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Fatigue Resistibility and Stimulus Strength Using Intraspinal Microstimulation vs. Intramuscular Stimulation in a Rat Model: Case Study

John Dube
College of Saint Benedict/Saint John's University

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PROJECT TITLE:

Fatigue resistibility and stimulus strength using Intraspinal microstimulation vs. intramuscular stimulation in a rat model: case study

Approved by:

Dr. Jennifer Schaefer

_____

Title: Assistant Professor of Biology

Dr. Clark Cotton

_____

Title: Adjunct Assistant Professor of Biology

Dr. Manuel Campos

_____

Title: Professor of Biology

Dr. William Lamberts

_____

Chair, Department of Biology

Dr. Anthony Cunningham

_____

Director, Honors Thesis Program

Introduction

Spinal Cord Injury (SCI) is a devastating neurological impairment that involves a disruption in the ascending and descending neural pathways of the spinal cord. The disruption of these neural tracts can lead to a permanent loss of sensorimotor and autonomic function in the body and thus impede an individual's ability to perform activities of daily living (ADLs). SCI affects nearly 130,000 individuals each year with 2-3 million individuals affected across the globe and health care treatments costs of nearly 1,000,000 dollars per patient in the first year alone (Jackson 2012). Due to the current inability to regenerate neural tissue post-injury, research has pursued alternate methods to help individuals with SCI regain locomotion, bladder control, and sexual functions (Musienko 2012, Yoo 2008). These methods called Functional Electrical Stimulation (FES) involve the use of a stimulator and the placement of electrodes in different parts of the body, dependent upon each respective method, to evoke movement in a target limb. Current practices of FES in clinical use to restore function include intramuscular stimulation, peripheral nerve stimulation, and epidural stimulation; however these are only the current manifestations of past neuroprostheses (NPs); another growing field is the research of intraspinal microstimulation (ISMS).

The use of electrical stimulation to achieve limb movement post SCI began in the early 20th century, with the use of an electrode to invoke movement in a spinally-transected cats and dogs (Philippson 1905, Sherrington 1910). The field would not advance much until the invention of the transistors in the 1960's, which would end in the culmination of peroneal nerve stimulation via surface electrodes to prevent foot drop (Liberson 1961). Stimulators like these and others, such as the ones that were triggered by voluntary muscle contraction were on the

market for 10 years until the age of the implanted NPs. NPs' was a product stemmed from the arrival of the pacemaker in the 1970's. The first NPs dorsal column stimulators, produced by Medtronic, were used to help control pain through means of gate control theory, supra-threshold stimulation amplitudes could block nerve pulses caused by pain, and the ability of neural tissue to remain within normal compliance parameters or spasticity (Waltz 1997). This era began the use to expand the use of FES systems to beyond that of locomotion. Deep brain stimulators for pain and extrapyramidal disorders would follow in the 1990's, with phrenic nerve stimulators for respiration and sacral root stimulators for bladder control also becoming used in the clinical setting (Kumar 1997, Benabid 1991, Elefteriades & Quin 1998). The rapid technological advancements have brought with them a number of new NPs to attempt to help individuals with SCI increase their ADLs, such as the four FES focused in this paper. This is not to say that other means have not been attempted, however this helps to show the evolution of FES systems and the evolution of what would evolution into the clinical use of intramuscular stimulation and peripheral nerve stimulation. Due to lack of technological advancement, these types of stimulation would be the most accessible and researched methods to evoke movements in SCI victims.

The investment into NPs has stemmed from the lack of neural regeneration post SCI. The inability to regrow neural tissue has pushed research to favor NPs in order to attempt to restore motor, sensory, or bladder function in SCI individuals. During the 1980's, researchers hypothesized that peripheral nerve tissue could be used to attempt to rebuild the broken neural connection in the spine (Richardson 1980). Research has shown that neural tissue regeneration is possible in a rat model, however, the success stemmed more from local neural circuits and was not comparable to a function restoration of the ascending and descending pathways response for

full body control (Jones 2001). There are numerous attempts to repair nerves through means of the implantation methods such as the use of scaffolding of biodegradable materials to stimulate nerve growth (Grahn 2014). Unfortunately, these methods have not been as successful as research would hope and alternatives are needed until nerve regeneration can come to fruition.

Literature review

FES systems are a current hot topic of research; however, missing from this literature is the use of comparative studies between ISMS and intramuscular stimulation and as such was the intent of this research. The limitations of each method have been recognized, however a direct comparison between methods is not present. The purpose of this project is to test the efficacy of ISMS compared to intramuscular stimulation, in a rat model, to test the fatigability and stimulation levels in the same locomotive movement.

As stated previously, SCI causes damage to the interneurons in the spinal cord and affects the activity of the peripheral and central nervous systems. The result can involve loss of sensation, limb movement, bladder control, and sexual function depending on the location and severity of injury (Musienko 2012).However, research has shown the neural circuitry pathways inferior to the injury are still intact (Bamford 2010). ISMS targets certain CPGs or motor units, located in the ventral horn of the grey matter in the spinal cord, to tap into the motor neurons that initiate functions such as walking and fire the appropriate muscles to achieve the desired movement (Bamford 2012, Mushahwar 2000). Research suggests that the motor neurons that control muscles of a movement, such as walking, are all interconnected in a neural network that enables the activation of one neuron to activate all the neurons in the CPGs. These CPGs control the coordination of such complex synergists muscle movements such as walking and are the

natural physiological way in which the brain activates the initiation of these actions. However, the use of these CPGs and ISMS to evoke movement is still underway and has not be achieved outside of a laboratory setting. There are a number of FES systems that are currently in clinical use.

Intramuscular stimulation involves the implantation of intramuscular or surface myoelectric electrodes into/on individual muscles, such as the vastus lateralis, rectus femoris, vastus intermedius, and the vastus medialias of the quadriceps muscle group, and the use of stimulation to evoke limb movement, such as standing (Bhadra 2001). Peripheral nerve stimulation uses a generic multi-channel stimulation device, capable of producing necessary amplitudes over 10mA, to stimulate a peripheral nerve, such as the tibial or common peroneal nerves, innervating a muscle, such as the gastrocnemius, or group of muscles by means of a nerve cuff electrode to cause contraction and produce a limb movement, such as plantar flexion or dorsiflexion (Schiefer 2013). As such, these methods are highly selective in terms of their ability to differentiate and activate muscle groups, making them highly favored as methods to help regain function post SCI.

Unfortunately, these methods of FES are also highly fatigable. Intramuscular and peripheral nerve stimulation depolarizes cell membranes of motor neuron axons in the nervous system to evoke muscle contraction. Due to the properties of action potentials and of motor neuron axon size, intramuscular and peripheral nerve stimulation fire the large and fatigable muscle fibers before small fatigue-resistant fibers, which produces a reverse order of motor recruitment that is against Henneman's size principle (Crago 1980). Larger-diameter axons that innervate larger motor units require less stimulation to propagate an action potential compared to smaller diameter axons due to the increase in space between the nodes of Raniver, which causes

an amplified transmembrane voltage increasing the spread of depolarization down the axon (Peckham 2005). The reverse motor recruitment pattern causes targeted large anaerobic muscles, responsible for quick powerful movements, to fatigue very rapidly due to constant stimulation. The result of this stimulation parameter is a rapid fatigue of mainly type II muscle fibers due to the depletion of creatine phosphate and glycogen storage reserves of which are the primarily molecules used for energy (Peckham 2005). These FES methods not only fatigue the muscles through reverse motor recruitment, but they also require higher levels of stimulation to achieve locomotion. The muscles and peripheral nerve axons that are fired through intramuscular and peripheral nerve stimulation means are much larger in size than other neural axons and therefore require larger stimulus parameters in order to incite similar contraction strength (Popovic 1999).

Intramuscular stimulation and peripheral nerve stimulation are two different FES systems that are currently in clinical use. Typical intramuscular systems consist of a large 10 or 8 channel stimulator with a number of electrodes attached. The types vary for internal to external stimulators with either surface electrodes or percutaneous (implanted) electrodes in the muscle. Intramuscular stimulation induces muscle contraction through extracellular electrical currents that cause depolarization in intact lower motor units (Bhadra 2001). The mechanism of muscle initiation based on electrode placement is extremely selective and allows for the ability to discrimination between muscle group and cause contraction of which ever groups are necessary for a given movement. These properties in addition to minimal infection rates and a small fracture rate make its use very practical (Knutson 2002). Until recently single function restoration was the primary focus of intramuscular stimulation. Recently the emergence of graded and logical hand grasp NPs have shown great promise in upper limb rehabilitation. NPs developed by Peckman et al. involves the use of these two stimulation parameters, graded and

logical, to provide a wide range of hand grasp actions, such as opening and closing of the hand (graded) and initiation of set functions for hand grasp configurations (logical) (Peckham 2002). The emergences of systems such as these are recent but have proven to be successful in limb movement.

The use of intramuscular stimulation has, however, its limitations. Early fatigue onset, reverse motor unit recruitment, and size limitations have all led to continued research on intramuscular stimulation or the need for innovative solutions for progress in SCI NPs (Troyk 2001). Due to the mechanism of stimulation by means contraction activation through the use of extracellular electrical currents, muscle fibers become fatigued more rapidly as cellular charge builds up, coupled with reverse motor recruitment the result leads to impractical use of intramuscular stimulus in situations of elongated duration. Lastly, current systems are extremely bulky and hard to don and doff these systems, requiring the continuation of research to achieve a practical intramuscular stimulation system (Popovic 1999). These limitations greatly reduce the ability for chronic use in patients.

Peripheral nerve cuff stimulation is another FES system that is currently in clinical practice for chronic use (Grill 1998). Peripheral nerve stimulation involves electrical current stimulus to depolarize nerve axons of motor unit pathways and thus cause muscle contraction. Much like intramuscular stimulation, peripheral nerve stimulation requires a large multichannel stimulator and penetrating electrodes, spiral electrodes that wrap around the nerve or flat interface nerve electrodes that cover the nerve and snap shut (Schiefer 2013). Due to this direct contact with the nerves, peripheral nerve stimulation is highly selective in muscle recruitment. Peripheral nerve stimulation has been successful in the application of rehabilitation for movements such as standing or walking with the stimulation of the femoral nerve through its

innervation of the primary hip flexors (Fisher 2008). Stimulation of the pudendal nerve has shown to help regain bladder function as well (Yoo 2008). The combination of selectivity and low electrode fracture rates as well as minimal invasiveness makes peripheral nerve a preferred NP for chronic long term use. However, again more research is required to advance peripheral nerve stimulation to more practical means.

Peripheral nerve stimulation suffers from many of the same short comings as intramuscular stimulation such as early fatigue onset and reverse motor recruitment. The stimulation of peripheral nerve motor neuron axons fire the largest axons first, due to the inverse relationship between stimulus amplitude and axon diameter, which fires the largest and most fatigable motor units and ends up leading to fatigue more rapidly. The practical use of peripheral nerve stimulation also requires a large stimulator and number of electrodes in order to invoke limb movement. These limitations are small in regard to the current uses of peripheral nerve stimulation, but must be overcome if fatigue-resistant movements are to become a reality.

Epidural stimulation stimulates interneurons in the spinal cord by means of a cylindrical surface electrode embedded in the sub-dural space in the vertebral column. Epidural stimulation relies on the depolarization of afferent neurons in the spinal cord to awaken sensory pathways that then activate the spinal motor units to cause muscle contraction. This is accomplished via sub-threshold or supra-threshold stimulation. Sub-threshold stimulation parameters are beneath the amplitude required for motor activation and, as such, establish a physiological state that enhances spinal circuitries excitability and allows for sensory-mediated movement (Gerasimenko 2003). Supra-threshold stimulation produces amplitudes above that required for direct motor activation to invoke muscle contraction and limb movement (Ichiyama 2008). Sub-threshold stimulation is a well-established technique for locomotor training in individuals with incomplete

SCI to recovery stepping (Dietz and Harkema 2004). The epidural stimulation allows for the activation of the sensory pathways responsible for movements such as walking. When exposed to a movement such as standing, epidural stimulation primes afferent neurons in the sensory pathway to travel up the spinal cord. Efferent pathways are then activated as a result and desired movements are then achieved. The incomplete injury allows increases the probability of the sensory signal reaching the brain and eliciting walking via the spinal cord.

Locomotor training is a well-established method for the recovery of stepping in individuals with incomplete SCI. The sensory information carried by afferent neurons helps to facilitate the formation of the proper neural circuitries needed to walk (Ahn 2006). Research has shown that the two important factors in locomotor training are the load receptor input, which are the proprioceptive and mechanoreceptor inputs from the lower leg to the spinal cord, and the hip joint afferents, which are the same but in the location of the hip joints. These two afferent pathways are responsible for the activation of the swing phase (hip) and the continuation of ongoing activity (load receptor) (Abraham 1985, Grillner 1978). There are a verity of factors that determine the success of locomotor training, such as age, time from injury, severity of injury, and a number of other factors. Due to the nature of locomotor training where improper weight bearing or stimulation can lead to success or disaster, the ability to gauge effectiveness of particular training regiments are unreliable and inconsistent (Fong 2009). These flaws lead to the need for a controlled and consistent robotic unit to assist with locomotion, but progress is still in the works (Fong 2009). Most NPs are able to use these neural networks due to the plasticity of the spinal cord and would be useless without these important properties. However, there are some that do not rely upon the spinal cord at all to accomplish limb movement.

Supra-threshold epidural stimulation, although, not as well established as intramuscular and peripheral nerve stimulation for locomotion, has successfully achieved full weight- bearing standing in a 23 year old man with complete paraplegia (Harkema 2011). The supra-threshold stimulus amplifies the sensory signal large enough that the desired motion of standing is activated through reflex pathways which propagate the desired action. The primary limitation of epidural stimulation focuses on the lack of selectivity to perform complex limb movement due to electrical spread and activation of agonist and antagonist muscle groups.

Traditionally used for the treatment of chronic pain, epidural stimulation has recently come to the forefront of FES system research, as demonstrated by the successful full weight bearing standing in a patient with complete paraplegia and the voluntary muscle contraction of the toes in a patient with complete motor function loss (Harkema 2011 & Angeli 2014). Epidural stimulation achieves muscle contraction from the activation of the neuromuscular pathways in the spinal cord. This is accomplished through the use of a surgically implanted electrode that lies on top of the dura mater of the spinal cord. As stated previously, the use of epidural stimulation in regards to locomotion utilizes two different types of stimulation parameters; sub threshold (10-200 μA) and supra threshold (20-300 μA) (Fong 2009). Sub threshold stimulation has been used to prime the neural networks in order that sensory triggered movements may be facilitated. A more natural motor unit recruitment pattern is facilitated because the afferent neurons cause depolarization of the motor pathways and produce movement (Musienko 2007). Supra threshold stimuli depolarize, through electrical spread, which is the event of electrical current flooding into surrounding tissue, the motor pools of the ventral horn of the gray matter to evoke limb movement (Fong 2009). Both of these methods have been successful in producing walking and standing, as well as improving neural plasticity in a feline and rat models.

Epidural stimulation is limited in practical usage as compared to other FES systems. First, due to the location of the electrode, epidural stimulation lacks the sensitivity to coordinate specific movements compared to intramuscular and peripheral nerve stimulation, although due to afferent and efferent pathway movements are coordinated in a more natural fashion. Electrode location also enhances the stimulation spill over, or essentially electrical spread, as compared with other FES systems due to the large number of neural pathways between the subdural space and the ventral motor horns and can produce unwanted movements due to activation of undesired neuromuscular pathways. Lastly epidural stimulation requires high stimulation parameters as compared to ISMS and therefore warrants caution. However, it should be noted that the natural motor recruitment pattern facilitated through epidural stimulation holds promise for chronic fatigue resistant movement.

ISMS targets the interneuron effector pathways between the brain and muscles to enact muscle contraction. However, ISMS differs in this regard from other FES methods as electrodes penetrate into the ventral horn of the gray matter in comparison to an epidural electrode that lies on top of the spinal cord dura mater. Research has suggested that ISMS triggers the activation of motor units in accordance with Henneman's size principle. Henneman's size principle states that recruitment of motor units follows an increasing fashion of smallest to largest as proportionate to the load (Henneman 1965). It is proposed that ISMS provides a more fatigue resistant method of locomotion by tapping into CPGs and hence firing motor neurons in a more natural fashion in a feline model (Mushahwar 2000). In addition, ISMS also uses lower levels of stimulation to achieve limb movement (Grahn 2014). As ISMS directly stimulates the motor pools of the ventral horn of the effector neurons, the stimulation distance is decreased significantly and

allows for stimulation parameters of micro amps to reach threshold level; much lower than any other FES system.

Although not in clinical use, ISMS holds promise for a solution to limitations of current NP devices. ISMS works much like epidural stimulation, as electrical current from an external stimulator through penetrating electrode that depolarize the motor unit in the ventral horn of the gray matter. The method of motor unit stimulation mimics the body's natural muscle recruitment from smallest motor unit to largest, according to Henneman's size principle (Mushahwar 2000). This activation mechanism thus provides better resistance to muscle fatigue than methods such as intramuscular or peripheral nerve stimulation (Bamford 2005). It is well-established that the stimulation of the ventral horn activates feline CPGs which allow for increased muscle fatigue resistance (Mushahwar 2000). ISMS has also shown the capability to perform single joint to whole limb synergistic movements through stimulation from one electrode (Mushahwar and Horch 2000).

Apart from the stimulation physiological advantages, ISMS also has a number of other advantages compared to other FES systems. The distance from contractile muscles and the protection of the spinal column provides a relatively stable and mechanically sound environment for electrode implantation for ISMS (Prochazka 2001). Also, due to the mechanism of stimulation, ISMS stimulation amplitudes are smaller (.04 to .12 mA) compared to peripheral nerve (0.1 to 5 mA) and intramuscular stimulation (20mA) to evoke relatively the same muscle contraction response due to the direct perforation of the neural tissue with the electrode. Lastly, ISMS requires fewer electrodes to cause muscle contraction and limb movement as other FES systems as its ability to tap into CPGs allows for a more controlled and coordinated muscle response because it follows the body's natural recruitment pattern, which allows for the use of

one electrode to initiate walking as compared to more for other methods due to the inability to fire more than one muscle.

ISMS does however have limitations that do not allow it to be practical for clinical use at this time. Due to the lack of consistent electrode delivery systems, ISMS can have poor selectivity in regards to electrode placement and as such can result in unwanted muscle contraction. ISMS electrodes are implanted by hand due to the lack of a stereotactic frame and therefore have a higher tendency to miss intended location by lateral movement and depth. Also the general lack of spinal cord mapping makes targeting precise neuromuscular pathways extremely hard. ISMS also involves invasive surgery in order to emplace penetrating electrodes in the ventral horn. Lastly, due to insufficient wireless electrical stimulators, ISMS is impractical for use outside of lab setting. However, it should be noted that limb movement has been achieved by ISMS from a distance of 10m through wireless transmitter which although still limits ISMS usage is a key step towards translation out of lab settings (Grahn, 2014).

Besides the methods mentioned in this literature review, there are a number of other methods in preliminary research that propose solutions to the limitations posed by these four FES systems. Optogentics is a new technique that utilizes the use of viral gene delivery to express Channelrhoposin-2 in cells of a given target area, which are then activated upon photo stimulation (Boyden 2005). Cell populations can then be turn on or off by means of light exposure through optical fibers in the targeted cell population. The use of closed loop optogentic control of the thalamus has proved to be useful in the disruption of seizures after cortical injury (Paz 2013). Optogentics has also been used to restore respiratory breathing via photo stimulation of targeted phrenic nerve cells (Alilian 2008). Research has been shown that initiation of locomotor-like activity has been produced through light activation of glutamatergic neurons in

the hindbrain and spinal cord (Hagglund 2009). Optogentic research shows great promise for a number of research areas including SCI, however due to the novelty it will take time to tell if it can bring new light to this problem.

Methods

Subjects

Studies were conducted *in vivo* in eight adult female Sprague-Dawley rats weighing 225-290 grams. Five out of these eight were used in this study; two were used as a SCI model, one for an intramuscular model, and two were used which had healthy intact spinal cord, three died due to analgesic and anesthetic complications. Rats were housed individually in standard conditions on 12-hour light/dark cycles with *ad libitum* access to water and food. All procedures were conducted in accordance with National Institutes of Health guidelines and approved by the Mayo Clinic Institutional Animal Care and Use Committee. A rodent SCI model was selected for this proof-of-principle study due to the retention of functional properties required for limb control and as an inexpensive alternative to feline and larger animal models.

Rodent model of Spinal Cord Injury

Two animals were used to as chronic SCI models. Animals were anesthetized with ketamine (80 mg/kg) and xylazine (5 mg/kg) and placed on a heating pad to maintain core temperature. The surgical procedure was as follows: The fourth thoracic vertebra (T4) was identified by means of counting down the spinous process from the anatomical landmark of the second thoracic vertebra found in between the shoulder blades. Upon identification of T4 a bilateral laminectomy was performed to reach the sub-dural cavity in order to expose the spinal cord. A complete transection of the spinal cord was then performed upon at the T4 level.

Following the verification of complete transection, the incision was closed by intramuscular and skin sutures, at which point the animal rested for seven day in order to allow time to recover from spinal shock. To prevent infection and ensure proper recovery, intraperitoneal (I.P.0 injections of buprenorphine (5µg/kg, twice per day) and intramuscular (I.M.) Baytril^{TM} (5 mg/kg, twice per day) were administered. Urinary bladders were expressed three times per day until reflex voiding was reached. To maintain joint flexibility and decrease spasticity, passive flexion and extension of the hind limbs were performed. Additionally a nociceptive paw pinch test was conducted to ensure complete SCI.

Intraspinal Microstimulation

Upon the completion of the seven day recovery period for two animals or day one for the two healthy controls, the animals were prepped and anesthetized. A bilateral laminectomy and durotomy were then performed to expose the lumbar spinal cord at the first through third lumbar (L1-L3) levels. The spine and hip were then stabilized with jaw clamp, hip pins, and a tail clamp, to ensure that the body was stable while allowing free movement of the limbs (Figure 1). Teflon-insulated Tungsten stimulating microelectrodes (127µm in diameter with 30-60µm exposed tip) were manually inserted approximately 1.8mm deep into the ventral horn of the gray matter of the spinal cord (Watson 2008).Electrode was inserted approximately 1mm lateral from midline beginning at the rostral level of the L1 vertebra with a reference electrode inserted into the lateral abdominal muscles. The electrode was removed and reinserted in 1mm increments caudally towards the third lumbar vertebra (L3) if desired hind limb movement was not achieved. Stimulation amplitude was linearly increased to maximum, 10 µA to 100 µA, at each electrode location while pulse width and frequency were sustained was 0.2 ms and 25 Hz, respectively, until desired extension of the hind limb was achieved through stimulation. Upon achievement of

desired extension, cyanoacrylate adhesive was used to fix the microelectrode to the spinal cord. Fatigue studies were conducted at sustained amplitudes of 70 μA, with a pulse width of 0.2 ms and frequency of 25 Hz for a total stimulation time of 30 seconds. A 30 second rest period was enacted after each trial for stimulation amplitudes and fatigue studies to ensure animal returned to normal. All stimulation studies were acute and lasted no longer than eight hours in accordance with the Institutional Animal Care and Use Committee protocol.

Intramuscular stimulation

One day after arrival of animals, the non- SCI animals were prepped and anesthetized. Non SCI animals were used for convenience as literature has shown intact and SCI animals evoke the same amplitude of response (Grahn 2014). At which point the animals were stabilized in same apparatus as the ISMS subjects, with the same hind limb freedom of movement. At this point, a stimulation needle was inserted into the median of the hamstring muscles, particularly the biceps femoris and semitendinosus muscles to invoke hind limb extension. When desired movement was achieved, electrode was fixed with a cyanoacrylate adhesive. Stimulation amplitudes were then linearly increased to maximum, from 300 μA to 1000 μA, at a constant pulse width of 0.2 ms and frequency of 50 Hz. Fatigue studies were conducted at sustained amplitudes of 700 μA, with a pulse width of 0.2 ms and frequency of 50 Hz for total stimulation time of 30 seconds. For each stimulation amplitude and fatigue trial, a 30 second recovery period was enacted to allow for conditions to return to normal.

Kinematic analysis

Opaque markers were placed on the hip beginning on the iliac crest, knee, and ankle joints, as well as the fifth metatarsal so that limb movement could be monitored with motion

analysis (Figure 2). The animals were placed on a mounted spinal unit (Kopf Instruments, Tujunga, CA) and a parasagittal view, at 50 frames per second using one 2048x1088 pixels ace GigE cameras (Basler, Ahrensburg, Germany), was used to capture limb movement. Templo 2D motion analysis system (Contemplas, Kempten, Germany) was used to analyze kinematic response to stimulation. Data was analyzed using Microsoft excel. Angle change was calculated by subtracting angle of limb during stimulation from baseline angle prior to start of stimulation.

Results

Stimulus Strength and Graded Response

Graded response of linearly increasing stimulation amplitude of ISMS exhibited proportional increased extension movement. The results observed a wide range of angle changes, depending on stimulus strength, of the hip and knee joints (Figure 3&4) in 100% of healthy intact models and 100% in SCI models. Strength of muscle contraction was measured as angle change from baseline. Although other methods are preferred, such as EMG, these methods were not available for analysis. EMG methods for data collection were conducted, however acquisition and subsequent analysis of data was unable to be performed. Angle change was used as an increase in muscle contraction, as measured through EMG, has been shown in the literature to be coupled with a proportional increase in the angle from baseline of the subject and the opposite holds true, a decrease in muscle contraction results in a decrease of angle change from baseline (Mushahwar 2000, Mushahwar 2002). The literature has also shown intact and SCI rats evoke similar amplitudes of muscle contraction during locomotion in ISMS, and therefore SCI model rats and intact ISMS fatigue and stimulus strength was combined (Grahn 2014).

Graded response for ISMS was conducted in 0.2ms stimulation periods with a linear increase in stimulation amplitudes between trials (10μA to 100μA). Peak angle change occurred at 90-100 μA for both hip and knee joints. Threshold amplitude to evoke movement was found to be at 40 μA for both joint angles. The hip joint evoked more than double the angle change of the knee joint for ISMS (50^{o}±9.5, 17^{o}± 6.5, respectively). The magnitude of angle changes, and therefore indirectly muscle contraction, was directly correlated with stimulation amplitudes as shown by Pearson's Correlation coefficients (R^2) of (0.96 and 0.97 for the hip and knee respectively $p < 0.05$) for both joints. The results show a positive proportional increase of angle change a result of increased stimulus strength.

The intramuscular stimulation is to be viewed as a case study due to the lack of subjects (n=1) and number of trails per subject (n=2). Intramuscular stimulation displayed a wide range of angle changes from baseline from graded responses due to linear increasing stimulation amplitudes (Figure 5&6) of the hip and knee joints in the one intramuscular rodent. Graded response of intramuscular stimulation was conducted in 0.2ms stimulation periods with a linear increase of stimulation amplitudes (300 μA to 1000 μA) between trials. Stimulation amplitudes were then increased linearly Threshold stimulation amplitude required to evoke movement was found at 225 μA for both hip and knee joints. Threshold amplitude was not included in figures 5&6 as only one trial was used to establish a threshold for each joint. The maximum angle change was observed 1000 μA for both joints. The hip joint, however, was more varied than the knee joint in regards to linear proportional increase of stimulus amplitude as denoted by the figures. The angle changes, again indirect measures of muscle contraction, were directly correlated with a proportional increase of stimulus amplitude as shown by Pearson's Correlation coefficients (R^2) of (0.7303 and 0.8533 for the hip and knee respectively, $p < 0.05$) for each

joints. The results suggest a positive linear relationship between stimulus strength and angle changed, however less conclusive than ISMS studies.

Fatigue studies

Fatigue studies comparing angle change of ISMS and intramuscular hip and knee joints in response to stimulation over a period of 30 s were measured as percent change from maximum angle to minimum angle during stimulation. The focus of this study was to measure fatigue over time and not the absolute value of the total angle change over time as the stimulation parameters were different between these two different stimulation types. Hip percent change of the angles through all of the trials equaled medians of 8.11^{o} and 2.40^{o} for ISMS and intramuscular stimulation respectively. Figures 7A, 7B, and 9 display a graphical representation of the individual trials and average angle changes, respectively, over time for the hip joint from baseline of the fatigue studies. Variation in the ISMS hip studies was drastically increased as compared to intramuscular stimulation. Both stimulation parameters have similar muscle force contractions as shown by the similarity in angle change. Figures 8A, 8B and 10 show a graphical representation of the individual trials and average angle changes, respectively, over time for the knee joint from baseline of the two stimulation parameters. Knee fatigue percent change of angles throughout all of the trials equaled medians 4.67^{o} and 6.58^{o} for ISMS and intramuscular stimulation respectively.

Discussion

The results have two main implications upon the questions addressed in this study; is there a linearly increase in muscle contraction as a result of an increase in stimulation amplitude of ISMS and one that is comparable to clinically use intramuscular stimulation. The second

being the fatigue resistance of ISMS as compared to intramuscular stimulation. The objective of both is to compare the efficiency of ISMS as compared to a clinically used FES system intramuscular stimulation in regards to the range of muscle contraction and fatigue resistance, which for this study was measured using angle change from baseline.

Graded response stimulation was shown to be consistent and repeatable as well show a positive linear relationship between stimulation amplitude and angle change, which is an indirect indication of muscle contraction force, in trials as shown by the R^2 values for the hip and knee joints (0.96 and 0.97). The range of angle change consisted of threshold to a maximum change of $50^o \pm 9.5^o$ for the hip joint, which was the high observed value in both ISMS and intramuscular stimulation studies conducted. Knee joint angle change range was considerably smaller at $17^o \pm 6.5^o$. The differences in magnitude between the two joints could be explain through means of the site of the electrode in the spinal cord in regards to vertebral level or gray matter level or the activation of agonist and antagonist muscles based on motor units that are activated by stimulation. If the electrode activated hip flexor muscle pathways as well as activation of hamstring and quadriceps muscles then we would observe such a trend as this where the knee angle change is significantly lower due to competition between agonist and antagonist muscle groups, but an overall increase in hip angle change as no competition exist due to sole hip extensor activation. The overall variance as observed with standard deviation, of the hip and knee angle changes is substantial but appears consistent at each stimulation parameter suggesting equal proportional increase between trials but a difference in overall angle change. The most likely explanation for the variance stems from the lack of selectivity of muscles activated by the ISMS electrode and as a result would lead to a difference in absolute angle change without affecting the slope.

In the intramuscular case study, the overall hip angle change reaches a maximum angle change of $26.8^{o}\pm0.5^{o}$ and displays a linear positive relationship in stimulus amplitude and muscle contraction force as shown by the Pearson's coefficient correlation (0.7303) with a considerable amount more variability between stimulation amplitudes; although it must be noted that this conclusion is weakly supported due to a low coefficient correlation and the lack of subjects. Knee angle changes in comparison surpass that of the ISMS knee joint changes with a maximum of $31.9^{o}\pm0.45^{\cdot}$ The knee joint for intramuscular stimulation showed a strong positive linear relationship in muscle contraction force as a result of stimulus amplitude as shown by the Pearson's correlation coefficient (0.8533) which although is stronger than hip angle. However, again it must be noted the lack of subjects and therefore the weak support of this conclusion due to the small sample size. The analysis of these results is not surprising. The high selectivity of intramuscular stimulation would explain the higher maximal knee joint angle change as stimulation directly activates the hamstring muscle group. This explanation would also account for the difference in hip angle change as well since intramuscular stimulation would primary stimulation the hamstrings and not true hip extensor muscles.

Due to low sample size, statistical analysis was not able to be achieved in the fatigue studies and as a result more qualitative measures were used. In the analysis of the average fatigue studies (figures 9 &10) the fatigue resistance, as measured by percent change of maximum angle appears to be fairly consistent between the two stimulation parameters as the slope of the lines for ISMS and intramuscular stimulation indicate. Examination upon the individual trails of each stimulation type of ISMS and Intramuscular help to shed some light on the explanation of this conclusion. Figures 7A and 8A show the extreme variability of ISMS not only between subjects but also within subjects. Rat 2 (figure 7A) shows the potential for great targeting and fatigue

resistibility of ISMS, however other trials such as rat 3 show a large degree of variability from the previous subject and within the subject. Hip angle change of intramuscular stimulation in the fatigue studies (figure 7B) was also shown to have less variation as compared to ISMS, but there is still a large degree of variation between the subjects. The analysis of knee angle changes over time from baseline (Figure 8A) shows a large variability between trials and subjects in ISMS, more so than the hip angle changes. The mechanism of CPG activation of ISMS helps to explain this phenomenon as the different electrode locations of the subject could have fired different motor units that lead to extension, but produced different knee angles based upon the muscles that were contracted. Knee angle change during stimulation for the different trials of intramuscular stimulation (figure 8B) was shown to have the lowest variability in this study. This is to be expected however, based upon the well-established literature and the mechanism of intramuscular stimulation.

The data conducted herein suggests qualitatively that the two stimulation parameters are not different in regards to the fatigue resistance. ISMS appears to not be more fatigue resistant then conventional intramuscular stimulation. Variability between different ISMS trials was significantly higher than intramuscular stimulation (figures 7A,7B, 8A, 8B), which is not a surprise as the delivery of ISMS is considerably less selective than intramuscular stimulation and as such might have an impact upon significance and findings of this study.

The equal rate of fatigue between ISMS and intramuscular can help to support ISMS as a potential use for SCI over conventional means such as intramuscular stimulation use as it offers a number of advantages and does not compromise muscle fatigue resistance. Due to activation of muscle through CPGs, limb movements appear more natural in coordination of muscle control and activation of synergistic muscle movements. A visual observation of ISMS shows the leg

move in a more controlled, smoother and coordinated movement as compared to the jerky and single muscle movement of intramuscular stimulation. Also, the location of implantation in the spine reduces potential damage to the tissue caused by mechanical strain as electrodes do not have to travel through joints or are in locations of muscle contraction such as those used in intramuscular stimulation (Prochazka 2001). Also, tissue damage to the spinal cord has also been proven to be minimal (Bamford 2010). With advancements in ISMS electrode delivery systems and improving mapping of spinal level circuitry, ISMS could progress to allow natural and coordinated for individuals with SCI.

The results of this study have concluded that the fatigue resistance of ISMS compared to Intramuscular stimulation is not significant upon observation of the fatigue studies. Although not the expected outcome, this conclusion points to ISMS as an equally capable method of FES as compared to current clinical devices with a number of distinct advantages. The field of ISMS is still relatively new and as such is still limited to the laboratory; however, continued research can help to establish repeatable and consistent studies to allow the translation to clinical use.

Future studies could be improved upon in a number of ways and methods. The next step to continue this research would be to establish EMG in addition to angle change to add strength to the data. Although, angle change can suffice as a measure of muscle fatigue and muscle contraction force indirectly, the combination of EMG data along with angle change would increase the validity of the results. As a measure of muscle electrical activity, EMG represents an ideal measure for fatigue and contraction force by allowing researchers to quantify muscle contraction force in real time. EMG in the combination with visual quantifiable angle change would allow researchers to not only see fatigue but also determine angle changes as a result of force contraction. Despite the obvious nature of this idea, the implications are important as

researchers could tune muscle contraction as a result of manipulating stimulus amplitudes to meet the specifics of complex movements and allow for a greater range of movements and more coordinated movements than the jerky and single dimensional movements of conventional methods such as intramuscular stimulation and peripheral nerve stimulation that we have today. With a range of stimulus parameters, movements could be activation enough muscle force to evoke an 180^{o} angle change to allow a patient to stand up or one that allows for muscle force that mimics that more of walking motions. From the combination of angle change and EMG, the tracking of gait angle changes as a result of muscle contraction could be mapped and used to further fine tune this devices for practical use. Other methods would focus on the consistency and selectivity of ISMS.

Future experiments would include a number of improvements to enhance the implantation and selectivity of these methods. As mentioned the selectivity and accuracy of ISMS are non-ideal compared to other methods in clinical use. To help alleviate the problems associated with a lack of selectivity, there are two main tools that will be needed. First, the development of a stereotactic frame in a rat model of ISMS. This would be a system that is improved to the current apparatus seen in figure 1, but one in which a coordinate system could be used so that an electrode could be implanted on a series of arms to a depth that would be measured and therefore allow for the replication of electrode placement in the exact location based off of the coordinates observed. A stereotactic frame is a system that would allow for improved selectivity of ISMS targets, such as the ventral horns, as coordinates that evoked desired movements could be recorded more precisely and easily repeatable. Stereotactic navigation would also bring the additional bonus of improving current mapping of spinal cord circuitry in to attempt to facilitate translational mapping to humans if possible. The use of a

stereotactic frame would be instrumental in the repeatable implantation of electrodes into the spinal cord.

Another device that would be essential to progress the current research and methods of ISMS is the development of a microelectrode array. A microelectrode array consists of some biologically compatible material to serve as a base from which columns of bundled electrodes protrude out from. The columns of electrodes consist of a number of different electrodes of various lengths to allow for different levels of stimulation, of any tissue such as the spinal cord. The benefits of such a method are tri-fold. Firstly, the implantation of an array as compared to an equal number of electrode insertions greatly decrease the amount of damage upon the implanted tissue as it takes only one perforation of the surface as compared to several. The vast number of electrodes and the various depths in a microelectrode array allow for increased mapping of the spinal cord. The time to implant an electrode and subsequent stimulation to map movement would be drastically decreased as the number of insertions decreases from a number of electrodes to one larger array which would substitute for many. Lastly, the use of a microelectrode array increases the efficiency of multi-electrode movements for synergist movement such as walking, as the single array allows for the coordination of a number of different electrodes that would be stimulate different motor units. The coordination of these different motor units would allow for controlled activation of muscles for each phase of walking. The ability to use one platform to evoke a complex movement as compared to the use of several electrode leads offers a distinct advantage compared to traditional means.

Conclusion

The current FES systems offer a number of different distinct advantages and disadvantages depending on the system. However, progress is still needed to help increase the ADLs of SCI victims. The current FES systems, although effective in limb locomotion, are hampered by the limitations of reverse motor unit recruitment and early onset fatigue. In order achieve a new level of care and treatment in SCI victims these limitations must be overcome or new methods must be utilized to ensure progress continues. One thing is for certain, research must continue in order to hone the current methods of intramuscular stimulation and peripheral nerve stimulation or advocating of new methods such as epidural stimulation or ISMS as new procedures to restore limb function to victims of SCI.

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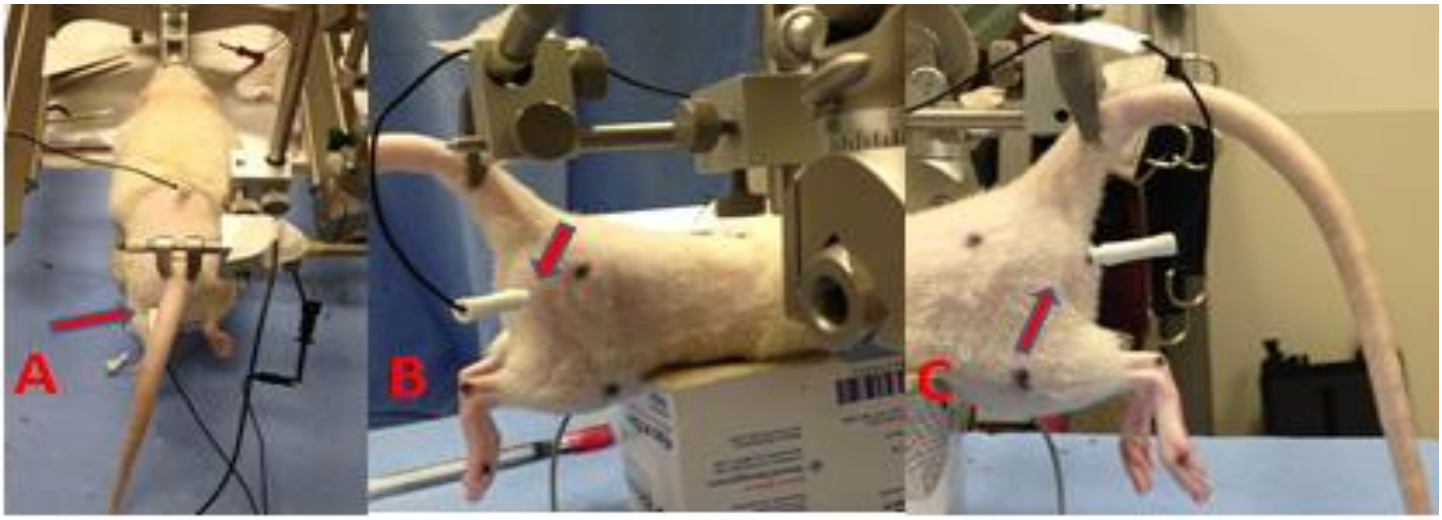

Figure 1. Intramuscular frame apparatus and Electrode placement in the Biceps Femoris muscle of the Hamstring muscle group in rodent model. A) Electrode location 1 Top view B) electrode location 2 Parasagittal view of right leg C) Electrode location 3 Parasagittal view of left leg. Electrode marked by red arrow in each letter.

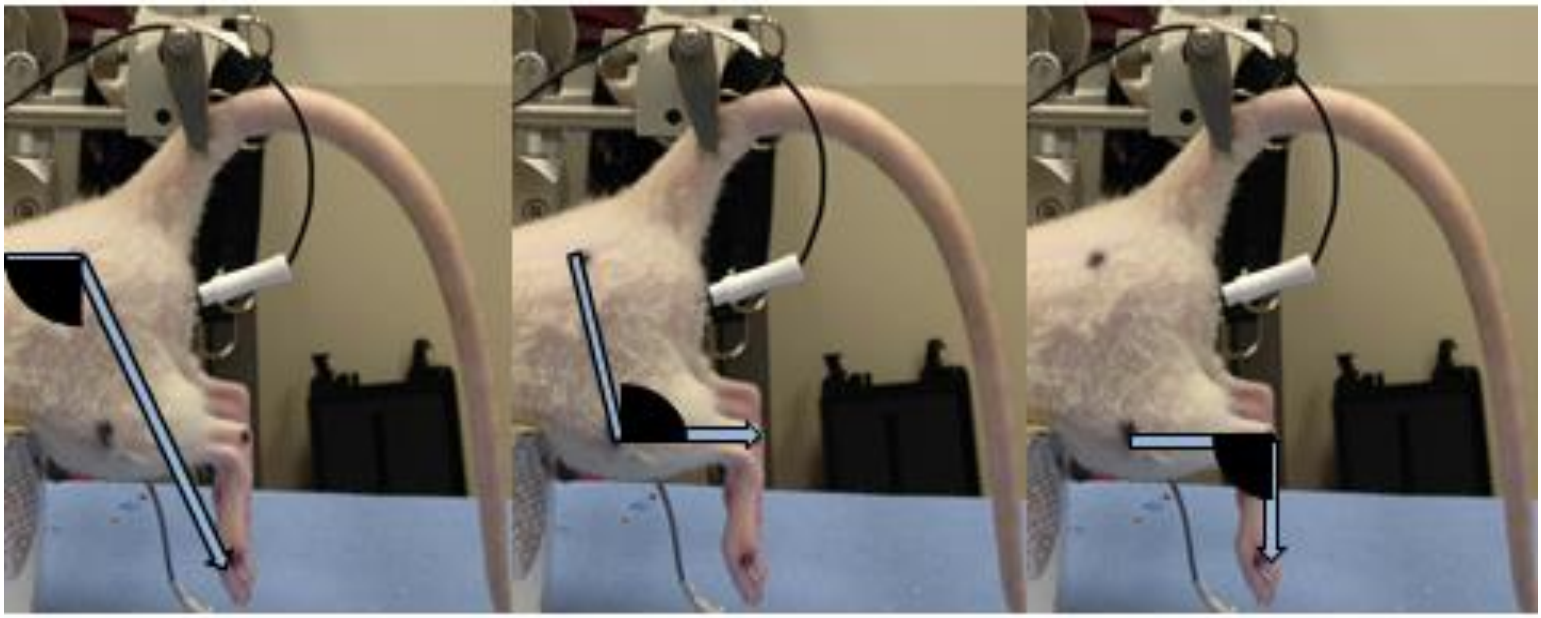

Figure 2. Kinematic angle analysis of hip angle (horizontal line on iliac crest to fifth metatarsal), the knee angle (iliac crest to knee joint to ankle joint), and the ankle joint (knee joint to ankle joint to fifth metatarsal)

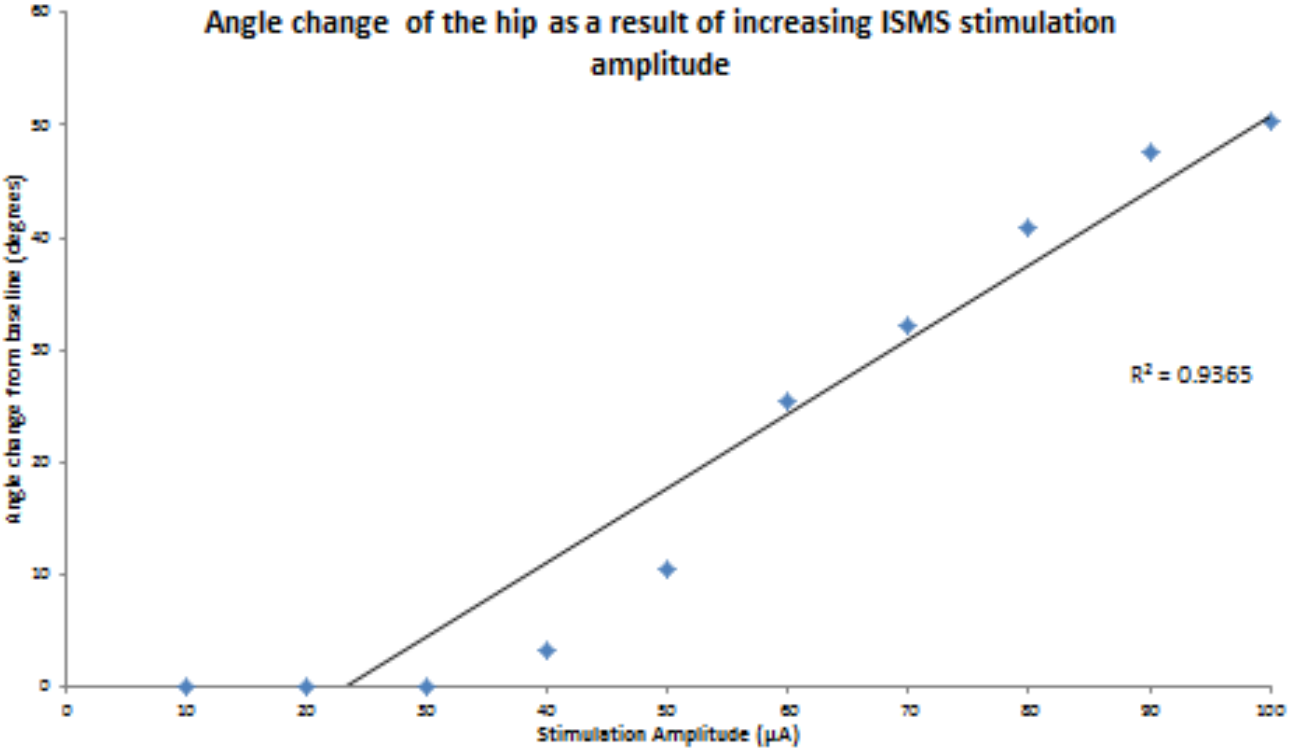

Figure 3. Angle change from baseline of the hip as a result of a linear increase in ISMS stimulation amplitude from 10 (μA) to 100 (μA) for a stimulation period of 0.2 seconds. Graph shows results of stimulation (blue) and linear regression line (black).

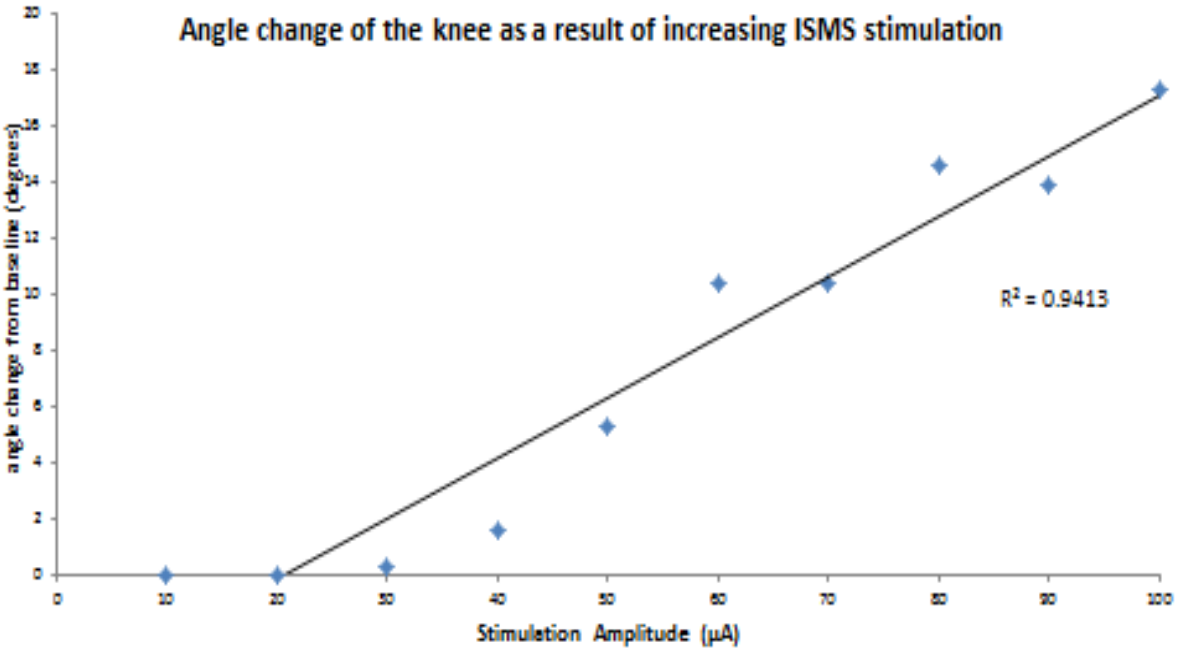

Figure 4. Angle change from baseline of the knee as a result of a linear increase in ISMS stimulation amplitude from 10 (μA) to 100 (μA) for a stimulation period of 0.2 seconds. Graph shows results of stimulation (blue) and linear regression line (black).

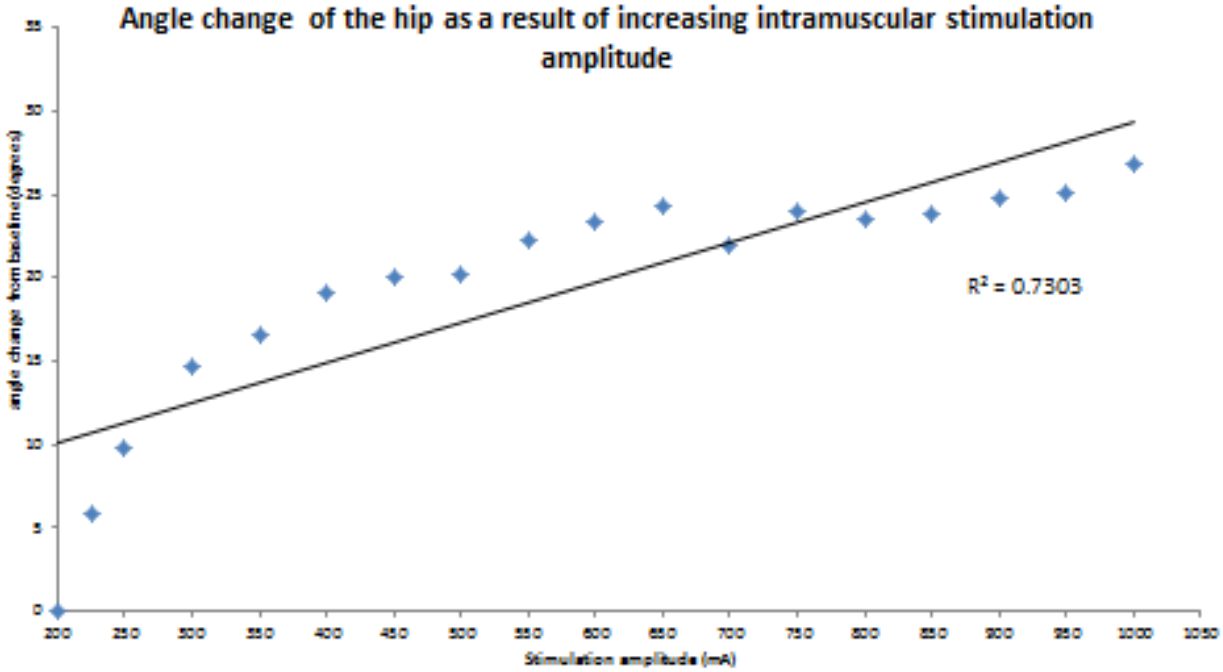

Figure 5. Angle change from baseline of the hip as a result of a linear increase in intramusular stimulation amplitude from 300 (µA) to 1000 (µA) for a stimulation period of 0.2 seconds. Graph shows results of stimulation (blue) and linear regression line (black).

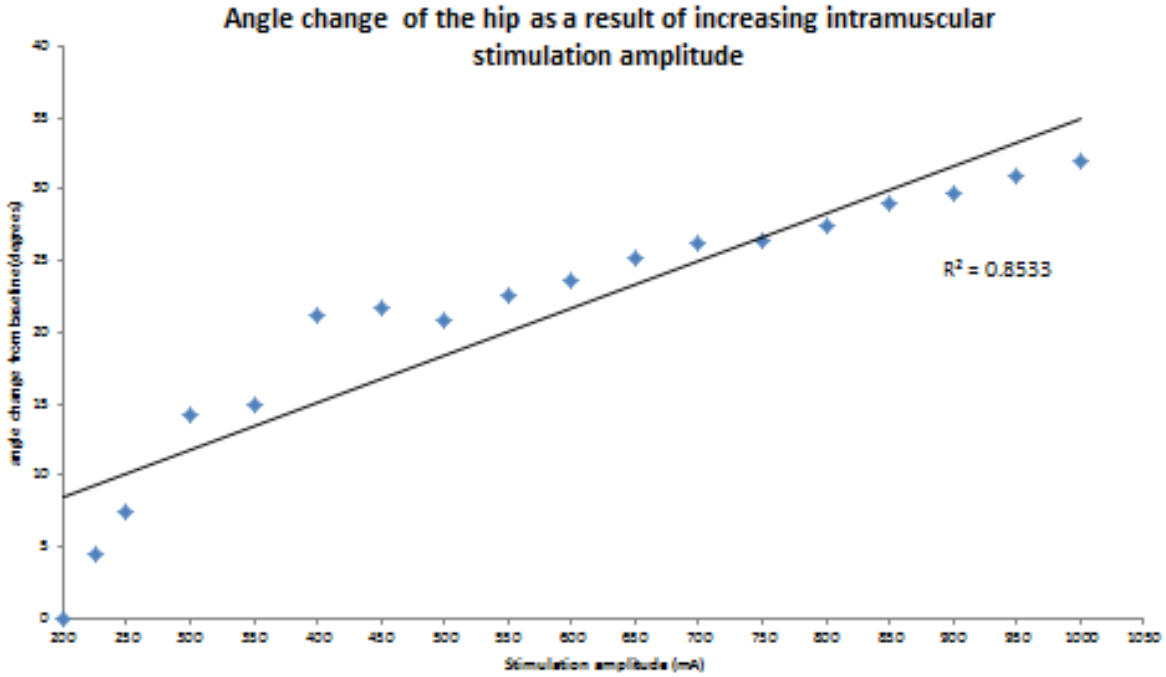

Figure 6. Angle change from baseline of the knee as a result of a linear increase in intramuscular stimulation amplitude from 300 (µA) to 1000 (µA) for a stimulation period of 0.2 seconds. Graph shows results of stimulation (blue) and linear regression line (black).

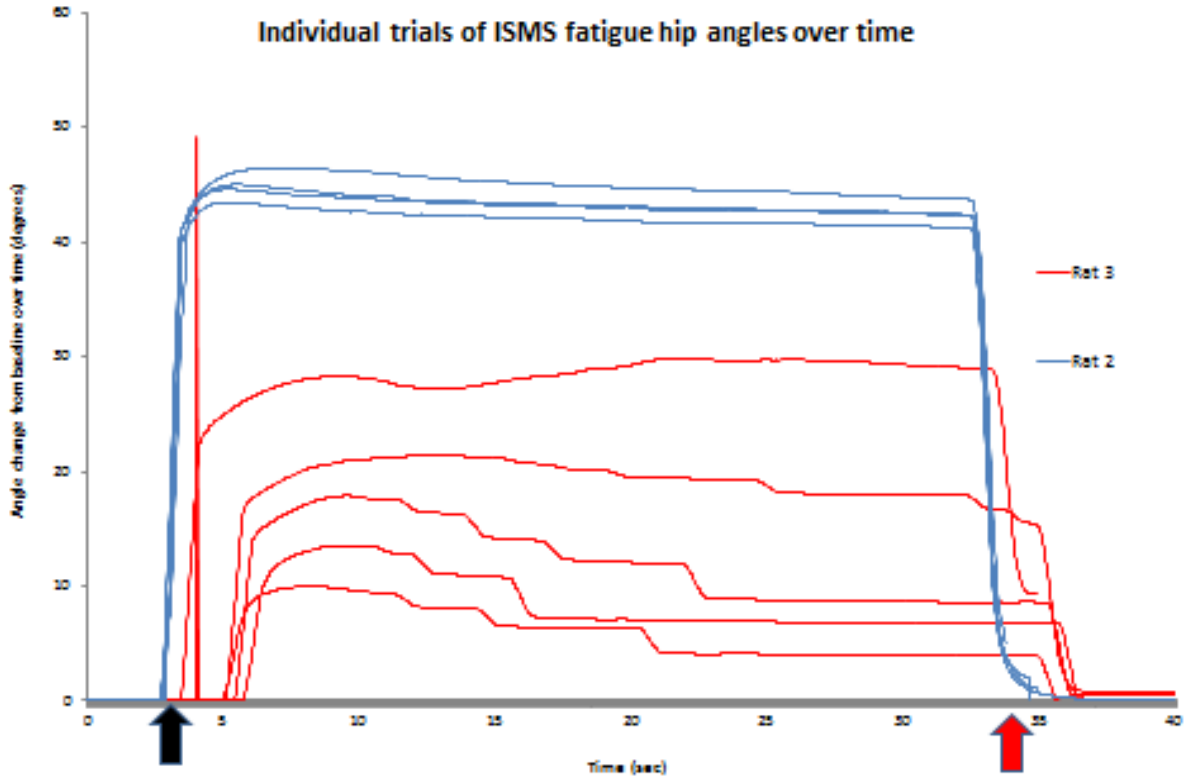

Figure 7A. Individual trials of ISMS (70µA) hip angle changes from baseline over 30 second stimulation duration. Arrows represent beginning (black) and end (red) of stimulations.

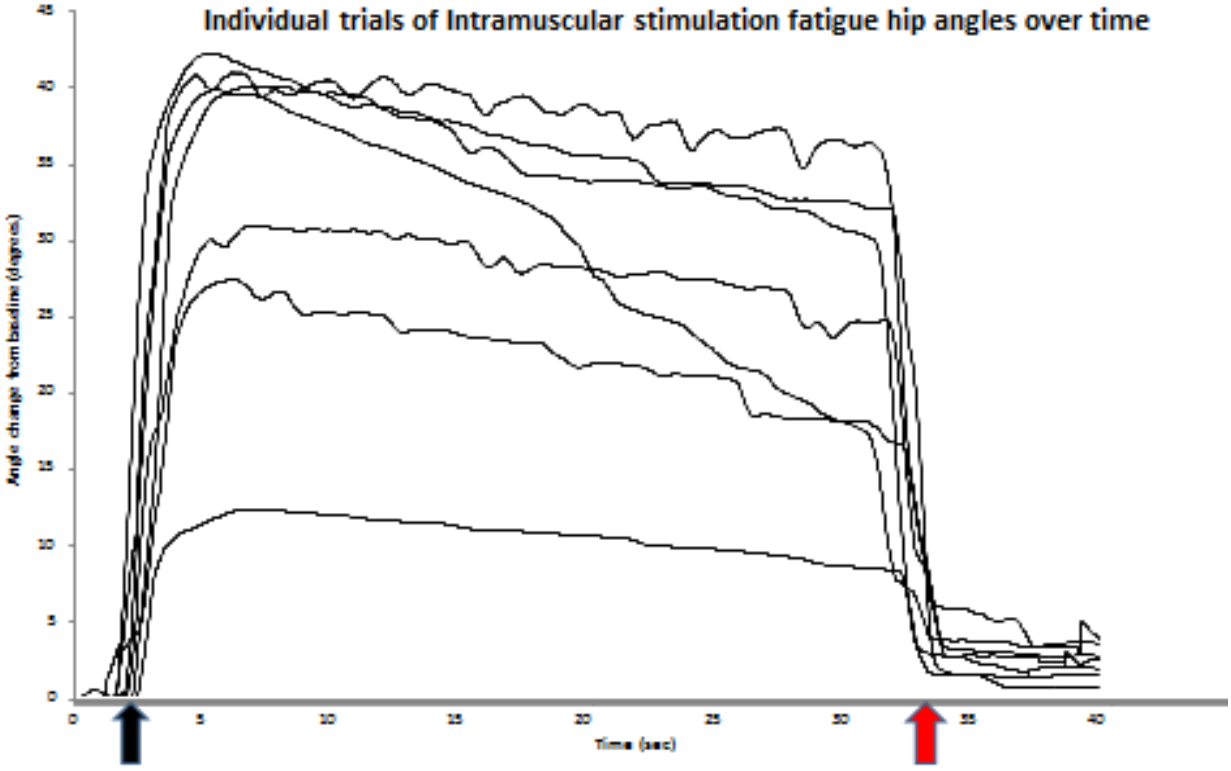

Figure 7B. Individual trials of intramuscular stimulation (700µA) hip angle changes from baseline over 30 second stimulation duration. Arrows represent beginning (black) and end (red) of stimulations.

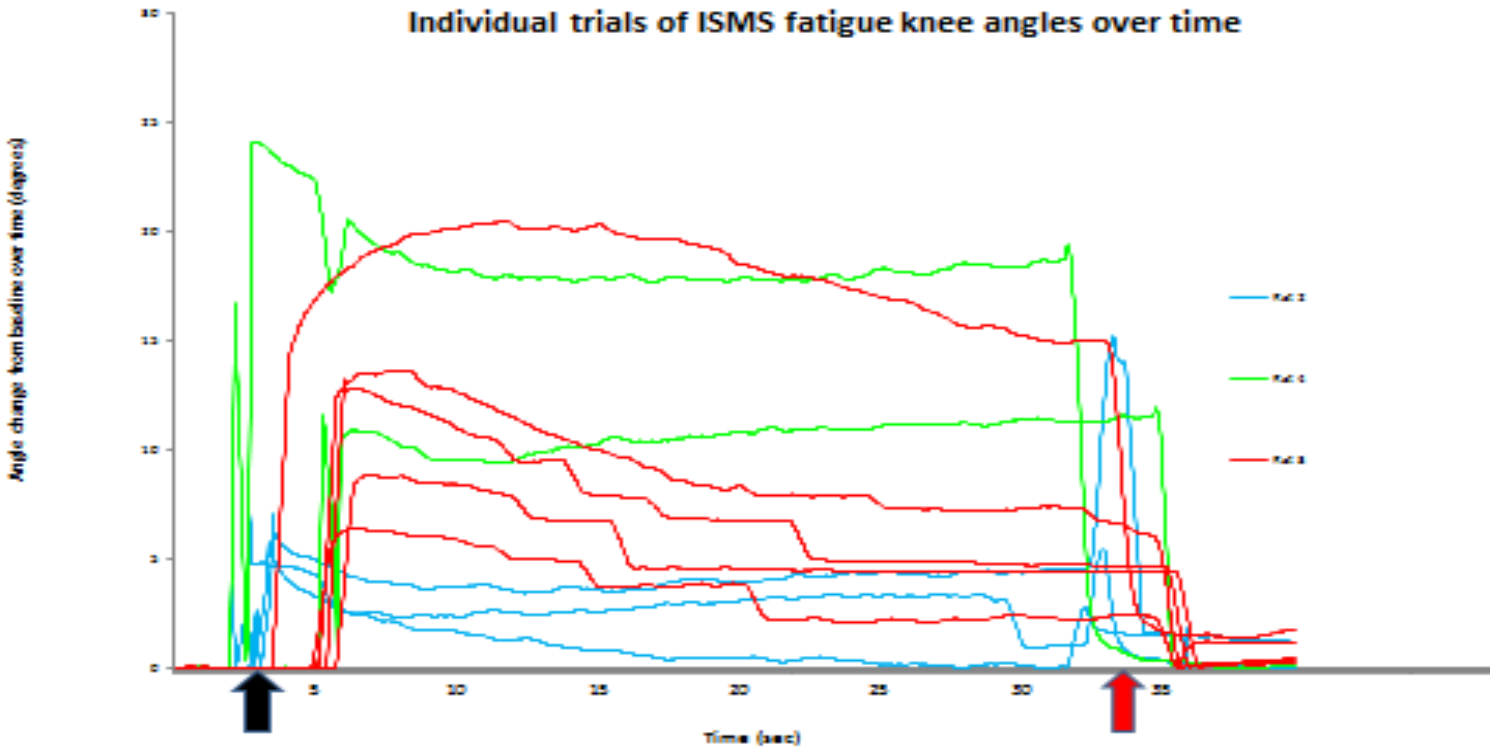

Figure 8A. Individual trials of ISMS (70µA) knee angle changes from baseline over 30 second stimulation duration. Arrows represent beginning (black) and end (red) of stimulations.

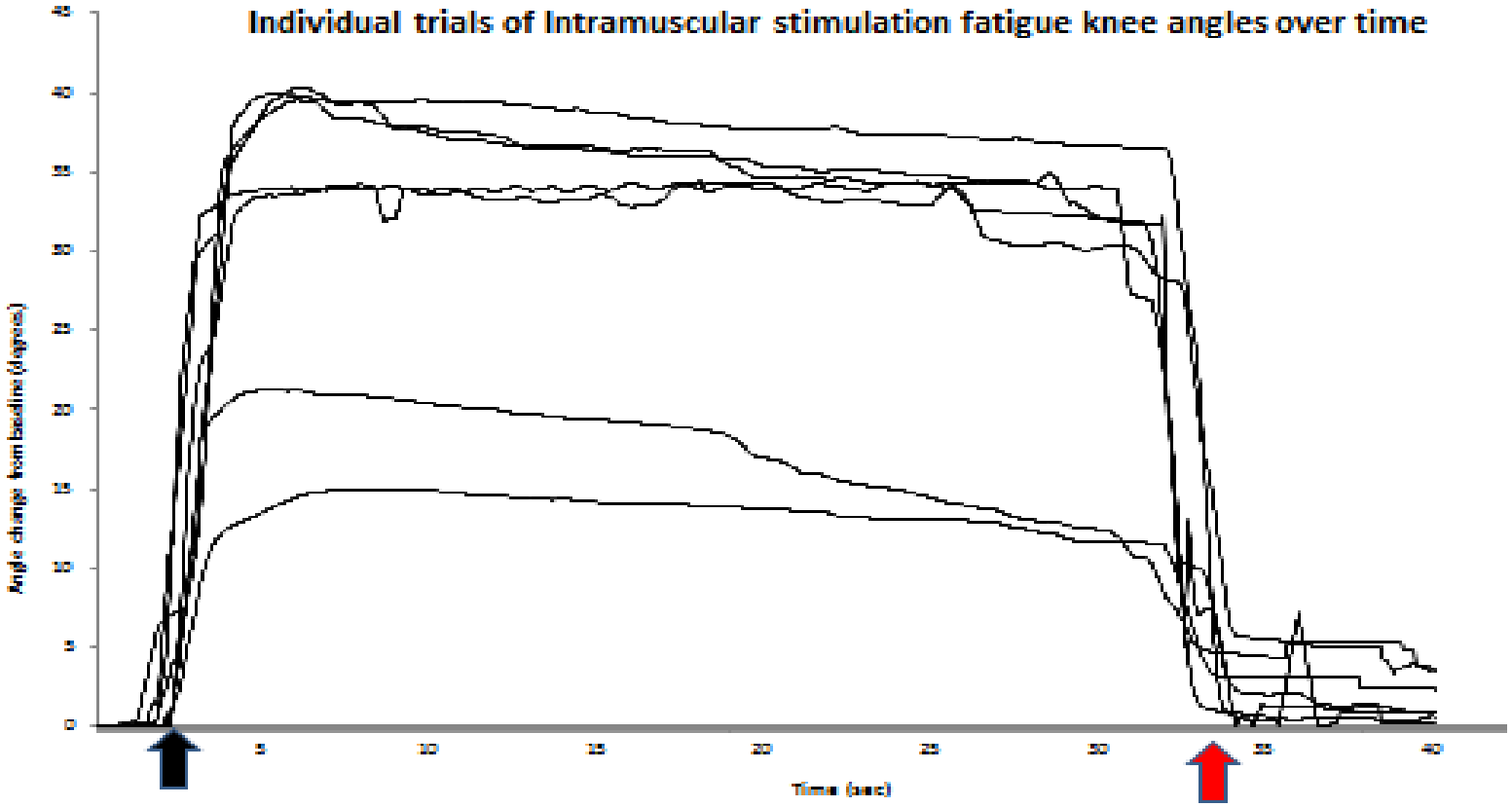

Figure 8B. Individual trials of intramuscular stimulation (700µA) knee angle changes from baseline over 30 second stimulation duration. Arrows represent beginning (black) and end (red) of stimulations.

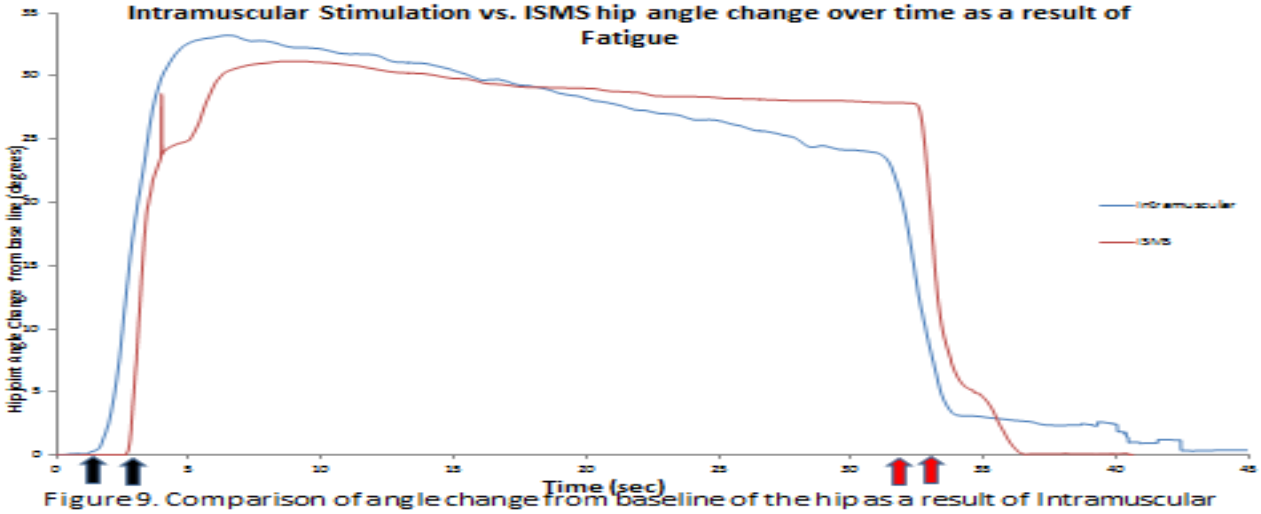

Figure 9. Comparison of angle change from baseline of the hip as a result of Intramuscular stimulation (700µA) and ISMS (70µA) over a stimulation period of 30 seconds. Arrows represent beginning (black) and end (red) of stimulations.

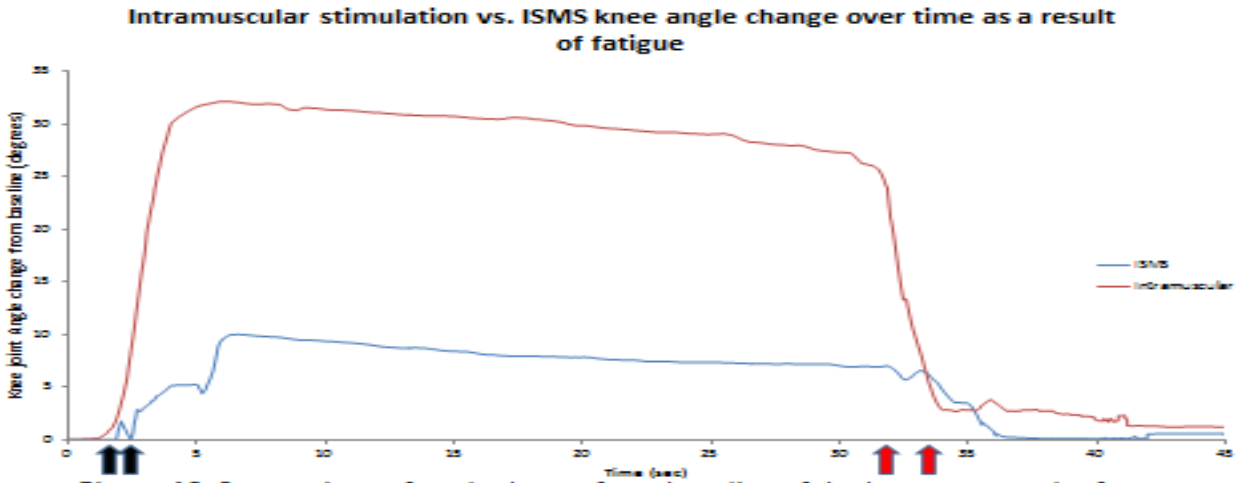

Figure 10. Comparison of angle change from baseline of the knee as a result of Intramuscular stimulation (700µA) and ISMS (70µA) over a stimulation period of 30 seconds. Arrows represent beginning (green) and end (red) of stimulations.

Table 1. List of abbreviations used in this paper

Name of term	Abbreviation
Spinal Cord Injury	SCI
Activities of Daily Living	ADL
Functional Electrical Stimulation	FES
Neuroprostheses	NP
Intraspinal Microstimulation	ISMS
Central Pattern Generators	CPG

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