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CHRONIC NEUROMUSCULAR ELECTRICAL STIMULATION OF PARALYZED HINDLIMBS IN A RODENT MODEL

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Abstract

Neuromuscular electrical stimulation (NMES) can be used to activate paralyzed or paretic muscles to generate functional or therapeutic movements. The goal of this research was to develop a rodent model of NMES-assisted movement therapy after spinal cord injury (SCI) that will enable investigation of mechanisms of NMES-induced plasticity, from the molecular to systems level. Development of the model requires accurate mapping of electrode and muscle stimulation sites, the capability to selectively activate muscles to produce graded contractions of sufficient strength, stable anchoring of the implanted electrode within the muscles and stable performance with functional reliability over several weeks of the therapy window. Custom designed electrodes were implanted chronically in hindlimb muscles of spinal cord transected rats. Mechanical and electrical stability of electrodes and the ability to achieve appropriate muscle recruitment and joint angle excursion were assessed by characterizing the strength duration curves, isometric torque recruitment curves and kinematics of joint angle excursion over 6–8 weeks post implantation. Results indicate that the custom designed electrodes and implantation techniques provided sufficient anchoring and produced stable and reliable recruitment of muscles both in the absence of daily NMES (for 8 weeks) as well as with daily NMES that is initiated 3 weeks post implantation (for 6 weeks). The completed work establishes a rodent model that can be used to investigate mechanisms of neuroplasticity that underlie NMES-based movement therapy after spinal cord injury and to optimize the timing of its delivery.

Keywords

Traumatic spinal cord injury; Rehabilitation; Movement therapy; Neuroprostheses; Neuroplasticity; Outcome measures

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1. Introduction

Several approaches have been utilized to tap into the plasticity of the nervous system to help improve recovery of locomotor function after incomplete spinal cord injury (Barbeau et al., 1999; Bouyer, 2005; Ramer et al., 2005). These approaches include rehabilitative therapies such as body weight supported treadmill training, robotic assist training, and functional electrical stimulation in combination with treadmill training. The mechanisms that underlie these therapeutic approaches are not yet well understood and the procedures for selecting the best time window for administering the therapeutic intervention are yet to be determined.

In neuromuscular electrical stimulation (NMES), low-level electrical current pulse sequences are used to stimulate nerves that innervate specific muscles to obtain functional movement by active contraction of muscles. Movement therapy that utilizes NMES has been used to restore or improve recovery of function after spinal cord injury, head trauma or stroke (Barbeau et al., 2002; Field-Fote, 2001; Sheffler and Chae, 2007). NMES may not only help provide a neuromotor retraining effect, but it can also improve muscle strength and endurance, thereby synergistically improving the chances of successful recovery (Martin et al., 1992; Mohr et al., 1997; Nash et al., 1997). The observed improvements in functional behavior promoted by NMES therapy may be mediated, at least in part, by molecular plasticity in the central nervous system since electrical stimulation of peripheral nerves causes upregulation of regeneration-associated genes, including brain-derived neurotrophic factor (BDNF) (Al-Majed et al., 2004; Geremia et al., 2007; Lynskey et al., 2008). However, the overall mechanisms mediating NMES-induced neural plasticity and recovery remain unclear.

Well characterized rodent models for incomplete spinal cord injury that mimic contusion injury in humans, traumatic brain injury and stroke have been developed (Metz et al., 2000; Thompson et al., 2005) and are being extensively used at the molecular, cellular and systems level to investigate the sequelae of neurotrauma, neuroprotection, and rehabilitation. Animal models of NMES-based movement therapy after spinal cord injury or other central nervous system trauma are required to perform controlled studies that assess the efficacy of the therapy and that investigate the molecular to systems level mechanisms of neuromotor plasticity underlying functional recovery.

We have recently developed rodent models for NMES induced forelimb reach-grasp-and release (Kanchiku et al., 2008) and NMES induced activation of the flexor and extensor muscles of the hip, knee, and ankle hindlimb muscles for repetitive hindlimb movement therapy (Ichihara et al., 2009). We have also developed an adaptive control algorithm for NMES in rodents that allows the implementation of multiple (up to 500 cycles) of repetitive reciprocal cyclic hip movements (Kim et al., 2009) or coordinated hip-knee movements (Abbas et al., 2008; Kim et al., 2007). Acute studies conducted in anesthetized rodents were utilized to evaluate these models.

To assess the effectiveness of NMES therapy on recovery of sensorimotor function, the therapy must be utilized in a chronic longitudinal study. For chronic NMES in the rodent model, the implanted electrodes must be biocompatible, electrically and mechanically stable, and reliably functional for several weeks. For use of NMES in a daily movement therapy paradigm, the electrodes should maintain these characteristics under conditions of repeated limb movement and NMES must yield desired joint angle torques and excursions. In this work, we report the results from a study conducted on awake, spinal cord transected rodents chronically implanted with custom intramuscular electrodes for electrical stimulation of hindlimb muscles to provide daily movement therapy. We assessed the ability to stimulate specific muscles in awake paraplegic animals and determined stimulation parameters that result in graded isolated muscle contraction and sufficient torque generation and joint angle excursion. By repeatedly

characterizing the recruitment response to electrical stimulation over several weeks, we assessed the long-term stability of the electrodes and stimulation paradigm.

2. Materials and Methods

The ability to implant and viably maintain intramuscular stimulating electrodes to achieve selective and daily muscle stimulation on a long-term chronic basis was assessed in seven, 2–3 month old (adult) female paraplegic Long Evans rats (Charles River, 200–250gms). Rats were housed individually in an AALAC accredited university animal care facility with a 12-hour light/dark cycle, with access to food and water *ad libitum*. All animals were treated in accordance with the U. S. Public Health Service Guide for the Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee at the Arizona State University approved all surgical and experimental procedures.

2.1 Minimally invasive surgical implantation of electrodes

Animals were anesthetized using sodium pentobarbital (40 mg/kg, ip). Presence of adequate anesthetic levels was assessed using toe pinch and visual monitoring of respiration. Under aseptic conditions, gas-sterilized, custom, monopolar intramuscular stimulating electrodes were implanted close to the motor points of flexor and extensor muscles of the hip (iliacus (IL), biceps femoralis anterior head (BF_h), knee (semitendinosus (ST); vastus lateralis (VL)) and ankle joints (tibialis anterior (TA); gastrocnemius medialis (GM)). Multiple muscles per hindlimb were implanted with one electrode per muscle. The choice of the muscles was guided by their contributions to joint movements during locomotion. We have also previously recorded electromyogram (EMG) activity from these muscles and related it to the joint angle kinematics during treadmill walking in the intact rodent (Thota et al., 2005). The EMG information along with the ability to chronically stimulate the muscles will hence provide the ability, in future studies, to develop the stimulation paradigms needed for complex coordinated multijoint movement therapy.

The stimulation electrode assembly used in this study and the procedures for identifying the motor points and anchoring the intramuscular electrodes were the same as previously utilized by us for implantation in forelimb muscles of anesthetized rodents for a reach-grasp-release task (Kanchiku et al., 2008), or stimulating hindlimb muscles of anesthetized rodents for a cyclic movement task (Ichihara et al., 2009). Briefly, the stimulation electrode assembly was custom-made using Teflon coated stainless steel lead wire with a bare tip, non-absorbable high tensile strength suture connected near the bare tip, 1.5–2 mm circular retaining discs for anchoring the electrode to the muscles, and in-line connectors on the free lead end of the electrode wire. The muscle was exposed through a skin incision and a 30 G needle inserted to find a location that when stimulated with 0.1–0.2 mA, 200µsec cathodic pulses at 100Hz provided maximal muscle contraction. Once the location was identified, the electrode-suture assembly connected to a curved eye needle was inserted adjacent to the 30 G straight needle, the latter removed and the curved needle used to thread the suture with the de-insulated electrode tip through the muscle. The location of the tip within the muscle was adjusted by pulling the two ends of the suture outside the muscle. The electrode assembly was anchored in place by suturing the retaining discs to the muscle surface. A 1 cm circular ground connector made from 0.003" stainless steel shim stock was sutured to the muscles on the back. The electrode lead wires from each of the muscles implanted were routed subcutaneously to an incision on the back where they and the ground electrode were connected via the inline connectors to subcutaneously routed leads from a custom head connector (Omnetics Inc.) fixed to the skull using screws and dental glue (Figure 1A). The in-line connectors were ensheathed in 1.47 mm silastic tubing and the ends of the tubing were sealed with silicon glue. The hindlimb incision sites were closed using non-absorbable 5–0 suture. Except for the head connector, the

entire electrode assembly was implanted, hence the incidence of infection was ameliorated. Proper electrode placement was checked by back-stimulating through the head-connector and the twitch threshold currents (minimum current required to elicit visible muscle activation) were obtained using a hand-operated stimulator (200 μ sec cathodic pulse bursts at 1 Hz).

During the same surgical session, a thoracic laminectomy was performed under sterile conditions and a 2–5 mm section of the spinal cord transected and removed at spinal level T9 to produce complete paraplegia. The muscles of the back were closed in layers using 5–0 absorbable suture and the skin using stainless steel staples. Non-absorbable sutures on the hindlimbs and staples on the back were removed about 1 week post surgery. During surgery, 0.9% sodium chloride was administered subcutaneously at a rate of 2.0cc every 2–2.5 hrs to avoid de-hydration and the rectal temperature monitored and maintained within a range of 36–38°C using a heating pad.

After surgery, the animals were allowed to recover from anesthesia and twice-daily administered antibiotic (Cephazolin 33mg/kg, ip) for one week post implant and analgesic (Buprenorphine 0.02mg/kg, sc) for 2–3 days post surgery. The implant sites were cleaned daily with Nolvasan wash until healed. Manual bladder expression and hydration with sub-cutaneous 0.9% sterile saline were performed twice daily as part of regular animal care. Regular weight checks and urinalysis were also performed and if necessary supplemental Nutrical^{TM} for weight maintenance or antibiotic regimen for bladder infection treatment utilized.

2.2 Assessment of electrode stability and functional recruitment of muscles

Awake animals were placed on a platform such that the head, forelimbs, and midline of the torso were supported. The head connector was engaged with a cable from the stimulator. The platform restrained excessive movement of the head and forelimbs while allowing the unloaded hindlimbs to either be connected to a force transducer for assessing joint angle torques during electrical stimulation or hang freely for assessing joint angle excursion or receive daily movement therapy (Figure 1B).

Assessment of mechanical and electrical stability of electrodes and of the ability to achieve appropriate muscle recruitment and joint angle excursion were performed by generating and evaluating strength duration (SD) curves, isometric torque recruitment curves and kinematics of joint angle excursion (Ichihara et al., 2009). The studies were conducted in two sets of animals. One set of animals (Group I; 7 passive muscles for 8 weeks) did not receive any NMES. The other set (Group II; 14 active muscles for 6 weeks) received coordinated cyclic stimulation of paired flexor and extensor muscles for a given joint (hip, knee or ankle) for three weeks for 15min/day, 5 days/week beginning the fourth week post implantation. Thus, for the first three weeks these muscles for Group II animals were also passive like those for Group I. The cyclic stimulation was provided with a train of biphasic pulses (cathodic first) at 75 Hz (pulse width of 40 μ sec/phase and pulse amplitude current of 1.5 x twitch threshold current at the 40 μ sec pulse width) repeated every 0.5 sec for a total duration of 15 minutes. The duration of the stimulation burst during each cycle of stimulation for each muscle was chosen to match the EMG activity we have previously recorded from these muscles during treadmill walking by intact, adult, female Long Evans rats (Thota et al., 2005). These durations were 90msec for the TA for ankle dorsiflexion, 255msec for the GM for ankle plantar flexion, 70msec for the ST for knee flexion, 220msec for the VL for knee extension, 130msec for the IL for hip flexion, and 200msec for the BF_h for hip extension.

2.2.1 Strength Duration Curves—In order to assess the suitability of the electrode implantation site (motor point) for selective recruitment of a muscle and stability of the implanted electrode, strength duration (SD) curves were obtained by determining the minimum current required to achieve a minimum muscle twitch (the twitch threshold current) in response

to stimulation by a single biphasic (cathodic first) current pulse. The responses to stimulation using pulse widths of 500, 300, 100, 70, 40, 20, 10 $\mu\text{sec}/\text{phase}$ were ascertained. A 5–10 second interval was interspersed between pulse stimulations to prevent cumulative muscle fatigue.

To determine the minimum muscle twitch on electrical stimulation either a force transducer (model 20E12A, JR3 Inc. Woodland, CA) that measured the forces caused by isometric contractions or a visual inspection of the limb segment with the targeted muscle were used. The visual inspection method requires little setup time and can hence be conducted easily on a repeat basis from week-to-week. The visual approach was compared with the force transducer approach of twitch identification to confirm the ability to accurately determine the twitch threshold current even in deeper muscles. In the force transducer approach, the lowest stimulus current at which a force of 0.01N (arbitrarily chosen small value) was detected in any of the three directions (X, Y and Z) was defined as the twitch threshold current. For the visual approach, the twitch threshold was defined as the lowest stimulus current at which a visible response was observed. The visual and force transducer twitch data for a particular muscle were collected on the same day for accurate comparison. A total of 23 such comparisons were made [GM (n=5), TA (n=5), VL (n=5), ST (n=5), BF_h (n=2), IL (n=1)]. The SD curves for these comparisons were obtained 7–10 days post implant (dpi) in most cases. However, to verify good correlation between the visual and force transducer approaches even during a later time point in the study, the SD curves were re-compared in two of the muscles at 18dpi and in four of the muscles at 51dpi. The person observing the twitch was blinded to the previous history of twitch threshold current values. Since the two methods of assessment had high correlation (see Results) the simpler and faster visual assessment technique was primarily used for threshold detection in all the other assessments.

For the seven Group I muscles [GM (n=3), TA (n=3), VL (n=1)] SD curves were obtained at least once per week for 8 weeks post implantation. SD curves for the fourteen Group II muscles [GM (n=2), TA (n=2), VL (n=2), ST (n=2), BF_h (n=3), IL (n=3)] were obtained once per week prior to initiation of NMES (control) and at least once a week beginning four weeks post implantation until the end of the sixth week post implantation. The SD data were averaged over the study period (Group I: 8 weeks; Group II: 6 weeks) per muscle for each rat. Mean $\pm$ standard error of the mean (SEM) values are reported. For each electrode, a coefficient of variation (CV=standard deviation/mean) for the twitch threshold current was calculated for each pulse width for the first 3 weeks (≤ 28 days for Group I implants and pre-stim for Group II implants) and all weeks thereafter (> 28 days for Group I implants and post-stim for Group II implants). From these an average CV (CV_{mean}) across all pulse widths for the first 3 weeks and an average CV for all later timepoints was determined. Comparisons of CV_{mean} using paired t-tests were considered statistically significant at $p \leq 0.05$. For each SD curve, we also defined the twitch threshold current for a 500 $\mu\text{sec}/\text{phase}$ pulse width as the rheobase current. To track the stability of the electrodes, the number of electrodes in each group that remained within a $\pm 50\%$ band of the initial values at day 7 post injury were determined. In addition, the twitch threshold current values were plotted across days post implant and significant changes (from zero) in the slope of the regression lines through these points determined. Since the rheobase is one feature of the SD curve and its variability does not indicate reflect the variability of the entire SD curve, a similar assessment was also done for the twitch threshold current at 40 $\mu\text{sec}/\text{phase}$ pulse width. This pulse width lies close to the “shoulder” of the SD curves and is typically smaller than the chronaxie value.

2.2.2 Isometric torque recruitment curves—The ability of the stimulation to recruit the muscles and achieve a graded torque was assessed by generating isometric torque recruitment curves. The isometric torque recruitment curves were obtained for a twitch response (single pulse stimulation) as well as for a tetanic response (multiple pulse stimulation). The tetanic response allowed us to determine the steady-state torque generated. Detailed mathematical

description for calculating the joint torques and the procedures for obtaining these measures in anesthetized rodents have been described previously (Ichihara et al., 2009).

To assess isometric joint torques, as previously described and illustrated in Figure 1B, the unloaded hindlimb with the muscle of interest was held in place using in-house custom designed braces and stoppers with the brace attached to a six degree-of-freedom force transducer (model 20E12A, JR3 Inc. Woodland, CA). In order to measure isometric hip joint torque, the rat's hip and knee angle were fixed at 90° and 110°, respectively, with a knee brace secured to the thigh and shank using porous tape. To measure isometric knee joint torque, the rat's shank was secured to a shank brace with the knee at 90° using porous tape and the rat's thigh was held in a fixed position with the hip at 90° using a plastic stopper. To measure isometric ankle joint torque, the rat's foot was secured to a foot brace with the ankle at 90° using porous tape and the thigh and shank were held in a fixed position using plastic stoppers with both the hip and knee at 90° (Figure 1B). The force transducer output (forces and moments) were acquired at 2500 samples/sec using custom-designed software (LabVIEW; National Instruments, Inc., Austin, TX) and the output monitored on a digital storage oscilloscope. The force transducer was calibrated to record a zero output when there was no stimulation. Offline analysis in Matlab® (Mathworks, Natick, MA) was used to calculate the isometric joint torques from the forces and moments sensed by the force transducer.

The twitch torque response was obtained by stimulating the muscle with single, symmetric, charge balanced biphasic (cathodic first) pulses at multiple pulse widths (500, 300, 100, 70, 40, and 20µsec/phase) and current amplitudes. The current amplitude was chosen in multiples of the twitch threshold current (T) for a 40µsec pulse (1T, 1.5T, 2T, 2.5T and 3T). A one-minute interval between pulse stimuli was used to prevent cumulative fatigue. To observe and compare the typical torque patterns generated by stimulating the different muscles, the torque was normalized with respect to body weight and the maximum torque obtained across all the pulse width and current values for that muscle. The normalized torque was plotted versus current amplitude for each pulse width to generate a set of isometric twitch torque curves. A set of twitch torque recruitment curves was obtained for both Group I [TA (n=1), GM (n=3), VL (n=1)] and Group II muscles [TA (n=4), GM (n=1), ST (n=2), VL (n=4)] one week post injury. A second set was obtained for a subset of Group I muscles (one each TA and VL and two GM) and Group II muscles (one each TA, GM and ST and two VL) five weeks post injury.

Isometric steady-state torque recruitment curves were obtained by multiple pulse tetanic stimulation using bursts of biphasic (cathodic first) pulses at either 50Hz or 75Hz delivered for 2.5 seconds. The average torque was calculated from the force and moment data over the last 0.5sec of the stimulation burst to eliminate the step response transients caused by the muscle dynamics. The stimulation was repeated with a one-minute interval between bursts. For each frequency, two current amplitude levels (1.5T, 3T) and different pulse widths (20, 40, 70, 100, 300 and 500µsec/phase) were used. Once again, the isometric torque obtained was normalized with respect to body weight and the maximum torque obtained across all the pulse widths, frequencies and current amplitude levels for that muscle. The normalized torque obtained at the two frequencies and current levels were plotted as a function of pulse width to obtain a set of isometric steady-state torque curves. A set of steady-state torque recruitment curves were obtained for both Group I [TA (n=3), GM (n=2), VL (n=1)] and Group II muscles [TA (n=3), GM (n=2), VL (n=2), ST (n=2)] one week post injury. A second set was obtained for a subset of Group I muscles (one each of TA, GM and VL) and a subset of Group II muscles (one each TA, GM, and VL and two ST) five weeks post injury.

Note that not all the muscles used for generating the SD curves were used for obtaining the joint torque measurements. Each torque recruitment assessment was considered independent of others and changes in torque recruitment that may be a function of time were not evaluated.

Mean $\pm$ standard error of the mean (SEM) values for the single pulse and multiple pulse stimulation paradigms are reported separately.

2.2.3 Joint angle excursion—During trials assessing the effects of muscle stimulation on joint angular movement, the brace and the force transducer were disconnected and the rat's hindlimb was left unloaded. The joint angle data for the stimulated unloaded limb was collected using a video-based kinematic system (Peak Performance Technologies, Inc. Centennial, CO) as described in detail previously (Ichihara et al., 2009; Thota et al., 2005).

The joint angle kinematics assessment was performed by stimulating Group I muscles [TA (n=3), GM (n=2), VL (n=1)] and Group II muscles [TA (n=4), GM (n=3), VL (n=4), ST (n=4), BF (n=1), IL (n=1)] one week post injury. The assessment was repeated five weeks post injury by stimulating a subset of both the Group I muscles (one each of TA, GM, and VL) and Group II muscles [TA (n=1), GM (n=1), VL (n=3), ST (n=3), BF_h (n=1), IL (n=1)]. Note that not all the muscles stimulated for obtaining the SD curves and torque recruitment curves were utilized for the joint angle measurements. Each assessment was considered independent of the others. The range of motion of the joint (difference between the maximum and minimum joint angle) obtained during stimulation was calculated and averaged for each muscle across time. Mean $\pm$ standard error of the mean (SEM) values are reported.

3.0 Results

3.1 Electrode stability (Strength Duration Curves)

Strength duration (SD) curves were used to evaluate the mechanical and electrical stability of the electrodes. As explained under Methods, initially in 23 implanted muscles, a visual and a force-transducer method were used to identify the twitch threshold current. As illustrated in Figure 2A the twitch threshold current measured from the two methods, irrespective of the pulse width of stimulation used, were virtually identical ($R^2 = 0.92$). The Root Mean Square Error (RMSE) for different pulse widths utilized, where error equals the difference between the visual twitch threshold current and force transducer twitch threshold current, is illustrated in Figure 2B. At the shorter pulse widths, the RMSE is higher. The visual twitch threshold current was lower than the force transducer threshold current level in 122 out of the 157 trials performed. These data indicate that the visual method is a reliable technique for threshold current detection.

To assess the stability of the implanted electrodes over time, the SD curves were generated every week over several weeks. The twitch threshold current values at each pulse width were compared. Figure 3(A–C) show the average SD curves for the Group I muscles (TA, GM and VL) over eight weeks post implant. Each curve represents a different electrode implant. At each pulse width the means and SEM twitch threshold current for the eight weeks over which the assessment was conducted is plotted. The SEM values of twitch threshold current across all pulse widths ranged from ± 0.03 to ± 0.70 mA for TA (Fig. 3A), from ± 0.01 to ± 0.53 mA for GM (Fig. 3B) and from ± 0.03 to ± 0.70 for VL (Fig. 3C). The SEM values are minimal indicating little change in threshold currents for all the muscles over the entire 8 weeks. The SEM was greater at the shorter pulse widths. Figure 6C shows the changes in CV_{mean} for each implanted electrode. One electrode had clearly become unstable, 3 had reduced CV_{mean} over time and 3 had slight increases in CV_{mean} . Figure 4 illustrates the rheobase current as a function of time for each of the muscles in all the different rats scaled as a percent of the value at day 7 post implant. Rheobase for 4 out of 7 electrodes remained within the $\pm 50\%$ band. 1 electrode in the TA exhibited a steady increase in rheobase, a 2nd had a jump in rheobase 2 weeks post injury, but the values decreased over time and one electrode in the VL was stable for 4 weeks but had high values when re-evaluated 6 weeks post injury. The slope of the regression line over the duration of the study for a given animal was not significantly different

from zero. Although not illustrated, the changes in the 40 μ sec/phase pulse width threshold current values were slightly greater than for the rheobase. Of the 4 electrodes with rheobase within the $\pm 50\%$ band across all weeks, 2 crossed the 50% band at 2 weeks post injury but then reduced to within the $\pm 50\%$ band.

The Group II muscles subjected to electrical stimulation 5 days/week showed SD curves similar to those for the Group I muscles over the 6 weeks of study (Fig. 5(A–F)). The SEM values of twitch threshold current across all pulse widths ranged from 0 to ± 0.23 mA for the ankle dorsiflexor (TA; $n=2$), from ± 0.02 to ± 1.06 mA for the ankle plantar flexor (GM; $n=2$), from ± 0.01 to ± 0.43 mA for the knee flexor (ST; $n=2$), from 0 to ± 0.24 mA for the knee extensor (VL; $n=2$), ± 0.01 to ± 0.2 mA for the hip flexor (IL; $n=3$), and from ± 0.02 to ± 0.66 mA for the hip extensor (BF_h; $n=3$). Fig. 6A illustrates the individual SD curves over multiple weeks for one electrode implanted in the TA that showed very repeatable threshold currents each week while Fig. 6B illustrates them for an electrode implanted in the GM that showed the worst repeatability amongst all implanted electrodes. Fig. 6D shows the changes in CV_{mean} for each electrode for Group II. Of the 14 implanted electrodes, one electrode had a very high CV_{mean} pre-stim and remained high post-stim. Post-stim CV_{mean} for 4 electrodes was at least twice the pre-stim value but for the remaining 9 electrodes CV_{mean} post-stim remained close to the pre-stim values. Figure 7 illustrates the rheobase current as a function of time for each of the implanted electrodes, scaled as a percent of the value at day 7 post implant. Rheobase for 8 out of the 14 electrodes remained within the 50% band or decreased over time. Rheobase for 1 electrode in the GM kept increasing until 28 dpi (also see SD curve in Fig. 5B). However, it reduced near day 42dpi. Rheobase for one electrode implanted in the ST and two implanted in the BF increased beyond the $+50\%$ band after 21, 28 and 35 dpi respectively. 2 out of the 14 muscles (one GM and one ST) had a regression slope that was significantly different than zero after the stimulation started. Although not illustrated, the changes in the 40 μ sec/phase pulse width threshold current values mirrored those for the rheobase. Thus, in general, as for the passive muscles the threshold currents remained consistent over several weeks and were not systematically altered by the daily stimulation.

In four muscles from Group I, initiation of daily electrical stimulation was delayed to 8 weeks post implantation and continued for another 4 weeks. For these muscles too, the SD curves obtained remained stable for the 4 weeks after electrical stimulation had been initiated (data not shown).

3.2 Isometric torque recruitment

3.2.1 Single pulse twitch stimulation—The twitch response of the muscle induced by single pulse stimulation can be modulated either by altering the width or amplitude of the stimulus pulse. Figures 8A, B, C and D illustrate the twitch torque obtained for different current amplitudes and pulse widths on stimulating the ankle dorsiflexor (TA), ankle plantar flexor (GM), knee flexor (ST), and knee extensor (VL) respectively.

Although there are variations in the specific response patterns of the different muscles, in general, for higher current amplitudes at a particular pulse width (identified by a unique marker), the torque generated is larger. Current amplitude levels greater than $1.5T$ are required to generate an increase in torque at a 20 μ sec/phase pulse width after which a monotonic increase in torque is observed. For a 40 μ sec/phase pulse width a monotonic linear increase in torque can be observed with increasing current amplitudes. For a 70 μ sec/phase pulse width a monotonic increase in torque followed by a plateau or absence of a set pattern is observed for higher current amplitudes. For a 100 μ sec/phase pulse width, decreases in torque at some of the joint can be observed at amplitudes greater than $1.5T$. For longer pulse widths of 300 μ sec/phase and 500 μ sec/phase, the recruitment curve is non-linear with steep increases or saturation of torque generated at higher current amplitudes. These data demonstrate monotonic

recruitment curves with a broad range, which are particularly useful in implementing NMES control.

3.2.2 Peak isometric torque using multiple pulse tetanic stimulation—Isometric torque values recorded during multiple pulse stimulation of the ankle dorsi flexor, ankle plantar flexor, knee flexor and knee extensor are shown in Figures 9A, B, C and D respectively. In general, there was a rise in the torque generated for longer pulse widths. However, at very long pulse widths (longer than 100 μ sec/phase) or high current amplitudes the torque generated sometimes decreased or perhaps due to spillover that activated non-synergistic muscles. Larger torque was produced at the 75Hz frequency for a particular current amplitude.

The SEM values observed for the isometric torque calculated over all the pulse widths, frequencies and current levels tested, range between ± 0.006 to ± 0.095 Nm kg⁻¹ for TA (n=6) (Fig. 9A), ± 0.006 to ± 0.095 Nm kg⁻¹ for GM (n=5) (Fig. 9B), ± 0.023 to ± 0.165 Nm kg⁻¹ for ST (n=2) (Fig. 9) and ± 0.0 to ± 0.045 Nm kg⁻¹ for VL (n=2) (Fig. 9D). The ankle torque values observed in our experiments with a 40 μ sec pulse width, 1.5T current level and at 75Hz frequency ranged from 0.035 to 1.0 Nm kg⁻¹ during dorsi flexion and 0.013 to 0.13 Nm kg⁻¹ during plantar flexion. Torque generated at the knee ranged from 0.15 to 0.19 Nm kg⁻¹ during knee extension and from 0.014- to 0.066 Nm kg⁻¹ during knee flexion.

3.2.3 Angular excursion on multiple pulse stimulation—In administering NMES movement therapy, it may be most effective to produce movement patterns with excursions that approximate those observed during locomotion. Figures 9 and 10 show joint angle excursions achieved during electrically stimulated movements in awake paraplegic animals with intact spinal reflexes. Joint angle range values obtained during multiple pulse stimulation are shown in Figure 9. An initial region of higher slope within the pulse width range of 20–100 μ sec/phase followed by a region of saturation or decrease in angle was observed. In figures 10B and 10C, at 75Hz stimulus frequency and 1.5T amplitude current, the increase in joint angle is relatively low for a pulse width of 40 μ sec/phase. The hip flexion joint angle range during activation of the iliacus decreased at the longer pulse width, amplitude and frequency combination (300/500 μ sec/phase, 3T, 75Hz) (Fig. 10E). The knee flexion joint angle range on activation of the VL also decreased at the higher current amplitude level of 3T and 500 μ sec/phase pulse width value at 75Hz (Fig. 10C). During activation of BF_h at 75Hz and 3T current amplitude, the hip extension joint angle range showed a steep increase at 500 μ sec/phase (Fig. 10F). The mean joint angle at the ankle with a 1.5T current level and at 75Hz frequency ranged from 0.35 to 66.75° during dorsi flexion and 0.54 to 66.28° during plantar flexion. Joint angle at the knee ranged from 0.27 to 30.99° during knee flexion and 0.18 to 34.45° during knee extension. Joint angle at the hip ranged from 0.6 to 20° during hip flexion and 0.55 to 16.74° during hip extension.. The SEMs for joint angle ranges over all the pulse widths, frequencies and current levels tested, varied from ± 0.352 to $\pm 8.0^\circ$ on stimulation of the TA (n=6) (Fig. 10A), ± 1.42 to $\pm 8.0^\circ$ on stimulation of the GM (n=5) (Fig. 10B), ± 0.213 to $\pm 2.718^\circ$ on stimulation of the VL (n=5) (Fig. 10C), ± 0.147 to $\pm 3.454^\circ$ on stimulation of the ST (n=4) (Fig. 10D), and ± 0.066 to $\pm 3.432^\circ$ on stimulation of for the BF (n=2) (Fig. 10E). Thus, these data indicate the range of motion that was measured at each joint for the different NMES pulse widths, at two different current levels and two different frequencies. The figures also help identify the range of pulse width parameters that allow for a monotonic increase in the joint angle values. This range of pulse-widths would be useful in the design of control algorithms for NMES. Figure 11, illustrates rhythmic flexion-extension of the hip, knee and ankle joints in a representative awake animal 21 days post transection and electrode implantation. As described under methods for the daily stimulation paradigm, alternating reciprocal stimulation was provided to paired flexor and extensor muscles for each joint using 75Hz, 40 μ sec/phase bipolar (cathodic first), constant amplitude pulses to obtain 2 Hz rhythmic joint angle

movement. Intralimb coordination was not implemented in this study and the periodic joint movements were obtained in independent trials.

4.0 Discussion

The long-term goal of this study is to develop a model of electrical stimulation assisted movement therapy in complete or incomplete spinal cord injured rodents. Such a model will allow us to investigate the molecular to systems level mechanisms underlying effectiveness of movement therapy on locomotor recovery. Development of such a model requires 1) accurate mapping of electrode and muscle stimulation sites, 2) the capability to selectively activate muscles to produce graded contractions of sufficient strength, 3) stable anchoring of the electrode within the muscles and 4) stable performance of the electrodes i.e. capability to generate graded responses of sufficient strength. For true locomotor therapy with partial weight support, it will ultimately be necessary to provide coordinated stimulation of muscles acting at the different joints under partial hindlimb loading. In this study, we chronically implanted custom designed electrodes in target hindlimb muscles of spinal cord transected rats and assessed the stability and performance of these implanted electrodes over several weeks under passive and active muscle conditions. Our electrodes remained well anchored in place, thereby assuring stable and reliable recruitment of the muscle over several weeks. We were able to achieve sufficient joint torques and angle excursions in awake animals and use the implanted electrodes to provide control of multiple muscles to provide cyclic joint angle movement.

4.1 Electrode design and implant procedure

For eliciting muscle activation, we could have used nerve cuffs or epimysial electrodes that are chronically implanted (Venkatasubramanian et al., March 2006). Nerve cuffs have been reported to have steep recruitment curves and be susceptible to performance degradation due to encapsulation (Aoyagi et al., 2004; Gorman and Mortimer, 1983; Singh et al., 2000). Both nerve and epimysial electrode implantation requires a more invasive surgery than implantation of intramuscular electrodes using a percutaneous approach. A modification of the percutaneous approach was utilized in this study and described by us in detail previously (Ichihara et al., 2009). It is important that the initial placement of the electrodes is very close to the nerve entry point into the muscle and that this location remains stable over weeks of movement therapy. Additionally, susceptibility to mechanical stresses and motion must not result in lead breakage and electrode migration. Like other chronically implanted electrodes, these electrodes too can become encapsulated, elevating stimulus thresholds to higher levels and in some cases result in neuromuscular damage (Prochazka, 1993; Scheiner et al., 1990). Our placement approach and electrode design enabled repositioning of the electrode within the muscle during the implantation surgery and the coiled configuration of the electrode lead provided elasticity during repeated movement thereby preventing lead breakage due to mechanical stress acting on the electrode. Sutures that attached the retaining discs to the muscle surface allowed anchoring for long-term mechanical stability. Thus, with this design and implant procedure, we were able to repeatedly and consistently position the electrode very close to the motor point. Out of the 14 Group II muscles in which electrodes were implanted, only one electrode showed an elevated initial threshold (Fig. 5E).

4.2 Electrode stability

Chronic intramuscular stimulation of the denervated tibialis anterior muscle has previously been utilized to investigate the ability of chronic stimulation to influence afferent sensory nerve regeneration in the self-anastomosed nerve innervating the denervated muscle (Marqueste et al., 2004; Marqueste et al., 2002). The procedure utilized very high voltage values (10 volts) presumably because the target tissue muscle fibers have a much higher threshold for activation by electrical stimulation. The studies of Marqueste et al. were not designed to evaluate the

stability of the electrical response over weeks nor to determine appropriate stimulation parameters. In our case of neuromuscular stimulation after paraplegia caused by spinal cord injury, as has been done for human subjects, the stimulation is not targeted to muscles but to the motor endpoints and hence very small levels of current is used for stimulating the nerve endings. In addition, multiple muscles with intact innervation were chronically stimulated for several weeks. In our study, there was a risk of electrode breakage or movement because muscles were stimulated daily to produce functional contractions and sufficient range of movement. The stability and reliability of the electrodes will have a strong impact on the potential viability of this chronic NMES-induced neuromotor therapy.

Stability of the implanted intramuscular stimulating electrodes over several weeks was assessed using strength duration curves. These curves graphically represent the characteristic excitability response of the muscle and show a typical non-linear hyperbolic relationship. As in our previous studies in acute experiments (Ichihara et al., 2009) and other published studies (Bostock et al., 1983; Mouchawar et al., 1989), to produce a similar minimum muscle twitch response stimulation using shorter pulse widths requires larger current amplitude pulses while stimulation using longer pulse widths requires lower current amplitude pulses (Fig. 3, 5). SD curves obtained from passive and active muscles remained stable over several weeks. SD curves obtained from muscles that were not subjected to NMES (Group I) remained stable over 8 consecutive weeks of the study duration. The standard errors recorded across all pulse widths were minimal and the rheobase current remained consistent over several weeks indicating little change in the threshold for muscle recruitment over the 8 weeks (Fig. 3, 4). SD curves of active muscles that underwent electrical stimulation therapy starting 3 weeks post implantation remained stable for a total of 6 weeks (maximum duration investigated) (Fig. 4) as did the rheobase current (Fig. 5). Stable SD curves were also obtained when electrical stimulation was initiated 8 weeks post implantation. The CV_{mean} before and after NMES was not statistically different and no obvious pattern of changes in the rheobase or “shoulder” current were apparent after NMES. The stability of each electrode is indicated by the consistency in the overall shape of the SD curves over time (low SEM values at each pulse width), the low values of CV, and the consistency of the values for the rheobase and twitch threshold current at $40\mu\text{sec/phase}$. Collectively these results suggest that electrode performance is not adversely affected by the mechanical stresses during the daily limb segment movement nor by the encapsulation response to the implanted electrode.

In this study, we have also established two methods of experimentally determining twitch threshold for generating the SD curves, a force transducer method and a visual method. The twitch threshold current detected visually was found to correlate very well with the twitch threshold value detected with the force transducer (Fig. 2A). In addition, the twitch threshold current detected visually was lower than that using the transducer method. Therefore, the visual method for threshold detection is a reliable and quick alternative to the force transducer method of threshold detection which can be time consuming and tedious.

4.3 Performance/functionality of electrodes

For developing a rodent model of long-term movement therapy, the implanted electrodes must not only be functional over weeks, but it is also important that the NMES system reliably produce graded control of force and sufficient joint angle excursion in a awake paraplegic animals with intact spinal reflexes. Additionally, to design of a control system for NMES a broad input parameter range over which a linear increase in joint torque/joint angle can be obtained would be beneficial. Hence, functionality of the electrodes, parameter ranges for requisite muscle recruitment and stability of the recruitment properties over weeks were assessed in awake animals.

Figures 8 and 9 indicate that graded recruitment of muscles can be achieved. All torque values are normalized to the maximum torque generated at the highest pulse width and current amplitude for that muscle. While the overall recruitment curve is non-linear, as previously reported during intramuscular stimulation in cats (Crago et al., 1980), a range of current amplitudes for different choice of pulse widths can lead to a monotonic increase in the torque generated. For a given current amplitude increasing the pulse widths within the range of 20 $\mu\text{sec}/\text{phase}$ – 100 $\mu\text{sec}/\text{phase}$ allows a monotonic increase in torque generated and angular excursion (Fig. 9, 10). Similarly, for a given pulse width within the 40 $\mu\text{sec}/\text{phase}$ – 100 $\mu\text{sec}/\text{phase}$ range, increase in current amplitude results in a monotonic increase in torque generated (Fig. 8). At high levels of pulse widths and current amplitude, torque levels generated and angular excursions obtained can plateau or decrease, the decrease is likely due to spill over that causes adjacent synergistic and non-synergistic muscles to be activated.

An overall assessment of the recruitment curves suggests that at a pulse amplitude of 1.5T, a linearly graded recruitment can be obtained with pulse width modulation during activation of the ankle dorsi flexor (TA) and knee extensor (VL). With pulse amplitude modulation, a linearly graded recruitment was obtained with a pulse width of 40 $\mu\text{sec}/\text{phase}$ during activation of ankle dorsi flexor (TA) and knee extensor (ST). These data suggest that, were a fixed pulse width to be used during current amplitude modulation, a stimulation pulse width of 40 $\mu\text{sec}/\text{phase}$ could be used with no or little spill over. Similarly, a fixed amplitude level of 1.5T would be suitable during pulse width modulation to obtain graded and linear recruitment. In general, the isometric recruitment curves for a particular muscle were similar across animals indicating the ability to obtain stable recruitment with every implant.

4.4 Additional studies and neuroprostheses for movement therapy

The results demonstrate that the NMES rodent model system provides many important features: successful implantation, selective activation, graded recruitment, strong contractions and sufficient movement ranges in awake spinal cord transected animals in which spinal reflexes can manifest themselves. In our rodent model, the chronically implanted electrodes of this system remain stable over 8 weeks in the absence of daily electrical stimulation. A delay of 3 weeks post implantation was utilized in this study to stabilize the electrodes within the muscles and stability was maintained in the presence of 15 minutes of daily electrical stimulation. Additional investigation will be needed to determine if this period for delaying stimulation therapy can be reduced in the rodent. On the other hand, in people with SCI where rehabilitation techniques such as treadmill training may be harder to implement early after injury, surface electrodes could be used for NMES, which would allow initiation of movement therapy early after injury. Early intervention with NMES, before initiating treadmill therapy, would be consistent with recent studies suggesting that rehabilitative training initiated soon after injury results in improved functional outcomes (Norrie et al., 2005; Scivoletto et al., 2005). NMES would also have other therapeutic effects by influencing muscle conditioning and recovery of muscle strength, thereby synergistically improving the chances of successful recovery.

To provide movement therapy that mimics locomotor type movement patterns it would be beneficial to obtain sufficient joint torques, angular excursion, and pattern of joint angle kinematics during electrically stimulated movements. Previously, hindlimb joint torques during overground walking have been estimated in the tailed tree shrew (*Tupaia glis*) (Witte et al., 2002). These approximate values of joint torque are 0.025 Nm kg^{-1} at the ankle, 0.014 Nm kg^{-1} at the knee and 0.015 Nm kg^{-1} at the hip and are likely typical for tailed species. Joint torque data for the rodent species during overground walking are not available and therefore we compared our isometric torque data to that from the shrew. We were able to generate body-weight normalized torques comparable to those observed in the tree shrew (see results).

Using NMES with a 1.5T current level, 75Hz frequency and different pulse widths the joint angle range of motion (flexion plus extension) at the ankle ranged from approximately 1° to 123° and at the knee from 0.45° to 65°. These values indicate that NMES can yield sufficient angular excursion matching that at the ankle joint (approximately 65°) and knee (approximately 51°) that we have previously obtained via 3D-kinematic analysis during treadmill walking in intact adult Long-Evans rats (Thota et al., 2005). Angular excursion of the hip was obtained during NMES in one rat (Figure 10). However, the actual values obtained at the hip cannot be compared to the hip angle range (approximately 22°) obtained during treadmill walking in the rat (Thota et al., 2005), since in the unloaded position utilized during NMES the hip was already in an extended position resulting in a very narrow extension range. Across multiple assessments with the different implants for the muscles acting on the ankle and knee joints, the joint angle patterns and range showed little variation.

We were also able to stimulate multiple muscles (flexor and extensor) to achieve rhythmic movement patterns during daily therapy. However, to achieve a desired complex shape of joint angle kinematics, appropriate intralimb coordination, and account for fatigue and the nonlinear muscle recruitment properties during NMES, an adaptive control algorithm that modifies either the current pulse amplitudes or pulse widths in real-time is needed (Riess and Abbas, 2001). A real-time adaptive control system was not utilized in this study but we have developed such a system and tested its ability in anesthetized rodent preparations (Kim et al., 2009; Kim et al., 2007). For therapy using stepping like patterns, NMES may have to be provided in the context of a partial-body weight supported system as is being done for people with spinal cord injuries (Field-Fote, 2001). Hence, we are developing a partial weight support system to allow some load bearing by the hindlimbs during the stimulation as well as cutaneous stimulation of the plantar surface of the foot. The NMES rodent model system can also be utilized for therapy after incomplete spinal cord injury and preliminary studies of short-term neuromuscular stimulation of unloaded hindlimbs in a contusion injury model that we have conducted using a fixed-pattern stimulation paradigm show promising functional recovery during non-weight supported treadmill walking (Jung et al., 2006; Mukherjee et al., 2005).

In summary, this study in conjunction with our previous work in anesthetized rodents (Ichihara et al., 2009; Kim et al., 2009; Kim et al., 2007) lays the groundwork for the use of chronic neuromuscular electrical stimulation based movement therapy in a rodent model of spinal cord injury. We have successfully implanted intramuscular electrodes in the target hindlimb muscles in spinal cord transected rats using a minimally invasive approach. We have assessed the stability and performance of these implanted electrodes over several weeks in awake rodents under passive and active muscle conditions. The minimal twitch threshold current remains stable under conditions of daily electrical stimulation and repetitive hindlimb mechanical movement. Torque recruitment patterns and joint angle curves obtained for a particular muscle were similar in all rodents. We were also able to arrive at a suitable stimulation protocol (75Hz with a pulse width of 40µsec/phase and current amplitude of 1.5T) that could provide sufficient torque and angular excursions required for hindlimb locomotion. Hence, by implementing an adaptive control algorithm for NMES, the model will allow the use of a movement therapy protocol that utilizes rhythmic, sequenced, repeatable, coordinated movements of the different limb segments with the generation of sufficient joint angle torque and excursion. Similar movement therapy protocols could also be extended to rodent models of traumatic brain injury or stroke. Future studies will thus allow assessment of the use and effectiveness of neuroprostheses for tapping into the endogenous neuroplasticity mechanisms for central nervous system recovery and repair after neurotrauma.

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Figure 1.

(A) Illustration of implanted electrodes and head connector. The implanted electrodes are subcutaneously routed to an Omnetics® nano-series head connector through inline Digi-Key® connectors. (B) Arrangement for assessing isometric ankle joint torque during electrical stimulation. Side and front views (inset) are shown. Awake rodent is allowed to sit on a platform. Stoppers and braces are used to stabilize the joints and a 6 degree-of-freedom force transducer (JR3) is used to measure moments and forces. Stimulation current pulses are provided via the implanted electrodes connected to the stimulator through the head connector.

Figure 2.

Comparison of the force transducer and the visual method of threshold detection. (A) Cross correlation between the force transducer threshold and the visual threshold. (B) The root mean square error (RMSE) between the two methods of threshold detection for multiple values of pulse width. A correlation coefficient of 0.9 and low RMSE values suggest that either method could be used with the visual method of threshold detection being practical and reliable.

Figure 3.

Strength Duration curves obtained from electrodes implanted in Group I muscles (no daily stimulation). The figures depict the Strength Duration (SD) curves generated on stimulation of (A) Ankle dorsi flexor, tibialis anterioralis (TA), (B) Ankle plantar flexor, gastrocnemius medialis (GM) and (C) Knee extensor, vastus lateralis (VL). Different lines with a unique marker represent different rats and each data point represents the twitch threshold current averaged over the entire eight weeks of study for the corresponding pulse width. The calculated $\pm$ SEM values indicated by error bars were not statistically significant suggesting little variation in the twitch threshold over time.

Figure 4.

Changes in rheobase current reflecting stability of implanted electrodes in Group I muscles (no daily stimulation training). The figures depict alterations in rheobase current (twitch threshold current at 500usec/phase pulse width) obtained on stimulation of (A) Ankle dorsi flexor, TA, (B) Ankle plantar flexor, GM, and (C) Knee extensor, VL over a period of 6–8 weeks. Each line represents a different rat identified by the same marker as used in figure 3. The dashed horizontal lines indicate $\pm 50\%$ of the initial value. The calculated slopes of the regression lines were statistically insignificant for all of the muscles, suggesting little variation in the twitch threshold over time.

Figure 5.

Strength Duration curves obtained from electrodes implanted in Group II muscles (daily stimulation training initiated 3 weeks post implant). The figures depict the Strength Duration curves generated on stimulation of (A) Ankle dorsi flexor, TA, (B) Ankle plantar flexor, GM, (C) Knee flexor, ST, (D) Knee extensor, VL, (E) Hip flexor, iliocus (IL) and (F) Hip extensor/knee flexor, biceps femoralis (BF) over a period of 6 weeks. Each line with a unique marker represents a different rat and each data point represents the twitch threshold current averaged over the entire six weeks for the corresponding pulse width. The calculated $\pm$ SEM values indicated by error bars on the figure were not statistically significant suggesting little variation in the twitch threshold over time even during daily movement therapy.

Figure 6. SD curves from Group II muscles over multiple weeks and coefficient variation of threshold current

SD curves obtained over 8 weeks on stimulation of electrodes implanted in (A) TA muscle and (B) GM muscle from Group II illustrating the responses obtained from a stable and a not stable electrode respectively. (C) Mean Coefficient of Variance (CV_{mean}) for threshold currents obtained on stimulation of individual electrodes in Group I and (D) Group II. The pre-stim duration for the Group II electrodes is the same as the ≤ 28 days period for Group I electrodes.

Figure 7.

Changes in rheobase current reflecting stability of implanted electrodes in Group II muscles (daily stimulation training initiated 3 weeks post implant, indicated by grey region). The figures depict alterations in rheobase current obtained on stimulation of (A) Ankle dorsi flexor, TA, (B) Ankle plantar flexor, GM, (C) Knee flexor, ST, (D) Knee extensor, VL, (E) Hip flexor, IL and (F) Hip extensor/knee flexor, BF over a period of 6 weeks. Each line represents a different rat identified by same marker used in figure 3. The dashed horizontal lines indicate $\pm 50\%$ of the initial value. The shaded gray area depicts the period in which NMES was being administered. The weekly SD curves for the electrodes identified by *TA and *GM are shown in Figure 6A and B respectively. The calculated slopes of the regression lines were not statistically significant for 12 out of the 14 muscles, suggesting little variation in the twitch threshold over time even during daily movement therapy.

Figure 8.

Isometric torque recruitment curves obtained by modulating the pulse amplitude during single pulse stimulation at different pulse widths of 20 μsec (●), 40 μsec (■), 70 μsec (▲), 100 μsec (○), 300 μsec (□) and 500 μsec (Δ)/phase. Isometric torque recruitment curves were generated at each of the joints by stimulating specific muscles. (A, B) illustrate torque at the ankle joint obtained on stimulating the ankle dorsi flexor (TA) and ankle plantar flexor (GM) respectively. (C, D) illustrate torque at the knee joint obtained on stimulating the knee flexor (ST) and knee extensor (VL) respectively. The torque values are normalized against the maximum torque obtained across all the pulse width and current values for the muscle stimulated. With current modulation a broader recruitment curve is obtained in comparison to that with pulse width modulation (see figure 8).

Figure 9.

Isometric torque recruitment curves obtained during multiple pulse stimulation. Recruitment curves were generated at the ankle joint (A-Ankle dorsi flexion (TA) and B-Ankle plantar flexion (GM)) and knee joint (C-Knee flexor (ST) and D-Knee extensor (VL)). The isometric torque was calculated at two different frequencies (50Hz (---) and 75Hz (—)) and at two different currents (1.5 times the twitch threshold at 40 μ sec ($\square$) and 3.0 times the twitch threshold current at 40 μ sec ($\bullet$)). Data are represented as Mean $\pm$ SEM. Repeated, similar graded torque patterns can be generated at every implant. At higher charge levels (higher current levels and/or longer pulse widths), the absolute value of the isometric joint torque saturates.

Figure 10.

Joint angle ranges obtained during multiple pulse stimulation. Joint angle ranges were calculated at the ankle joint (A: Ankle dorsi flexion and B-Ankle plantar flexion), knee joint (C-Knee flexion and D-Knee extension) and the hip joint (E-Hip flexion (IL) and F-Hip extension) on stimulating the TA, GM, ST, VL and BF respectively.. The joint angle range was calculated at two different frequencies (50Hz (----) and 75Hz (—)) and at two different currents (1.5 times the twitch threshold at 40usec/phase (□) and 3.0 times the twitch threshold current at 40usec/phase (●)). Data are represented as Mean $\pm$ SEM. At higher charge levels (higher current levels and/or longer pulse widths), the measured joint angle range saturates.

Figure 11.

Joint angle curves obtained during reciprocal flexor-extensor stimulation. Data from an awake paraplegic (T9 transection) rat obtained 21 days post electrode implant. Intramuscular stimulation electrodes were implanted in the flexor and extensor muscles of the hip (iliacus, biceps femoralis), knee (semitendinosus; vastus lateralis) and ankle joints (tibialis anterioris; gastrocnemius medialis). Each joint angle flexor-extensor pair was stimulated reciprocally to achieve a 2 Hz period excursion using constant current biphasic pulses (40 μ sec/phase at 75Hz).