showed in a single case that such multi-electrode stimulation of one muscle (anterior deltoid) was sufficient to briskly

and repeatedly elevate the arm against gravity, an action that, as far as we are aware, has not previously been realized using FES alone.

Limitations. A limitation of the present study was that the maximum output capacity of the stimulator used was about 20 mA/channel. It should be pointed out, however, that the maximum current capacity of the most advanced implanted FES systems presently used in human patients is 20 mA (Memberg et al. 2014). Nevertheless, it is possible that had greater currents been delivered through single electrodes in the present experiments, greater forces might have been evoked and thereby diminished the relative advantage of using dual-electrode stimulation. However, with increased current intensity delivered at a single site, the radius of the effective current field could exceed the boundaries of the targeted muscle and begin to activate neighboring muscles while leaving distant portions of the target muscle un-activated. As such, perhaps an additional benefit of multi-electrode stimulation is that the activating current fields can be more readily sculpted to the oblong shape of most muscles.

Another limitation of the present study is that the test subjects were monkeys, not humans, and we only tested two subjects. As such, we had no ready way to determine the magnitude of the evoked forces relative to the maximum voluntary force capacity, as is commonly done in healthy human studies. On the other hand, because we were interested to examine the maximum force capacity produced by electrical stimulation, the pain associated with the high stimulation currents makes it practically intolerable for intact human subjects and also provokes complex nociceptive reflexes leading to inadvertent co-activation of other muscles that could compromise force measurements (Marsden et al. 1983; Martin et al. 2008). Therefore,

we used general anaesthetics in macaque monkeys to enable fully reversible, functional paralysis while eliminating pain perception associated with high stimulation currents. In addition, we did not detect overt signs of spinal reflexes triggered by the stimulation, viz. co-activation of other muscles or sustained contraction of the target muscle following cessation of stimulation (Lagerquist et al. 2009). We cannot, however, rule out activation of brief reflexes (such as H-reflexes) under isoflurane anaesthesia (Leis et al. 1996). Another important feature of this method was that the animal was maintained in an upright, seated position, akin to its natural posture enabling relatively unrestricted motion of the upper limb. As such, this method would seem to be one that could be used to repeatedly and safely evaluate aspects of FES delivered with percutaneous or chronically implanted electrodes in macaque monkeys.

We used only two animals in keeping with ethical concerns to reduce the number of non-human primates tested in experiments. Each animal was tested during multiple sessions and the data from each session were more or less treated as independent samples from the set of all possible responses to stimulation of a given muscle. As such, some caution is warranted in extrapolating the results beyond the individual monkeys. However, the consistency of responses within monkeys and similarity of responses across the two monkeys provides some confidence that the results are likely generally representative.

The fatigue tests used in the present investigation involved target forces that were relatively weak (perhaps 10 – 15% of maximum capacity). While such low forces might seem too weak to be functionally practical, most natural muscle activity in healthy subjects rarely exceeds 15% of maximum voluntary capacity (Klein et al. 2010). Therefore, force levels in this

range are critical to the performance of activities of daily living. In a spinal-cord-injured individual, however, typical daily activities (e.g. lifting a coffee cup) would likely require substantially higher percentages of force capacities because of muscle atrophy. Nevertheless, if fatigue is evident at these low force levels (as shown in the present study) then this will significantly impact the utility of FES systems. Consequently, understanding the nature of fatigue in response to electrical stimulation even at low forces will be important for advancing the effectiveness of FES. While such heightened fatigue with FES has been ascribed to reverse recruitment (i.e., selective recruitment of high-threshold, fatiguable motor units, Kubiak et al. 1987; Trimble and Enoka 1991), a number of investigations have shown that orderly activation is more likely than reverse with electrical stimulation (Knaflitz et al. 1990; Feiereisen et al. 1997; Thomas et al. 2002; Farina et al. 2004).

False Plateaus. A widely used method to elicit putative maximal responses of human skeletal muscle using electrical stimulation is to progressively increment stimulus intensity delivered to a single electrode until a plateau is observed in the evoked responses. Stimulus strength is then typically increased by 20 – 50% above this level to ensure stimuli are 'supramaximal' (Merton 1954; Bigland-Ritchie et al. 1979; Rutherford et al. 1986; Stein et al. 1992; Fuglevand et al. 1993; Bilodeau 2006; Parise et al 2001; Lagerquist et al 2009; Seigler et al. 2016; Millet at al 2011, Maffiuletti et al 1994). In some instances, however, this approach may lead to stimulation at submaximal levels because of the presence of false plateaus as often encountered in the present study. Indeed, emergence from a false plateau often required stimulus strengths 250% higher than that just needed to reach the plateau. We assume that such false plateaus were mainly due to the activation of a subset of nerve branches close to the stimulating

electrode and that substantially higher currents were then needed to activate more distant nerve branches. The validity of this assumption, however, warrants investigation.

Interestingly, in an important previous study, Crago et al. (1980) did not observe clear-cut plateaus in the current-force relationship of denervated soleus in the anesthetized cat. Some reduction in the slope of the current-force relationship was occasionally seen at intermediate intensities but not to the degree observed in the present investigation (see Figs. 3 and 4). This discrepancy could be due to differences in the masses (and associated volumes) of the two muscles tested (~ 3 g for cat soleus, Nelson 1968, ~7.5 g for macaque anterior deltoid, Cheng and Scott 2000). Notably, however, Crago et al. (1980) often showed saturation of force output at the highest intramuscular stimulus intensities tested that were below the maximum force capacity of the muscle (measured in response to whole nerve stimulation). Those results are consistent with the overall observations of the present study.

Fatigue. Somewhat contrary to our expectations, interleaving stimulus pulses to two intramuscular electrodes at relatively low rates to each electrode (17.5 Hz) did not improve force maintenance during prolonged stimulation compared to synchronous stimulation at a higher rate (35 Hz). Both methods led to about a 70% decline in force, a 50% reduction in force area, and about a doubling in relaxation time over 4 minutes of intermittent stimulation. There would seem to be at least two theoretical reasons why lower frequencies of stimulation, by itself, should tend to lessen fatigue during prolonged activity for tasks that involve an initial submaximal target force (as was used here). First, because of the force-frequency relation, the forces exerted by the activated muscle fibers using lower stimulus frequencies will be less than that occurring with

higher frequencies. Therefore, in order to achieve the initial target force, more muscle fibers will need to be recruited (using higher stimulus currents), and consequently, the load will be more broadly shared across a greater number of muscle fibers. Conversely, with higher frequencies of stimulation, the load burden is carried by fewer muscle fibers, all of which must contract more intensely in order to achieve the initial target force. This latter situation would seem likely to promote more rapid fatigue.

Second, at the extreme, high stimulation frequencies can lead to impairment of excitation-contraction coupling and cause relatively rapid fatigue (Naess and Storm-Mathisen 1955; Jones et al. 1979; Metzger and Fitts 1986, McDonnall et al. 2004). The slightly greater fatigue in one monkey to 35-Hz interleaved stimulation might have occurred due to a high net frequency of 70 Hz imposed on the subset of muscle fibers activated by both electrodes. While we did not quantify the degree of overlap in activation of muscle fibers by the two electrodes for the fatigue experiments, the results of the force summation experiments (Fig. 5B) suggests an upper limit of ~ 50% overlap in fibers activated by the two electrodes.

Previous studies that have used interleaved stimulation to assess fatigue have yielded results not entirely consistent with the present investigation. For example, in the noteworthy work by Yoshida and Horch (1993), fatigue responses in the cat gastrocnemius were studied in response to single- or dual-electrode intraneural stimulation. Stimulation at 60 Hz, either with a single electrode or synchronously delivered to two electrodes, led to substantially greater fatigue than with interleaved stimulation to two electrodes at 30 Hz. Unfortunately, no tests were performed using *synchronous* stimulation at 30 Hz. As such, it is unclear whether the enhanced

fatigue resistance seen in the Yoshida and Horch (1993) study with 30-Hz interleaved stimulation was due to interleaving the stimulation *per se* or was simply due to the use of lower stimulus frequencies.

Similarly, McDonnall et al. (2004) compared fatigue responses in cat gastrocnemius to stimulation with single or four intraneural electrodes. Stimulation at 60 Hz synchronously to all four electrodes caused greater fatigue than with interleaved stimulation to four electrodes at 15 Hz. Importantly, they also showed that interleaved stimulation at such low frequencies (15 Hz) markedly attenuated the unfused ripple evident with single electrode stimulation at 15 Hz. Puzzlingly, however, the degree of fatigue was *greater* with interleaved stimulation at 15 Hz compared to that associated with single electrode stimulation at 15 Hz.

In a number of recent studies using human subjects, fatigue resistance during FES has been shown to be consistently and significantly enhanced using low frequency stimuli delivered in an interleaved way to multiple surface electrodes compared to higher frequency stimuli delivered to a single electrode (Popović and Malešević 2009; Malešević et al. 2010; Nguyen et al. 2011; Popovic´ Maneski et al. 2013; Sayenko et al. 2014; Lou et al. 2016; Bergquist et al. 2016; Bergquist et al. 2017; Laubacher et al. 2017). These impressive results clearly demonstrate that distributed stimulation is more efficacious in maintaining muscle output than single site stimulation. Yet, it is not entirely clear from these studies as to whether interleaving of stimulation by itself helps to minimize fatigue or whether it is primarily the use of lower stimulus frequencies during interleaved stimulation. Nevertheless, interleaving stimulus pulses across multiple electrodes using low stimulus frequencies will help minimize the large force

fluctuations that otherwise would occur using synchronized stimulation. This is an important benefit in terms of output stability for FES.

In the present study, we observed little difference in fatigue resistance using interleaved versus synchronous stimulation. Indeed, when using the same stimulus frequency (35 Hz), interleaved stimulation caused slightly greater fatigue than synchronous stimulation in one animal. Importantly, the lack of an effect on fatigue using interleaved stimulation at 17.5 Hz versus synchronous stimulation at 35 Hz is at variance with some of the previously mentioned studies. There are perhaps two factors that might account for this discrepancy. First, the relative difference in stimulation frequencies used here for interleaved vs. synchronous stimulation (17.5 Hz) was modest compared to that in other studies (30 – 45 Hz) (Yoshida and Horch, 1993; McDonnall et al. 2004; Nguyen et al. 2011). And second, as pointed out by Yoshida and Horch (1993) and Wiest et al. (2017), the benefits in terms of fatigue resistance using interleaved multi-electrode stimulation diminishes as the degree of overlap in muscle fibers activated by the electrodes increases. Here the degree of overlap may have been substantial in comparison to the previous investigations.

Force Augmentation with Multiple Electrodes. And finally, despite relatively wide recognition that single electrode stimulation is unlikely to activate all motor units within a muscle (e.g. Vanderthommen and Duchateau 2007; Memberg et al. 2014), there are few previous studies that have evaluated the force-generating capacity of multisite versus single-site intramuscular stimulation. In one study, Maffiuletti et al. (2014) found that the maximum evoked force in healthy human quadriceps using three surface electrodes was greater (44% of

maximum voluntary contraction force) than that produced by a more conventional dual-electrode configuration for the quadriceps (36% of maximum voluntary contraction force). Part of the increased force was due to the higher tolerance in the subjects for larger current intensities using the three-electrode configuration possessing a larger overall electrode surface area than the conventional configuration. In the present investigation, where stimulation discomfort was not a complicating factor, we found dual-electrode stimulation produced about 50% greater force than single-electrode stimulation.

Practical Implications. Given the greater force output achieved with dual-electrode compared to single-electrode stimulation in the present study, it would seem appropriate to consider including multiple electrodes per muscle in chronically implanted FES systems. Furthermore, an advantage of using simultaneous stimulation with two (or more) electrodes is that distant muscle regions that are below threshold for activation by any one electrode can be brought above threshold due to summation of the otherwise sub-threshold electric fields (Mortimer 1981). Practical factors, however, must also be taken into account including the increased surgical complexity, hardware needs, and power requirements for a multi-electrode approach. Nerve cuff, rather than intramuscular electrodes, would seem a viable alternative (Sweeney et al. 1990; Tyler and Durand, 2002; Schiefer et al. 2013) but these also have some drawbacks including lack of muscle selectivity and possibility of nerve damage (Grill and Mortimer, 2000). Perhaps the most efficacious approach at present would be to use a combination of electrode designs (single intramuscular electrodes for small muscles, multiple intramuscular electrodes for large muscles, and cuff electrodes where applicable), , similar to that

recently used in implanted upper limb (Memberg et al. 2014) and lower limb neuroprostheses (Guiraud et al. 2014).

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APPENDIX B:

MITIGATION OF EXCESSIVE FATIGUE ASSOCIATED WITH FUNCTIONAL ELECTRICAL STIMULATION

Mitigation of Excessive Fatigue Associated with Functional Electrical Stimulation

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ABSTRACT

Restoration of motor function in paralyzed limbs using functional electrical stimulation (FES) is undermined by rapid fatigue associated with artificial stimulation. Typically, single electrodes are used to activate muscles with FES. However, due to the highly distributed branching of muscle nerves, a single electrode may not be able to activate the entire array of motor axons supplying a muscle. Therefore, stimulating muscle with multiple electrodes might enable access to a larger volume of muscle and thereby reduce fatigue. Accordingly, we compared the endurance times that ankle dorsiflexion could be sustained at 20% maximum voluntary force using feedback controlled stimulation (25 Hz) of human tibialis anterior (TA) using one or four percutaneous intramuscular electrodes. In addition, we measured endurance times in response to direct stimulation of the nerve supplying TA and during voluntary contraction. In all sessions involving electrical stimulation, an anesthetic nerve block proximal to the site of stimulation was used to isolate the effects of stimulation and alleviate discomfort. Endurance time associated with stimuli delivered by a single intramuscular electrode (84 ± 19 s) was significantly smaller than that elicited by four intramuscular electrodes (232 ± 123 s). Moreover, endurance time in response to nerve stimulation (787 ± 201 s) was not significantly different that that produced during voluntary contraction (896 ± 272 s). Therefore, excessive fatigue associated with FES is probably due to the inability of conventional FES systems to enlist the full complement of motor axons innervating muscle and can be mitigated using multiple electrodes or nerve-based electrodes.

INTRODUCTION

Functional electrical stimulation (FES) is a rehabilitative technology that serves to restore motor function in paralyzed individuals. FES takes advantage of the retained excitability of motor axons that innervate most paretic skeletal muscles. This enables induction of muscle contraction through artificial electrical stimulation delivered by surface electrodes, intramuscular electrodes, or by electrodes that encircle peripheral nerves supplying muscles. The utility of FES, however, is undermined because of the rapid muscle fatigue that occurs during FES (Bhadra & Peckham 1997; Mizrahi 1997; Kesar et al. 2008; Doucet et al. 2012; Guiraud et al. 2014; Ibitoye et al. 2016; Barss et al. 2018). While a component of this accelerated fatigue is due to peripheral adaptations that occur in chronically paralyzed muscle (Grimby et al. 1976; Stein et al. 1992; Martin et al. 1992; Shields 1995; Butler & Thomas 2003; Thomas et al. 2003), FES-induced contractions also fatigue rapidly in able-bodied subjects (Naess & Storm-Mathisen 1955; Binder-Macleod & Snyder-Mackler 1993; Karu et al. 1995).

One reason proposed to account for rapid fatigue with FES is that the normal recruitment order of motor units, from weakest and most fatigue resistant toward the strongest and most fatigable, is disrupted. This is thought to occur because extracellular stimulation favors activation of the larger diameter axons (Blair & Erlanger 1933; McNeal 1976; Rattay 1986; Fang & Mortimer 1991; Grill & Mortimer 1995) that innervate strong, fatigable motor units (Wuerker et al. 1965; Jami & Petit 1975; Zajac & Faden 1985). In addition, everything else being equal, axons closest to the stimulating electrode are those most readily activated by electrical stimulation (Mortimer 1981; Grill & Mortimer 1995). Because axons of varying diameters

appear to be intermingled within motor nerves and muscle, there would be no particular spatial bias favoring activation of one type of motor unit over another (Thomas et al. 2002). As a consequence, investigators have suggested that electrical stimulation tends either to invert the normal recruitment order (Parker et al. 1986; Kubiak et al. 1987, Sinacore et al. 1990; Trimble & Enoka 1991; Binder-Macleod & Snyder-Mackler 1993, Yoshida & Horch 1993; Heyters et al. 1994; Mizrahi 1997; McDonnall et al. 2004; Navarro et al. 2005; Sheffler & Chae 2007; Malešević et al. 2010) or to activate motor units in a relatively random way (Knaflitz et al. 1990; Binder-Macleod et al. 1995; Feiereisen et al. 1997; Bickel et al. 2011; Barss et al. 2018). It should be noted, however, that some studies have shown little disruption in normal recruitment order with electrical stimulation (Thomas et al. 2002; Farina et al. 2004).

A second reason often cited as a possible cause for rapid fatigue with FES is related to the synchronized discharge of motor units induced by peripheral electrical stimulation (Binder-Macleod & Snyder-Mackler 1993; Karu et al. 1995; Mizrahi 1997; Chou et al. 2008; Downey et al. 2015; Popović & Malešević 2009; Malešević et al. 2010; Rohm et al. 2013; Sayenko et al. 2014; Lou et al. 2017; Barss et al 2018; Zheng & Hu 2018). Such synchronization can lead to marked fluctuations in evoked force, which in turn, can itself provoke fatigue because of the additional work required by the contractile apparatus repeatedly shortening against series elastic elements in muscle (Garland et al. 1988; Sandercock 2006). To minimize force fluctuations (which also compromises force control), stimulus frequencies can be increased. Yet excessively high stimulus frequencies can also promote rapid fatigue (Naess & Storm-Mathisen 1955; Jones et al. 1979; Metzger & Fitts 1986; Jones 1996, McDonnall et al. 2004). Therefore, some FES investigators have turned to asynchronous stimulation (Lind & Petrofsky 1978; Yoshida &

Horch 1993; Wise et al. 2001; McDonnall et al. 2004; Malešević et al. 2010; Nguyen et al. 2011; Maneski et al. 2013; Sayenko et al. 2014; Downey et al. 2015; Bergquist et al. 2016, 2017; Laubacher et al. 2017; Lou et al. 2017), an approach originally described by Rack and Westbury (1969), wherein different sets of motor units are activated sequentially at relatively low rates using multiple electrodes. Such asynchronous (or interleaved stimulation) can produce reasonably smooth muscle force despite low stimulus rates delivered to each set of motor units that, on their own, would cause markedly unfused contractions (Rack & Westbury 1969; Wise et al. 2001, Sandercock 2006).

What is puzzling, however, is that the advantage of asynchronous over synchronous stimulation practically disappears for stimulus rates above ~10 Hz (Rack and Westbury 1969; Sandercock 2006). And while those studies involved cat soleus, the average contraction time of cat soleus (76 ms, Nelson 1969) is briefer (and hence, the fusion frequency higher) than that found in many lower limb muscles of humans (e.g. 81 ms for tibialis anterior, Marsh et al. 1981; 87 ms for quadriceps, Bergstrom & Hultman 1990; 104 ms for triceps surae, Marsden & Meadows 1970). Yet, many interleaved FES protocols involve frequencies ≥ 10 Hz (Malešević et al. 2010; Nguyen et al. 2011; Maneski et al. 2013; Sayenko et al. 2014; Bergquist et al. 2016, 2017; Lou et al. 2017). Indeed, we have recently shown that there was no difference in the degree of fatigue induced with interleaved versus synchronous stimulation when delivered to two different locations in a muscle using stimulus frequencies > 15 Hz at each electrode (Buckmire et al. 2018). Therefore, it seems possible that the documented improvement in fatigue resistance using interleaved stimulation compared to single site stimulation (Malešević et al. 2010; Nguyen et al. 2011; Sayenko et al. 2014; Downey et al. 2015; Bergquist et al. 2016, Laubacher et al.

2017; Lou et al. 2017) was not primarily because of the asynchronous activation per se. Rather, given the widespread distribution of motor nerve branches within human muscle (Amarali et al. 2007; Mu and Sanders 2010; Won et al. 2011; Yu et al. 2016), multi-site stimulation may simply enable access to more of the muscle fibers within a muscle (Buckmire et al. 2018).

To test this possibility, here we compared the duration that submaximal isometric contractions of human tibialis anterior could be sustained when feedback-controlled electrical stimulation was delivered through a single intramuscular electrode to that delivered synchronously through multiple electrodes. In addition, in separate sessions we also measured contraction duration evoked by direct stimulation of the peripheral nerve proximal to its entry into tibialis anterior (i.e. at a site where motor axons are spatially constrained) and during voluntary contractions.

We found that multi-electrode stimulation markedly extended the endurance time of submaximal contractions over single-electrode stimulation. Moreover, the duration of contractions induced by electrical stimulation delivered directly to the peripheral nerve was no different than, and in some cases longer, than that achieved during voluntary contractions. These findings indicate that the rapid fatigue associated with conventional FES is unlikely to be primarily caused by synchronized discharge or disrupted recruitment order of motor units but rather because only a fraction of the motor units can be readily enlisted using single electrodes placed in or over muscle.

METHODS

Subjects and Muscle. Five healthy human subjects (1 female, 4 male), ages 20 to 58 were included in this study in accordance with human subjects guidelines and approved by the University of Arizona institutional review board. Each subject participated in four experimental sessions (separated by ≥ 2 days) involving sustained isometric contraction of the tibialis anterior muscle. The tibialis anterior (TA) was selected for this study because it is readily accessible for intramuscular stimulation and it generates the preponderance of the dorsiflexion torque at the ankle. In addition, the nerve supplying TA (deep peroneal neve) is reasonably accessible for stimulation while the major nerve (common peroneal nerve) giving rise to the deep peroneal nerve can be anesthetically blocked several centimeters proximal to the deep peroneal nerve, thereby isolating TA for study.

Force and EMG measurements: Subjects were seated in a dental chair with their knee extended and their right foot secured to a custom-built footplate instrumented with a transducer to measure isometric force during dorsiflexion. The footplate rotated freely about an axis aligned approximately co-linear with the talocrural joint axis of the ankle. Once the foot was secured with Velcro straps, the footplate was rotated such that it held the ankle in a plantar-flexed position. An isometric force transducer (Grass FT-10, Warwick, RI, USA using custom-built heavy-duty springs inserted into the housing of the transducer) was then attached to the distal end of the footplate (22.5 cm from the axis of rotation of the footplate) that resisted ankle dorsiflexion. The knee was held in an extended position with a wide strap that ran over the anterior surface of the distal thigh and was tightened and secured to the chair. Bipolar surface

electrodes (4 mm diameter, ~ 5 cm inter-electrode separation) were placed on the skin over the TA and over the triceps surae to record electromyographic (EMG) activity. EMG signals were amplified (x 1000, band-pass filtered 30 to 1000 Hz., Grass Technologies Product Group, Astro-Med Inc; West Warwick Rhode Island). Force and EMG signals were digitally sampled (1000 and 4000 samples/s, respectively) by a computer-controlled data acquisition system (Power 1401, Spike2, Cambridge Electronic Design, Cambridge England).

Electrical Stimulation: Current-regulated stimuli (0.25 ms duration, rectangular, monophasic, cathodic pulses) were delivered to TA or deep peroneal nerve through percutaneous tungsten microelectrodes (250 μm shaft diameter, 1 – 5 μm tip diameter, 2 – 4 mm of insulation removed from the tip, 30 mm total length, Frederick Haer, Bowdoin Maine, USA) using a programmable multi-channel stimulator (STG4008 MultiChannel Systems, Reutlinger, Germany). Surface electrodes (Covidien/Kendall, Pediatric cloth ECG Hydrogel Electrodes H59P, Medtronic, Dublin, Ireland) placed over the tibia or the lateral malleolus of the fibula served as common return electrodes for electrical stimulation. Current pulses delivered by the stimulator were digitally sampled (12 kHz) by measuring the voltage drop across an in-series resistance (~150 Ω).

Anesthetic block: Strong electrical stimulation can be painful. Such painful stimuli can trigger spinal reflexes and descending activity that interferes with measures of force from the target muscle. Furthermore, some subjects may not tolerate the high stimulus intensities delivered over prolonged periods needed for tests of muscle endurance. Therefore, we used an

anesthetic block of the common peroneal nerve supplying the TA to largely eliminate sensory feedback associated with the stimulation and to fully paralyze the TA.

Under ultrasound guidance, 10 -15 ml of 1.5% Mepivacaine was administered to the peri-neural space surrounding the common peroneal nerve at a site ~ 8 - 10 cm proximal to the head of the fibula. Complete anesthetic block was confirmed by the subject's inability to voluntarily generate detectable dorsiflexion force. This occurred within ~ 20 min of the injection in all cases but one. In that one case where a complete nerve block was not achieved, the experimental session was terminated and the subject returned on a different day during which the nerve block was successful. As a precaution, prior to the nerve block, an intravenous line was placed into a peripheral vein in the upper extremity to administer fluids or medications in the unlikely event of anesthetic toxicity. No such events occurred in any of the subjects tested. Following experiments involving the nerve block, subjects wore a plastic ankle cast to prevent foot drop for a period of about 4 – 5 hours until the paralysis resolved.

Procedure: Subjects participated in four experimental sessions in random order, one session involving sustained voluntary contraction of the TA and three sessions involving sustained stimulation of the TA with a nerve block present. For sessions using electrical stimulation, in one session a single intramuscular electrode was used to deliver stimuli, in a second session four intramuscular electrodes distributed throughout TA and each controlled by a separate stimulus channel were used to deliver stimuli, and in a third session stimuli were delivered by a single electrode placed adjacent to the deep peroneal nerve just distal to the fibular head.

In each session, subjects first performed three brief (~ 2 s duration) maximum voluntary contractions (MVC) of ankle dorsiflexion with about 60 s between trials. The largest force exerted among the three trials was deemed the MVC force. For the voluntary fatigue task, subjects observed a target force of 20% MVC displayed on a computer screen and matched that force by isometric dorsiflexion of the ankle. To keep subjects motivated, subjects were verbally encouraged throughout the contraction to sustain the target force for as long as possible (e.g. Fuglevand and Keen 2003). The task was terminated when the force continuously remained below the target force for a period of 5 – 10 s.

For sessions involving intramuscular stimulation, sterilized microelectrodes were inserted through alcohol cleansed skin and into TA following induction of paralysis by the nerve block. For experiments involving a single intramuscular electrode, the initial electrode placement was at a proximal site ~ 1/3 of the length of the muscle and ~ 2 cm lateral to the tibial ridge. This site was selected based on nerve dissection (Watt et al. 2014) and surface EMG-array studies (Barbero et al. 2012) indicating that this site approximates the location where major branches of the deep peroneal nerve typically penetrate the TA. For experiments involving four electrodes, one electrode was inserted at this proximal site, two electrodes were placed at ~ 50% of the length of TA with one located ~ 1 cm lateral to the tibial ridge and the other ~ 3 cm lateral to the ridge, and the fourth electrode was inserted at a distal site ~ 2/3 of the length of muscle and inserted into the midline of TA.

Each electrode was initially inserted to a depth of about 5 – 8 mm below the skin. Brief (1 s) trains of stimuli (5 mA, 25 Hz) were delivered and the evoked force recorded. The electrode

was then advanced in ~ 2 mm steps to a maximum depth of ~ 30 mm with stimulation repeated at each step. Electrode depth was estimated at each stimulation site by measuring the length of the electrode extending above the skin surface. The insertion site was marked with ink and then the electrode was removed and reinserted at sites ~ 1 – 2 cm proximal, distal, medial, and lateral to the original insertion site and the process repeated. Following this survey, the electrode was then reinserted at the site that evoked the largest force in response to the stimulus train. Stimulation was repeated at this site to confirm similar levels of evoked force as detected originally. If needed, small adjustments to the electrode depth were made to ensure robust force responses were evoked. In cases involving four electrodes, this process was repeated for each electrode. The time needed to place all four electrodes was usually about 1 hr. Because of concerns that the anesthetic might begin to wear off, we did not carry out additional procedures to assess the degree of independence of each electrode by stimulating each electrode separately and in various combinations with other electrodes and measuring the degree of force summation.

For sessions involving nerve stimulation, a single tungsten microelectrode was inserted at an oblique angle to the skin immediately distal to the head of the fibula in order to approach the deep peroneal nerve. The electrode position was manually adjusted until strong dorsiflexion forces were elicited in response to 1-s trains of 1 mA pulses delivered at 25 Hz. We often also observed toe extension during stimulation of the nerve indicating activation of extensor hallucis longus and extensor digitorum longus. This was largely unavoidable because axons to those muscles are also carried in the deep peroneal nerve.

Once electrodes were in place, single stimulus pulses were delivered to each electrode separately and the associated twitch forces were recorded with amplitude incremented in 1 mA steps from 1 mA to 32 mA and with a 2-s delay between pulses. The associated current – twitch force relationships were evaluated immediately to identify the operating range of currents for each electrode for the upcoming fatigue task.

Prior to the fatigue test, a few 1-s trains of stimuli (25 Hz) were delivered to identify the stimulus pulse amplitudes needed initially to elicit the 20% MVC target force. For the case involving four electrodes, stimulus amplitudes were identified separately for each electrode that evoked ~ 5% MVC force (assuming that the forces would sum near linearly). A custom-designed Matlab (Mathworks, Natick MA) program was then used to provide on-line feedback control of stimulus pulse amplitude during the fatigue task.

The inputs to the program included the target force (20% MVC), the starting current levels (based on those identified with 1-s trains), the upper current levels (based on the current – twitch force relationship), the force exerted by the subject sampled in real time , and a gain factor. Force was sampled in parallel by two data acquisition systems: one dedicated exclusively for feedback control (120 samples/s, USB 6001, National Instruments, Austin, TX) and one for general data acquisition and storage that was used for off-line analyses (1000 samples/s, CED Spike2). The gain factor in this simple proportional feedback system was used to transform the detected error between target and a six-point (50 ms) moving average of the actual force into a current adjustment scaled to the operating range of the electrode. We used a nominal gain value of 0.25 indicating that 25% of the full current range would be added to the ongoing current in the

case of an error representing 100% of the full force range. In brief tests before the fatigue run, if overt force oscillations developed, we reduced the magnitude of the gain. If, on the other hand, evoked force was slow to approach the target, we increased the gain. For the majority of cases tested, however, a gain of 0.25 worked reasonably well.

The commanded adjustments in current amplitude were then dispatched every ~ 240 ms to the MultiChannel Systems stimulator that delivered continuous 25 Hz (0.25 ms pulse duration) stimuli to the electrode(s). A stimulus frequency of 25 Hz was selected because it evokes fused force responses and is within the upper range of motor unit firing rates of TA recorded during voluntary contractions (Connelly et al. 1999; De Luca & Hostage 2010). In the case of multielectrode stimulation, the timing of the pulses were offset by 1 ms across electrodes to help prevent summation of otherwise sub-threshold electric fields (i.e. 'subliminal fringe') at sites relatively distant from the electrodes (Mortimer 1981, Branner et al. 2001). For example, we found in some experiments that precisely synchronized stimulation (i.e. without the 1 ms delay) could lead to overt plantar flexion that was not evoked by any electrode alone when stimulating using maximal intensities. Feedback-controlled stimulation was maintained until the evoked force was clearly below the target force by at least 10% for ~ 5 – 10 s despite escalating current intensities.

Data Analysis: For each fatigue trial, we used a custom-written program (Spike2) to measure the endurance time as the duration from when the force initially came within 10% of the target force until the time when force fell 10% below the target for more than 5 seconds. A one-way repeated-measures analysis of variance (ANOVA) was performed to determine whether

endurance time varied significantly with different fatigue protocols. Mann-Whitney rank sum post-hoc test was used to evaluate differences in endurance times between fatigue protocols with Bonferroni correction for multiple comparisons. The level of statistical significance was set at $P < 0.05$ and data are reported as means ± one standard deviation (SD).

RESULTS

Current – Twitch Force Relation. Figure 1 shows example twitch force responses to escalating current-amplitude stimuli delivered during separate trials to each of four intramuscular electrodes placed in different locations in TA in one subject during a single session. In this example, a small twitch was detected for the lowest current delivered (1 mA) on electrodes 1, 2, and 4, while 3 mA of current was needed to elicit a detectable twitch on electrode 3. Peak twitch forces of 17.4, 17.1, 17.4, and 15.7 N were attained at 31, 32, 29, and 30 mA for electrodes 1 – 4, respectively. The difference in peak forces between the electrode that evoked the largest twitch and that which evoked the smallest was 9.8%.

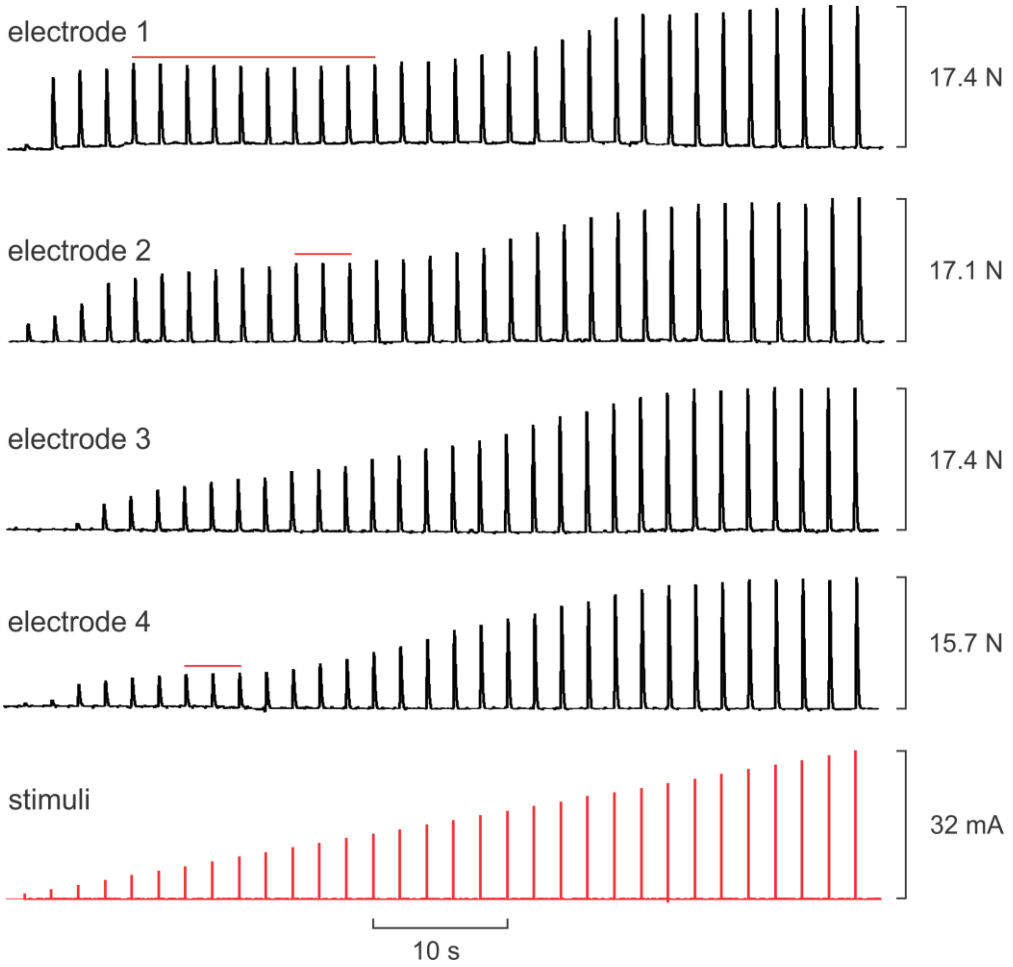

Figure 1. *Example dorsiflexion twitch force responses (upper four traces) to increasing stimulus current pulses (bottom trace) delivered through four intramuscular electrodes placed in different locations within tibialis anterior in one subject. Stimulation through each electrode was performed in separate trials but traces have been aligned for compact display. Red horizontal lines indicate intermediate plateaus wherein force saturated across a range of increasing stimulus intensities.*

Across all subjects and all cases involving one or four intramuscular electrodes, the average threshold current for evoking a detectable twitch response was 1.6 ± 1.0 mA while the average current associated with peak twitch force was 27.4 ± 5.7 mA (range 9 - 32 mA). For the

case of four intramuscular electrodes, the average percent difference across electrodes evoking the largest and smallest peak forces was 25.6 ± 18.1% (range 9.8 – 51.6%). During nerve stimulation we did not get clear measures of the threshold currents due to the large increments (1 mA) used for the current – twitch force assessment. In all subjects during nerve stimulation, 1 mA (the smallest value tested) always evoked a strong twitch (> 50% of the peak twitch force) while the current associated with peak twitch force was ≤ 5 mA.

Intermediate Plateaus. While twitch forces tended to progressively increase with current above threshold up to the current associated with peak force, there were often intermediate plateaus wherein evoked force saturated across a range of increasing currents. Such plateaus are highlighted with red horizontal lines in the examples shown in Figure 1. Force responses to stimuli delivered by electrode 1, for example, saturated across a nearly 3-fold increase in stimulus intensities (from 5 – 14 mA). We quantified the prevalence of such intermediate plateaus using a method we described previously (Buckmire et al. 2018). Namely, we calculated the percentage change in force associated with each 1 mA increment in current for all of the current– twitch force sequences involving intramuscular electrodes. We then identified the number of cases for which the change in force fell below 5% for two or more consecutive steps in current and which was then followed by increases in force above 5%. For all 25 intramuscular electrode sequences tested (20 from 4-electrode experiments, 5 from 1-electrode experiments), 80 % exhibited one or more intermediate plateaus. The average span of currents over which twitches saturated during these intermediate plateaus was 4.7 ± 3.4 mA (range 2 – 14 mA).

Fatigue: The target force for all fatigue trials was set at 20% of the MVC obtained in each session. Across all sessions and subjects, the average MVC force was 218.8 ± 32.2 N (49.2 ± 7.2 N•m of torque). There was little variation in MVC force across sessions for individual subjects (average coefficient of variation = 5.6 ± 3.9%). Figure 2 shows example force responses obtained in a single subject during sustained voluntary effort (Fig. 2A), stimulation of TA with a single intramuscular electrode (Fig. 2B), stimulation of TA with four intramuscular electrodes (Fig. 2C), and stimulation of the deep peroneal nerve supplying TA (Fig. 2D). The TA surface EMG is shown in Fig. 2A, whereas the feedback controlled stimulus currents are shown in Figs. 2B – 2D. For clarity, only one of the four stimulus-current signals is shown in Fig. 2C. All four panels in Fig. 2 are depicted using the same time base so their durations can be directly compared.

During the voluntary contraction (Fig. 2A), EMG activity progressively increased reflecting increased motor unit recruitment and rate coding needed to compensate for diminishing force capacities of the active muscle fibers. Eventually, however, the increased drive to the muscle was insufficient to maintain the target force and the contraction was halted. Across all five subjects, the average value of the rectified TA EMG signal measured over the last 10 s of the trial was 71.6 ± 34.5 % (range 47.8 – 132.1%) larger than that measured over the initial 10 s. The endurance time for the voluntary contraction shown in Fig. 2A was 740 s.

In the session involving stimulation with a single intramuscular electrode (Fig. 2B), ~ 10 mA of current was needed at the outset to achieve the target force. The stimulus current then increased rapidly up to its assigned upper limit of 24 mA under feedback control in order to

maintain the target force. Despite the increasing current, force decayed slowly over much of the trial and stimulation was halted when the force was < 90% of the target level. The endurance time for this trial was 80 s. When four intramuscular electrodes were used to deliver stimuli (Fig. 2C), force was maintained five times longer (endurance time 448 s) compared to stimulation with a single electrode. At the outset, the evoked force overshot the target but with

feedback control, the stimulus intensities delivered to the four electrodes were rapidly adjusted and the evoked force was then stably maintained at the target force. Stimulus current then increased almost linearly over much of the trial except near the end when current amplitude rapidly accelerated in an attempt to maintain the target force in the face of weakening output of the active muscle fibers. Despite this increase in stimulus intensity, additional force was not generated and the trial was halted when force dropped below 90% of the target even though current had not reached the upper limit on any of the four stimulus channels.

The most remarkable trial was that associated with nerve stimulation (Fig. 2D). In this case, the endurance time (993 s) far surpassed (by 34 %) that of the voluntary contraction (Fig. 2A). The stimulus currents involved were much lower than that used for intramuscular stimulation and the amplitude only gradually increased over much of the trial except a sharp escalation near the end. In one other subject, endurance time of nerve stimulation exceeded that of the voluntary contraction by 42%. As can be seen in Fig. 2D, force fluctuations were evident throughout the trial but grew in intensity during the latter two-thirds of the trial. These force fluctuations were seen in all subjects and were dominated by a 4.2 Hz oscillation. It was only discovered later that these oscillations were due to an inadvertent lengthening of every 5^{th}

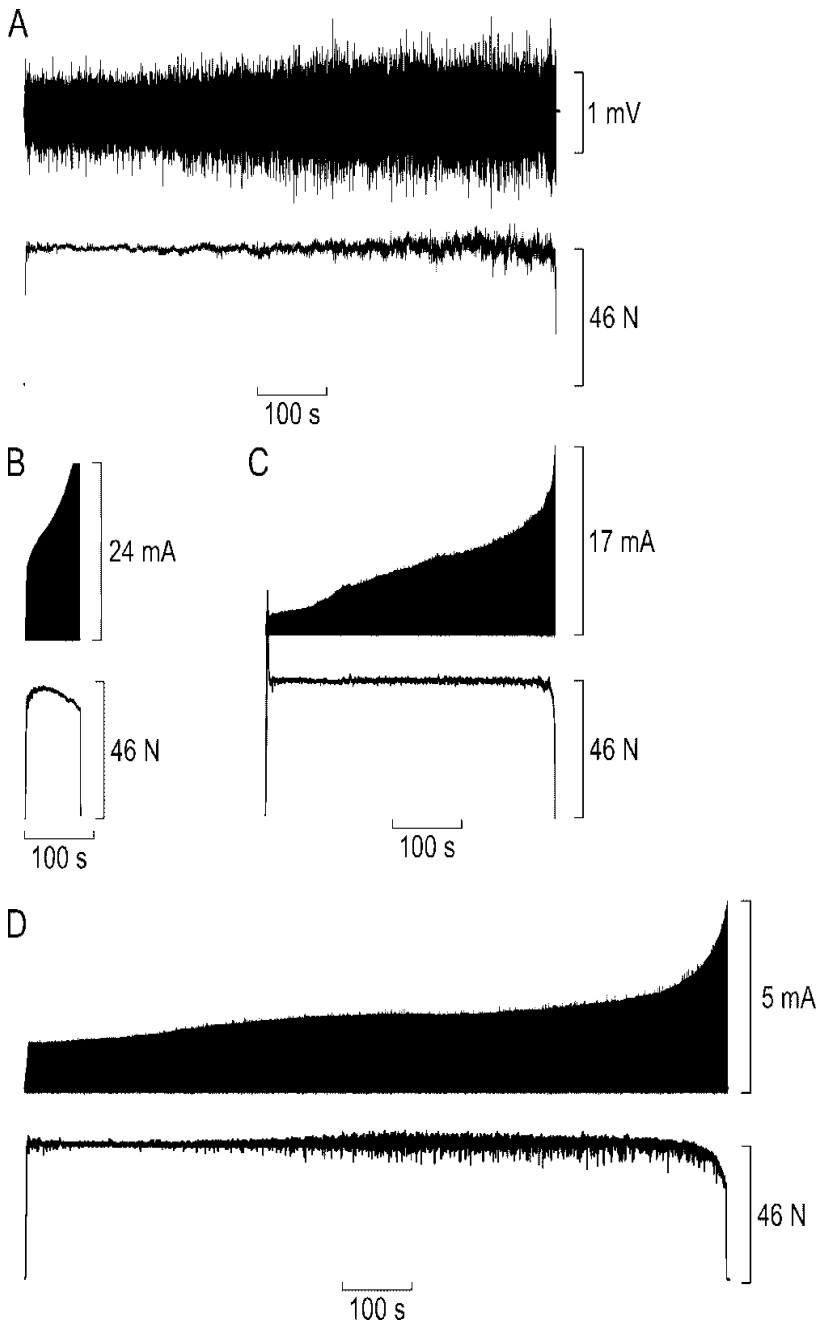

Figure 2. *Example force responses (bottom traces) recorded in a single subject during four sessions involving A) voluntary contraction, B) intramuscular stimulation of tibialis anterior (TA) with a single electrode, C) intramuscular stimulation of TA with four electrodes, D) stimulation of deep peroneal nerve. In A), the top trace shows the surface EMG signal recorded from TA. In B), C), and D), the top trace indicates the feedback controlled stimulus current delivered to the electrodes. In C), only one of the four stimulus current signals is depicted for clarity. All four stimulus current signals associated with the trial shown in C) increased exactly in parallel but the absolute values were different.*

interpulse interval (occurring at cycle period of ~ 240 ms), presumably due to buffering delays in the computer – stimulator interface. As such, the average stimulus rate was ~ 22 Hz rather than 25 Hz for all subjects.

The mean (SD) endurance times for the five subjects across the four fatigue tasks is shown in Figure 3. ANOVA indicated a significant effect of fatigue task on endurance time ($P < 0.001$). Post hoc analysis indicated no significant difference ($P = 0.46$) in the mean endurance times between voluntary (896 ± 272 s) and nerve stimulation tasks (787 ± 201 s). The mean endurance time associated with stimuli delivered by a single intramuscular electrode (84 ± 19 s) was significantly ($P < 0.01$) smaller than that elicited by four intramuscular electrodes (232 ±

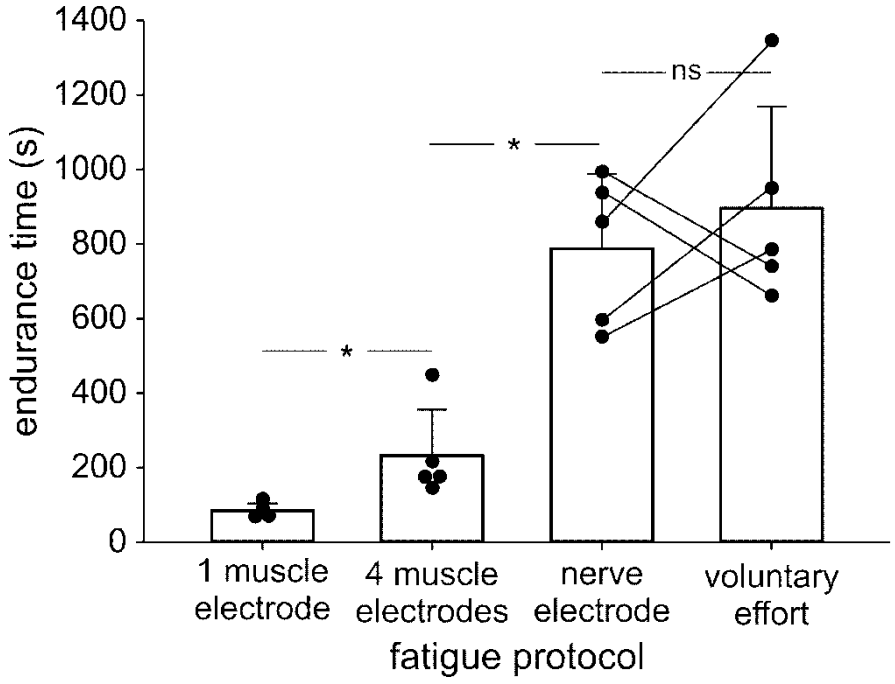

Figure 3. *Mean (SD) and individual values (dots) of endurance times of ankle dorsiflexion at a 20% MVC force target in response to feedback controlled stimulation of tibialis anterior with one or four intramuscular electrodes, feedback controlled stimulation of the deep peroneal nerve, or during voluntary contraction. ns – non-significant difference (P = 0.46). * - significant difference (P< 0.01).*

123 s). All other between-task comparisons were significant ($P < 0.01$).

DISCUSSION

Here we have shown that the rapid fatigue associated with electrical stimulation of muscle can be partially mitigated by increasing the number of intramuscular electrodes used to activate muscle. Furthermore, stimulating the nerve proximal to where it enters muscle produced a target force that could be sustained as long as, and in some cases longer, than that produced during voluntary contraction. We conclude, therefore, that the excessive fatigue associated with FES must primarily be due to the inability of conventional FES systems to enlist the full complement of motor units within muscle. Moreover, these results indicate that neither altered motor unit recruitment order nor synchronized motor unit activity can account for much of the fatigue seen with FES as both of these factors presumably were in play in the present experiments.

A likely explanation as to why a single stimulating electrode (as has been the convention in many FES systems) is unable to activate all the motor units in a muscle is because of the widely distributed arrangement of nerve branches within muscle. A long-held view is that nerves typically enter muscle at a single location to innervate muscle fibers along a constrained central region referred to as the innervation zone (Coërs & Telerman-Toppet 1977; Lee et al. 2012; Behringer et al. 2014; Jahanmiri-Nezhad et al. 2015). Yet, many anatomical studies have clearly shown extensive and complex ramification of nerve branches prior to entry into (Sunderland and Hughes 1946) and throughout large expanses of skeletal muscle (Amarali et al. 2007; Mu and Sanders 2010; Won et al. 2011; Yu et al. 2016). Because of the steep decay in the

electric field with distance from a stimulating electrode (McIntyre & Grill 2002; Rattay 2004), it may be challenging in practice to deliver sufficient current to excite all of the widely dispersed nerve branches, particularly in large muscles such as the tibialis anterior. As such, using more electrodes situated in different regions of a muscle should enable electrical access to a larger subset of the nerve branches. In support of this idea, we have recently shown that the maximum force that could be evoked using intramuscular electrodes was always greater when using multiple compared to a single electrode (Buckmire et al. 2018).

Indeed, the prevalence of intermediate plateaus in evoked force responses to increasing stimulus intensities observed here (Fig. 1) and previously (Crago et al. 1980; Cameron et al. 1998; Buckmire et al. 2018) probably reflects the presence of widely separated nerve branches within muscle. Namely, the initial increase in force with increased stimulus intensity likely arises due to progressive activation of more motor axons contained within nerve branches in the vicinity of the electrode. Eventually, however, most motor axons in such nearby branches might be recruited and thereafter, no additional force would be elicited over a range of increased stimulus strengths (Cameron et al. 1998). At some point, however, sufficient current could be delivered such that other distant nerve branches begin to be activated, leading to an additional rise in evoked forces with increasing current. In the present experiment, there was no way to be sure that additional intermediate plateaus might have been detected had we delivered currents higher than the 32-mA maximum allowed by our stimulator.

It is possible that secondary increases in evoked force after a plateau might have been due to activation of neighboring synergist muscles, such as extensor hallucis longus or extensor

digitorum longus, both of which contribute to ankle dorsiflexion. Yet, we only rarely detected toe extension during intramuscular stimulation of tibialis anterior, which would have been indicative of activating those synergists. Furthermore, such intermediate plateaus were observed in response to intramuscular stimulation of cat hindlimb muscle when no muscles, other than the target muscle, were attached to the force transducer (Crago et al. 1980; Cameron et al. 1998).

In the context of fatigue resistance, the ability to engage more motor units with multiple electrodes is beneficial. A larger reserve of motor units that can be called upon (by increasing stimulus strength) as force declines in earlier activated motor units will enable a given target force to be maintained for a longer duration. Indeed, when we stimulated the deep peroneal nerve (at a site where most of the motor axons supplying tibialis anterior are bundled together), no evidence of excessive fatigue was found. Moreover, in two of the five subjects tested, the duration over which the target force could be sustained with such nerve stimulation exceeded that associated with voluntary effort. Although some previous studies have shown that the extent of fatigue to be similar during sustained maximum voluntary contractions and maximal nerve stimulation (Merton 1954; Jones et al. 1979; Bigland-Ritchie et al. 1979; Marsden et al. 1983), we are unaware of previous cases for which fatigue resistance of electrically evoked contractions surpassed that of voluntary effort.

While caution against over-interpretation of these two cases is certainly warranted, some consideration as to why such supra-endurance arose in these cases seems worthwhile. First, it is possible that the two subjects simply did not exert themselves fully to sustain the voluntary contraction for as long as possible. Indeed, those two subjects had the briefest endurance times

associated with the voluntary contraction (see Fig 3). Yet, both subjects showed substantial increases in TA EMG during the voluntary fatigue task indicative of increasing exertion. Indeed, the subject who showed the greatest increase in endurance time with nerve stimulation compared to voluntary contraction also exhibited the greatest increase (>100%) in TA EMG during the voluntary task.

Thus, if taken at face value, it is then important to ask why such supra-endurance has not been observed previously. Perhaps one reason is that few other studies have used feedback control during electrical stimulation to determine the duration over which a given target force can be maintained. Rather, most fatigue studies involving electrical stimulation measure the change in force in response to a fixed stimulus intensity applied over a set duration (e.g. Yoshida & Horch 1993; Thomas et al. 2002; Lou et al. 2017; Buckmire et al. 2018). Because it is difficult to 'clamp' the intensity of voluntary drive during fatiguing contractions, it is not possible to directly compare such electrically evoked contractions to that produced voluntarily. On the other hand, voluntary contractions naturally lend themselves to visual feedback control of a displayed target force and as such, can be compared to that produced by electrical stimulation under force-feedback control, as was done here. It should be said that under open-loop stimulation involving fixed stimulus intensities, the same set of motor units would be activated throughout a stimulation bout. In this case, the tendency of extracellular stimulation to favor activation of higher threshold, fatigable motor units would indeed contribute to more rapid loss in force than that associated with activation of motor units that mimics that which occurs naturally.

A second possible reason for the absence of such observations previously is that few such studies have used anesthetic nerve blocks as we used here to isolate the effects of electrical stimulation. Intense electrical stimulation delivered to muscle not only activates motor axons but also engages an array of sensory axons including nociceptors. The associated sensory signals can provoke spinal reflexes and perhaps even descending inputs leading to unregulated contraction of agonists and antagonists, which in turn contaminates the force signals meant to detect the effect of electrical stimulation alone (Lagerquist et al. 2009). Furthermore, subjects may not readily tolerate the pain associated with prolonged intense stimulation and investigators may avoid imposing such discomfort on human volunteers.

An additional possible reason relates to the fortuitous selection of TA as the target muscle in the present study. The deep peroneal nerve supplying TA arises as one of two main branches (the other being the superficial peroneal nerve) of the common peroneal nerve. From this bifurcation point, there typically is about a one centimeter span of the deep peroneal nerve before it gives rise to the first of multiple branches destined for the TA along ~ 20 cm of length of the nerve (Sunderland and Hughes 1946). This span of the nerve was targeted for stimulation in the present experiments. What is advantageous about this site is that it also carries axons supplying the two other ankle dorsiflexors, extensor hallucis longus and extensor digitorum longus. Therefore, stimulation at this site engaged all of the ankle dorsiflexors (as evidenced by toe extension as well as ankle dorsiflexion during nerve stimulation), just as would likely occur during voluntary dorsiflexion. Consequently, the total muscle mass involved in voluntary and nerve stimulation experiments was reasonably similar.

It should be noted, however, that the branching patterns of the peroneal nerves are highly variable across human cadaver specimens (Sunderland and Hughes 1946; Aigner et al. 2004). For example, in 6 of 20 specimens, the first branch to the TA arose from the common peroneal nerve above its bifurcation to the deep and superficial nerves (Sunderland and Hughes 1946). Therefore, a portion of the TA would not have been activated with nerve stimulation in the present study in subjects with such a branching arrangement. In addition, the superficial peroneal nerve runs almost adjacent to the deep peroneal nerve in the region targeted for stimulation such that the distance between the centers of the two nerves may be as small as 5 mm (Aigner et al. 2004). It is possible, therefore, that with increasing stimulus intensity during the fatigue task, some portion of the superficial peroneal nerve supplying the peroneus muscles, may have been activated. Indeed, in some subjects, we visually observed contraction of the peroneus muscles during the later stages of the fatigue protocol involving nerve stimulation. Because the peroneus muscles contribute to ankle plantarflexion, their activation would tend to curtail dorsiflexion endurance time. Therefore, these two factors (possibility of not activating all nerve branches to TA and possibility of activating antagonists), may have limited the measured endurance time in response to nerve stimulation in some of the subjects.

The results of the nerve stimulation experiments also bear on fundamental questions related to the contribution of the central nervous system (CNS) to voluntary muscle fatigue. The observation that endurance time in some cases was longer with electrical stimulation than during voluntary contraction strongly suggests some degree of failure of the CNS to fully engage muscle during prolonged activity in those cases. There is a significant body of work that

supports this contention and numerous mechanisms have been proposed to account for such fatigue-related impairment of CNS drive (see reviews by Gandevia 2001 and Taylor et al. 2016).

Finally, based on the findings of the present study, it would seem appropriate to consider using multiple stimulating electrodes (particularly for large muscles), and where possible, to stimulate the peripheral nerves supplying muscle for FES applications in paralyzed individuals. Of course, this must be weighed against the increased complexity of the control system and associated hardware, and added surgical challenges for implanted systems (Memberg et al. 2014). For therapeutic interventions involving surface electrodes, using more than one active electrode would also seem beneficial. Indeed, the efficacy of interleaved stimulation among multiple surface electrodes suggests this to be the case (Malešević et al. 2010; Nguyen et al. 2011; Maneski et al. 2013; Sayenko et al. 2014; Downey et al. 2015; Bergquist et al. 2016, 2017; Laubacher et al. 2017; Lou et al. 2017). The results reported here and previously (Buckmire et al. 2018) indicate that the improved fatigue resistance associated with interleaved stimulation is most likely related to the use of multiple electrodes (providing access to a greater volume of muscle) rather than the asynchronous activation induced by the interleaved protocol. Additional studies will need to be performed to determine the degree of improved fatigue resistance using multiple electrodes or nerve stimulation for a variety of tasks including intermittent contractions and different target forces. Likewise, it will be especially important to evaluate the degree of improved fatigue resistance in individuals with spinal cord injuries given the changes in fiber type composition and atrophy that often occur with paralysis. Nevertheless, we expect such an approach to increase both the strength and endurance of electrically evoked contractions and thereby enhance the capability of FES to restore movement in paralyzed individuals.

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