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NEUROMUSCULAR ELECTRICAL STIMULATION OF THE HINDLIMB MUSCLES FOR MOVEMENT THERAPY IN A RODENT MODEL

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Abstract

Neuromuscular electrical stimulation (NMES) can provide functional movements in people after central nervous system injury. The neuroplastic effects of long-term NMES induced repetitive limb movement are not well understood. A rodent model of neurotrauma in which NMES can be implemented may be effective for such investigations. We present a rodent model for NMES of the flexor and extensor muscles of the hip, knee, and ankle hindlimb muscles. Custom fabricated intramuscular stimulating electrodes for rodents were implanted near identified motor points of targeted muscles in ten adult, female Long Evans rats. The effects of altering NMES pulse stimulation parameters were characterized using strength duration curves, isometric joint torque recruitment curves and joint angle measures. The data indicate that short pulse widths have the advantage of producing graded torque recruitment curves when current is used as the control parameter. A stimulus frequency of 75Hz or more produces fused contractions. The data demonstrate ability to accurately implant the electrodes and obtain selective, graded, repeatable, strong muscle contractions. Knee and ankle angular excursions comparable to those obtained in normal treadmill walking in the same rodent species can be obtained by stimulating the target muscles. Joint torques (normalized to body weight) obtained were larger than those reported in the literature for small tailed therian mammals and for peak isometric ankle plantarflexion in a different rodent species. This model system could be used for investigations of NMES assisted hindlimb movement therapy.

Keywords

isometric joint torque; muscle recruitment and strength duration curves; intramuscular electrodes; joint angle measurements; rehabilitation; hindlimb

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1. Introduction

The National Spinal Cord Injury Statistical Center reported in 2006 that approximately 250,000 people live with spinal cord injury (SCI) in the United States, with 11,000 new cases annually. Of these approximately 34.1% have an incidence of incomplete tetraplegia and 18.5% have incomplete paraplegia (Center, 2006). Incomplete cervical or thoracic injuries lead to impaired mobility. Similar loss of mobility can occur after traumatic brain injury or stroke. In an effort to promote recovery of motor function several rehabilitation techniques such as body weight supported treadmill training, robotic assist and functional electrical stimulation in combination with treadmill or robotic assist training are being utilized e.g. (Colombo et al., 2000; Hesse et al., 2004; Hicks et al., 2005). All of these techniques aim to tap into the neuroplasticity of the injured system and promote beneficial recovery. In human studies, neural plasticity can be documented using either functional outcome measures or non-invasive imaging techniques such as PET or fMRI. Animal models, however, can be used for more detailed investigation of the mechanisms that underlie therapy-induced neural plasticity. 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 rehabilitative therapy effectiveness. In this manuscript, we describe development of a rodent model of functional neuromuscular electrical stimulation based hindlimb movement therapy.

Functional neuromuscular electrical stimulation (NMES) is a technique that applies sequences of low-level electrical current pulses to nerves that control muscles in order to generate functional movements. It has long been used as a neuroprosthesis to restore or improve recovery of function after spinal cord injury, head trauma or stroke (Barbeau et al., 2002; Field-Fote, 2001; Riess and Abbas, 2001; Sheffler and Chae, 2007). NMES can also be utilized to provide coordinated repetitive movement therapy (Lynskey et al., 2008; Sheffler and Chae, 2007) and some studies have found this approach to be beneficial for promoting recovery of motor function in people with incomplete spinal cord injury or stroke (Daly et al., 2006; Popovic et al., 2006). In people with incomplete SCI, the use of electrical stimulation to elicit the flexion withdrawal reflex of the lower limb during body weight supported treadmill training enhances locomotor recovery (Bajd et al., 1999; Barbeau et al., 2002; Field-Fote, 2001). Unlike passive exercise with most robotic assist therapies, electrical stimulation based movement therapy uses muscle force to generate movement. In addition to any neuromotor retraining effect, it may 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).

Electrical stimulation of peripheral nerves can promote upregulation of regeneration-associated genes, including brain-derived neurotrophic factor (BDNF), and promote regeneration in axotomized spinal cord motoneurons (Al-Majed et al., 2000; Al-Majed et al., 2004; Geremia et al., 2007). Thus, 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. However, the mechanisms mediating NMES-induced neural plasticity and recovery are unclear and a rodent model could be a useful tool to investigate and optimize this technique. Furthermore, the effectiveness of techniques that combine NMES therapy with other rehabilitation interventions could be further assessed using a rodent model.

Initial development of this NMES model required the design of electrodes suitable for use in rodents and the identification of appropriate stimulation electrode implantation sites in rodents. The implanted electrodes should provide sufficient recruitment of motor units to achieve suitable muscle contractions. Here, we report the results of a detailed investigation of the effect of stimulation pulse parameters (pulse width, amplitude and frequency) on stimulated muscle

responses in anesthetized adult intact rodents. Electrodes were implanted in several hindlimb muscles that are typically active during locomotion and the ability to obtain sufficient isometric joint torque and angle excursions was examined.

2. Materials and Methods

The development of the rodent model for neuromuscular electrical stimulation included the use of a recently designed electrode, surgical identification of the motor points of different hindlimb muscles, and the assessment of muscle recruitment properties as a function of stimulation pulse parameters. All studies were conducted under an Institutional Animal Care and Use Committee approved protocol.

2.1 Electrode Design and implantation

In ten intact, adult, female Long Evans rats weighing 220–250 gms, anesthetized with sodium pentobarbital (40 mg/kg, ip), tracheotomized and breathing room air, custom designed stimulating electrodes were implanted in muscles acting at the hip (biceps femoralis, n=2; Iliacus, n=1), knee (semitendinosus, n=2; vastus lateralis, n=2) or ankle (tibialis anterioris, n=2; gastrocnemius medialis, n=2) joint. To conduct the detailed investigation of the effects of the different pulse parameters, in this study, data was collected from up to 2 implanted muscles per hindlimb from one rat. Level of anesthesia was assessed using toe pinch and maintained using additional ip 15 mg/kg doses every 2–2.5 hrs. 2.0 cc of 0.9% sodium chloride was administered every 2–2.5 hrs to avoid de-hydration. Rectal temperature was monitored and maintained within a range of 36–38°C using warming pads and an infrared heating lamp.

The electrode design and implant methods are similar to those described recently by us for use in neuromuscular stimulation controlled movement of the forelimb (up to four muscles implanted in the same forelimb) for reach-grasp-release (Kanchiku et al., 2008). Multi-joint flexion extension of the elbow and digits could be achieved by coordinated activation of the four muscles of the same forelimb implanted with electrodes. A similar design was also utilized for implanting up to four muscles to achieve hip flexion and extension for both hindlimbs in incomplete spinal cord injured rats provided daily training therapy (Jung et al., 2006). Briefly, a custom monopolar intramuscular stimulation electrode assembly (illustrated in Figure 1A) was made using Teflon coated stainless steel wire, 5-0 non-absorbable suture and 1.5 mm retaining discs. The monopolar design allowed ease of implantation into small or difficult to access muscles. Figure 1B is a schematic representation of the muscles targeted for stimulation, their motor points and the electrode implant procedure. A de-insulated lead sutured to the muscles on the back acted as a ground (return) electrode.

2.2 Isometric Torque measurement

Figure 2 illustrates the setup, force sensor axis orientation and parameters used to measure isometric torque during stimulation of different motor points. The rat was placed on a horizontal base plate, mounted on a custom frame placed on an air table (Vibraplane 2212, Kinetic Systems Inc., Boston MA). The unloaded hindlimb with the muscle of interest was held in place using in-house custom designed braces and stoppers. During data collection, the limb segment with the targeted muscle was strapped to a 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 (Orthletic, 1.3cm Professional Medical Products Inc, Greenwood, SC) (Figure 2A). 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 (Figure 2B). 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 2C). The force transducer and the platform were fixed on two Jiffy-Jack® apparatus positioners (Cole-Parmer Instrument Company, Vernon Hills, IL). The orientation of the end-point force vectors and the joint torques are dependent on the limb geometry (Lan and Crago, 1992). As explained in Warren et al. (Warren et al., 2004) and illustrated by our own study of 3D-kinematics of treadmill walking in Long-Evans rats (Thota et al., 2005), there are multiple reasons to clamp the knee and ankle at 90°. The limb posture is chosen to reflect the positions at which the joint is likely to have peak demands during walking. A knee angle of approximately 90° lies slightly higher than midway between maximum flexion and extension (60° to 111° internal knee angle during treadmill walking in Thota et al. 2005) and occurs during late mid-stance and early push-off when the ankle plantarflexors are active. Ankle angle of approximately 90° occurs about between the maximum dorsiflexion and plantarflexion angles (55° to 120° internal angle during treadmill walking in Thota et al. 2005). A hip angle of approximately 90° occurs at foot contact during treadmill walking (Thota et al. 2005), which is the point in the gait cycle that is likely to require peak hip extension moment. The plateau of the length-tension relationship of the rat gastrocnemius has been shown to occur at the combination of the knee and ankle joint angles chosen (Ettema, 1997; Warren et al., 2004).

A voltage pulse generator (Master-8, A.M.P.I., Jerusalem, Israel) was used to trigger an isolated pulse stimulator (2100, A-M system, Carlsborg, WA) that was configured to generate either single or multiple constant current pulses. The outputs of the force transducer (forces and moments in three directions-Fx, Fy, Fz, Mx, My and Mz) were simultaneously sampled using a computer equipped with a data acquisition board (model PCI-MIO-16E-1, National Instruments; Austin, TX) and displayed on an oscilloscope (Tektronix TDS 3034) to enable real-time monitoring of force output. The JR3 sensor outputs were acquired at 2500 samples per second using custom-designed software (LabVIEW; National Instruments, Inc., Austin, TX). The calibrated force and moment data were analyzed offline using custom-designed software that was developed in MATLAB® (Mathworks, Natick, MA) to calculate joint torques.

2.3 Data analysis

2.3.1 Strength duration (SD) curves—In order to assess the suitability of the electrode implantation site (motor point) for selective recruitment of a muscle, strength duration (SD) curves were generated by plotting the twitch threshold current required (strength levels) versus the pulse widths (duration of the stimulating pulse). The minimum current that generated a force ranging from 0.017–0.03N in any of the X, Y or Z directions was considered as the twitch threshold current. Single, symmetric, charge balanced, biphasic (cathodic first) current pulses of different amplitudes (low to high) were delivered. Decreasing pulse widths from 500–100µsec in steps of 100µsec and 100–10µsec in steps of 10µsec were used. A 60-sec interval was present between consecutive pulse stimuli to prevent carryover effects of the stimulation on muscle recruitment.

From the SD curves, the rheobase (twitch threshold current at very long pulse duration (here 500µsec)) and a range of chronaxie (stimulus duration at the point where the twitch threshold current is twice the rheobase) were defined in order to assess the excitability of the muscle. Low values of rheobase and chronaxie would indicate that the electrode is positioned close to the motor point. In longitudinal studies evaluating stability of electrode placement and recruitment characteristics these value could be monitored.

2.3.2 Isometric Torque Recruitment Curves—Isometric torque recruitment curves (Figure 5) were generated to assess recruitment characteristics of the implanted electrodes and

characterize the effects of altering stimulation parameters. 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. The role of the frequency of stimulation on tetanic responses was also examined.

Specifically, the twitch response was obtained by stimulating the muscle with single, symmetric, charge balanced biphasic (cathodic first) pulses. Since the response of the muscle induced by electrical stimulation depends on the amount of charge (pulse amplitude $\times$ pulse width) delivered to the tissue, twitch torque was altered by modulating either of these parameters. While keeping the pulse width constant at the highest level (500 μ sec), current amplitudes were altered from 1–10mA in steps of 1mA. Pulse widths were then decreased (500, 300, 100, 70, 40 and 20 μ sec) and the process was repeated for each pulse width value. A 60-sec interval was present between consecutive pulse stimuli. 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. The normalized torque was plotted versus the different current amplitudes for each pulse width to generate a set of isometric twitch torque curves.

Isometric steady-state torque recruitment curves obtained by tetanic multiple pulse stimulation were generated using the steady-state step response method (Durfee and MacLean, 1989). The muscle was activated with a stimulus burst (consisting of symmetric, charge balanced, biphasic, cathodic-first pulses) at a fixed current amplitude level for a period of 2.5sec. 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 (Durfee and MacLean, 1989). The frequencies used for stimulation were 25Hz, 50Hz, 75Hz and 100Hz. Four different current amplitudes as multiples of the threshold current at 40 μ sec (the lower end of the chronaxie range) were chosen.

Torque calculations: The forces (F_x, F_y, F_z) and moments (M_x, M_y, M_z) in the three directions X, Y and Z obtained during stimulation were measured by a 6 degree-of-freedom JR3® sensor, custom designed to be sensitive to low torque values. The orientation of forces and moments in the JR3® sensor are illustrated in Figure 2. The reference point for all loading data is the geometric center of the sensor. In order to calculate the joint torque (ankle, knee, or hip), forces and moments were translated and rotated from the reference point, A, to the joint center represented by the point C (Figure 2). Since there is no direct rigid link between point A and point C, the forces and moments were first translated and rotated to the point B and then further translated and rotated from point B to point C. The orientation of the transducer with respect to the animal during ankle, knee and hip joint torque measurement is illustrated in Figure 2.

The translation and rotation of forces and moments from point A to point B is given by,

$${}^B_W = {}^B\Gamma_A {}^A_W \quad (1)$$

w consists of forces (F) and moments (M) acting on the sensor and ${}^B\Gamma_A$ is the transformation matrix that transforms the forces and moments from frame B (${}^B_W$) to frame A (${}^A_W$).

$$w = \begin{bmatrix} F \\ M \end{bmatrix} = \begin{bmatrix} F_x \\ F_y \\ F_z \\ M_x \\ M_y \\ M_z \end{bmatrix} \quad {}^B\Gamma_A = \begin{bmatrix} {}^B R_A & 0_{3 \times 3} \\ [\hat{r}] {}^B R_A & {}^B R_A \end{bmatrix} \quad (2)$$

R is the Rotation matrix and $\hat{r}$ is a 3×3 skew symmetric matrix corresponding to the distance vector from point A to point B given by:

$$R = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad \hat{r} = \begin{pmatrix} r_x \\ r_y \\ r_z \end{pmatrix} \quad (3)$$

The ankle, knee and hip braces were aligned with the X axis and therefore, the rotation matrix is the Identity matrix. r_x , r_y and r_z are the distances in the X, Y and Z directions. The torque was calculated about the Y axis, hence $r_y = 0$.

A similar transformation was done from point B to point of interest C to arrive at the final torque (in the Y direction) as:

$$T = F_x dz + F_z dx + M_y \quad (4)$$

2.3.3 Joint angle measurement—During trials assessing the effects of 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 (Thota et al., 2005). Three cone-shaped reflective markers made from 3M infrared reflective tape were placed on the bony protrusions of the anterior rim of the pelvis, head of the greater trochanter, and the lateral malleolus. A strip was placed on the toe (fifth metatarsal head). Since, skin slippage at the knee can be considerable, an infrared LED was affixed to the bone (lateral head of the femoral condyle), using Teflon-coated stainless steel wire. The infrared cameras of the PEAK MOTUS system were focused to capture the locations of reflective markers during limb movements produced by electrical stimulation. The video records were analyzed offline to determine the joint angles.

3. Results

3.1 Anatomy of target muscles and motor point location

Guided by published anatomical data in the albino rat (Greene, 1955) and our own extensive anatomical dissection studies in the Long Evans rat, we were successfully able to locate implantation sites that enabled selective stimulation of target muscles in the rodent hindlimb. Figure 3 illustrates the detailed anatomy of the muscles and the sites that were identified as suitable for electrode implantation. Table 1 provides a list of abbreviations describing the different muscles identified during anatomical dissection. The tibialis anterioris (TA) was targeted for implantation to achieve ankle flexion. This muscle is situated beneath the biceps femoralis for knee flexion (BF_k). It has its origin on the margin of the lateral condyle, tibial tuberosity (TT) and ventral crest of the tibia. The deep peroneal nerve innervates the TA close to the femoral head (FH) (Figure 3A). In Figure 3A, the BF_k was removed and the TA elevated

off the tibial head for better illustration of the motor point. The gastrocnemius (GM), which is innervated by the tibial nerve and can be activated to produce ankle plantar flexion, is made up of two heads, the medial and lateral. We targeted the medial gastrocnemius for stimulation which arises from the medial epicondyle of the femur and from the medial fabella. In Figure 3B, the overlaying gracilis muscle was removed and the GM elevated to illustrate the tibial nerve innervation and motor point location.

The vastus lateralis (VL), which was selected to provide knee extension, arises at the greater trochanter and the third trochanter and inserts by the ligamentum patellae into the tuberosity of the tibia. It is innervated by the posterior division of the femoral nerve. In order to illustrate the muscle and its innervation, the BF_k and the gluteus superficialis were removed in Figure 3C. The semitendinosus (ST), which was targeted for knee flexion, arises by two heads, the principal and accessory heads. Of interest to our study, for targeted stimulation, is the principal head arising from the posterior part of the sciatic tuber. It inserts onto the medial side of the leg at the distal end of the tuberosity of the tibia. The principal head is innervated by the tibial division of the sciatic nerve. In Figure 3D, for illustration the BF_k was removed and the lateral and medial views show the innervating nerve and the electrode insertion approach (needle insertion direction).

The iliacus (IL) and the biceps femoralis anterior head (BF_h) were targeted for hip flexion and extension, respectively. The iliacus arises from the fifth and sixth lumbar vertebrae and is inserted into the lesser trochanter of the femur. A branch of the anterior division of the femoral nerve innervates the muscle (Figure 3E). For illustration, the external abdominal oblique (EO) muscle was cut and elevated in order to expose the IL and identify its motor point. The biceps femoralis has three parts; the anterior head, posterior head and the accessory head. The anterior head arising from the last sacral and first caudal vertebrae inserts into the distal end of the femur and hence on stimulation does not cause knee flexion. A branch of the common peroneal nerve innervates the anterior head and this head was targeted for achieving hip extension (Figure 3F).

3.2 Strength duration curves

The excitability thresholds of all of the implanted electrodes were determined through SD curves shown in Figure 4. The curves show a typical nonlinear hyperbolic relationship in which stimulation pulses with shorter pulse widths require higher current amplitude to reach threshold. Typical rheobase values ranged from 0.07mA to 0.35mA. The average rheobase value across all muscles was calculated to be 0.16mA $\pm$ 0.02. The chronaxie values ranged from 40–90 μ sec.

3.3 Isometric torque recruitment by twitch stimulation

Figures 5A, 5B, 5C, 5D, and 5E illustrate the torque generated in response to single pulse twitch stimulation of the ankle dorsiflexor (TA), ankle plantarflexor (GM), knee extensor (VL), knee flexor (ST) and hip extensor (BF_h), respectively. In general, for a given pulse width, increase in current amplitude (and hence total charge) resulted in higher recorded torque values and plateau was sometimes observed at higher current values. When charge levels were very high (current amplitude=9mA or 10mA; pulse width=300 μ sec or 500 μ sec), a reduction in the torque sometimes occurred. For example, in the extreme case for TA stimulation, the torque actually reversed direction. The reduction in joint torque (or reversal in direction) is likely due to spillover and recruitment of adjacent antagonist muscles. The higher amplitude ranges for the longer pulse widths of 300 μ sec and 500 μ sec were not tested in the GM as spillover was visually observed.

Similarly, for a given current amplitude, increase in pulse width (and hence total charge) resulted in greater torque. In all muscles, the recruitment curve had a monotonically increasing portion over a range of current amplitudes. For many muscles, a 40 μ s pulse width provided a wide current amplitude range over which torque gradually increased. Larger pulse widths of 300 μ sec or 500 μ sec resulted in a steeper recruitment curve for the knee muscles (VL and ST) and, in the case of the BF_h, the twitch torque response cycled between positive and negative values as current amplitude was increased. The results thus suggest a complex relationship between charge levels and the activation of agonist and antagonist muscles. While not illustrated, qualitatively similar torque recruitment responses were obtained until spill over from the additional rodent strength-duration curves are shown in Figure 4.

3.4 Effect of stimulation frequency on fusion of muscle contraction

Across the set of muscles tested, fused contractions were observed at the higher stimulation frequencies (75 Hz, 100 Hz) but not at the lower stimulation frequencies (25 Hz, 50 Hz). For example, Figure 6A illustrates the ability to obtain fused contractions by varying the frequency of stimulation of the ankle plantarflexor, GM. The force response obtained in the z-direction for the initial 0.5sec of stimulation is shown. Stimulation was provided for 2.5sec with a pulse width of 500 μ s at 1.5 times the twitch threshold current obtained with a 40 μ s pulse width. At frequencies of 25Hz and 50Hz, the contractions were not fused; however, at 75Hz and 100Hz, the torque traces were relatively smooth, thus indicating a fused contraction. A similar response was obtained for all other muscles at all current levels and pulse widths tested (not shown).

3.5 Steady-state step responses to multiple pulse stimulation

Figure 6B illustrates the typical steady state force traces observed during steady-state step response to multiple pulse stimulation delivered for 2.5 s, at different pulse widths and fusion frequency of 75Hz. Note that higher values of pulse width (higher charge) produced higher values of force, but the relationship was nonlinear.

To determine steady state isometric torque, the torque calculated from the forces and moments was averaged over the last 0.5 s of the 2.5 s stimulation burst. Typical steady state isometric torque obtained during multiple pulse stimulation at various frequencies for different muscles is shown in Figure 7. The data represents recruitment curves for the different muscles (n=1 for each muscle).

The amount of torque generated at a 25Hz stimulation frequency was relatively small at all pulse widths (Figure 7). For the higher stimulation frequencies of 75Hz and 100Hz, the recruitment curves usually consisted of two regions; a region in which torque increased linearly with pulse width followed by a “plateau” region in which torque was relatively constant.

Figures 7A, 7B, 7C, 7D and 7E illustrate the steady state isometric torque obtained during stimulation of the ankle dorsiflexor (TA), the ankle plantarflexor (GM), the knee extensor (VL), the knee flexor (ST), and the hip extensor (BF_h). At a given pulse frequency, steady-state torque was related to pulse width in a nonlinear manner and, in general, pulse trains at higher stimulation frequencies resulted in higher torque production. At the lower frequencies of 25Hz and 50Hz, which did not produce fused contractions, increases in pulse width had little effect. At 75Hz and 100Hz, where fused contractions were observed, there was a graded increase in torque within a pulse width range of 10–100 μ s that was followed by a significantly slower rise in the torque at larger pulse widths, for example for TA, VL, and ST. For both 75Hz and 100Hz stimulation, the recruitment curves exhibited a steeper slope within the pulse width range of 10–100 μ s for the TA, VL, ST and BF_h muscles than for lower stimulation frequencies. At the highest charge level (500 μ s pulse width and 100Hz stimulation frequency), an additional non-monotonic increase in the torque was observed in some cases, for example in the TA and

BF_h. This may be due to spillover of charge to adjacent synergistic muscles. The maximum steady state torque produced by stimulation of the ankle dorsiflexors and knee flexors were 13 Nm/kg and 10 Nm/kg respectively while the maximum torque produced during activation of the ankle plantarflexor, knee extensor, and hip extensor were 5 Nm/kg, 13 Nm/kg, and 2 Nm/kg respectively.

3.6 Joint angle excursion during steady-state step response

To generate locomotor type patterns it will be necessary to obtain sufficient angular excursion during the electrical stimulation. The joint angle curves and corresponding stick figures shown in Figure 8 illustrate this ability to achieve movements in a specific joint direction using multiple-pulse stimulation at 100Hz, pulse widths within the lower range of chronaxie (40µs–70µs), and current amplitude of 1.5 times the twitch threshold current at 40µs. Figure 8A shows ankle joint angle changes (35° excursion) when the ankle dorsiflexor (TA) was stimulated while Figure 8B shows joint angle changes (35° excursion) when the ankle plantarflexor was stimulated (GM) in an unloaded freely hanging limb. The hip and knee joint angle change during ankle muscle activation is minimal. Selective activation for knee flexion (Figure 8C, ST stimulated for 30° excursion) or knee extension (Figure 8D, VL stimulated for 21° excursion) can also be obtained. The excursion ranges observed are similar to the ranges observed during treadmill walking by intact rats (Thota et al., 2005).

4. Discussion

In this study we have presented a rodent model for neuromuscular electrical stimulation of hindlimb muscles. The model could be utilized in an animal movement therapy protocol to investigate the putative mechanisms that underlie improvements in sensorimotor function. We have identified the anatomical location of motor points for several flexor and extensor muscles of the rodent hindlimb and we have described detailed anatomical and surgical techniques to identify these motor points. Selective and graded recruitment in the target hindlimb muscles can be achieved by using customized intramuscular stimulating electrodes and appropriate ranges of stimulation parameters. We have characterized the 15 effects of altering the pulse stimulation parameters (pulse width, amplitude and frequency) on the recruitment curves. These recruitment curves provide guidelines for selecting stimulation parameters (or stimulation parameter ranges) to be used in NMES movement therapy protocols. The recruitment curves suggest that short pulse widths have the advantage of producing less steep recruitment curve when current is used as the control parameter. A stimulus frequency of 75Hz or more produces fused contraction in the hindlimb muscles of the rat. Sufficient and selective angular excursions could be obtained by stimulating the target muscles.

4.1 The intramuscular stimulating electrode

Multiple types of electrodes have previously been utilized for providing NMES and use of each electrode type has advantages and disadvantages (Venkatasubramanian et al., 2006). Surface electrodes are non-invasive but require higher threshold current levels and cannot provide selective and repeatable stimulation. Epimysial or intramuscular electrodes placed on or in the muscle, and intraneural or extraneural electrodes placed within or adjacent to a motor nerve are invasive but require lower current injection and may be better suited for longer-term chronic use. In cats, nerve and intramuscular stimulation have been shown to produce movement of up to 60% of the passive range of motion at the joint in all directions. Nerve cuff electrodes have been reported to exhibit steep recruitment characteristics, require sophisticated approaches to provide selective stimulation and can be susceptible to encapsulation. On the other hand, intramuscular electrodes have been effectively utilized in a number of studies to provide selective activation of individual muscles or muscle groups in both humans and in animal models. Existing intramuscular electrodes are not suitable for implantation in the smaller sized

muscles of the rodent, hence we used an intramuscular stimulating electrode assembly that we designed to be sized appropriately and that utilizes a surgical approach that is suitable for rodents (Kanchiku et al., 2008). The intramuscular stimulating electrode was fabricated from Teflon coated stainless steel in order to provide high tensile strength and low electrical resistivity. The coiled configuration was used to reduce strain on the implanted electrode during limb movement. The retaining discs and the suture attached to the electrode helped to anchor the electrode within the muscle. The suture also allowed repositioning of the electrode to optimize proximity to the motor point. Such a design would also allow for corrective repositioning of the electrodes in a chronic implant, if necessary.

4.2 Mapping electrode implantation and muscle stimulation sites

Stimulation sites closer to the motor point require lower charge levels for activation. Using published data on rat anatomy as a guide (Greene, 1955) we initially conducted extensive anatomical investigation and dissection studies to identify the main neural innervation to the target muscle. This study contributes to further identification and mapping of the motor points using electrophysiology to determine the appropriate electrode implantation sites. In earlier studies with intramuscular electrodes implanted in the tibialis anterior of the cat, the rheobase current was reported to be 5.2mA and the chronaxie 85 μ s; for electrodes implanted near the peroneal nerve, the rheobase was identified to be 0.38mA and chronaxie 55 μ s (Crago et al., 1974). We were able to achieve low rheobase currents with our implantation technique. The rheobase value of 0.16mA ($\pm$ 0.02 SEM) obtained from the SD curves (Figure 4) suggests that we were able to implant the electrodes very close to the motor point. Similar SD curves were obtained across the different muscles, thus suggesting that electrodes were consistently and reliably implanted at suitable locations.

4.3 Muscle recruitment and generation of joint angle torque

For use in a movement therapy protocol, it will be important that the NMES system be able to reliably produce graded control of force. A broad input parameter range over which a linear increase in joint torque can be obtained would be beneficial for the control system. We therefore investigated the effects of the stimulation parameters (pulse width, amplitude and frequency) on the recruitment characteristics. Twitch isometric recruitment curves were generated by single pulse stimulation (Figure 5) and steady-state isometric torque curves were generated by multiple pulse stimulation (Figure 7).

In general, for a particular pulse width value, higher stimulus amplitudes and higher stimulus frequencies generated higher torques, but both types of recruitment curves were nonlinear. During steady-state step response, a graded and qualitatively linear increase in torque was obtained at 75Hz stimulation frequency within a pulse width range of 10–100 μ sec. At the higher charge levels (high pulse width, amplitude or frequency), a decrease or saturation in the recruitment pattern was observed. Since there can be close anatomical location of nerves or nerve branches innervating adjacent muscles in the rodent, a high charge injection could cause spillover to these adjacent nerves thereby causing muscles innervated by them to contract. If the effect of these extraneously contracting muscles is antagonistic to the targeted muscle a net reduction in torque generation will occur. For example, the complexity in the recruitment curve at the higher charge levels (Figure 5E, Figure 7B, Figure 7E) could be due to spillover to both non-synergist and synergistic muscles. Such recruitment characteristics have previously been observed with intramuscular (Gruner and Mason, 1989), intrafascicular (Nannini and Horch, 1991), nerve (Grill and Mortimer, 1996) and spinal cord (Mushahwar and Horch, 2000) stimulation techniques in cats.

The data also suggest that short duration stimulus pulses have the advantage of producing less steep recruitment curves when current amplitude is used as the control parameter (Figure 5).

To achieve low or intermediate levels of joint torque, it may be advantageous to use short pulse width value and modulate pulse amplitude to get graded control of muscle contraction as has been noted before (Crago et al., 1974; Nannini and Horch, 1991). With small pulse widths there is a better selectivity in recruitment of different nerve fibers (Grill, 2004). This can facilitate better gradation in force output. The charge required for excitation decreases as duration decreases, thereby reducing power requirements and reduced electrode corrosion and tissue damage. All of these considerations favor use of smaller pulse widths for stimulation. For higher levels of joint torque, larger pulse width values may be required to generate sufficient muscle contractions.

The amount of torque generated is also dependent on the position of the limb as defined by muscle length and joint angle. Angles defined for torque measurement at the ankle, knee and hip in this experiment were chosen to maximize torque generation. Though care was taken to appropriately position the limb, it is not possible to achieve exact angular placement across different animals. Therefore, though similar torque patterns were observed for a particular muscle, the absolute value of the torque generated varied. Thus, while maximum values obtained are reported, the data were interpreted in terms of the normalized torque values.

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 at the ankle, 0.014 Nm/kg at the knee and 0.015 Nm/kg at the hip and are likely typical for tailed species (Witte et al., 2002). Data for the rodent species during overground walking are not available and therefore we compared our isometric torque data to that from the shrew. The angular torque values (normalized to bodyweight) that we were able to generate during tetanic stimulation for several combinations of pulse-widths and frequencies (see results) were considerably larger than those reported for the tree shrew. In another study using anesthetized Sprague-Dawley rats (Warren et al., 2004), peak values for stimulated responses of ankle plantarflexors were reported to be approximately 0.7 Nm/kg. Using our electrodes, we were able to achieve more than 5 times that value, thus suggesting that this electrode design and placement technique are effective.

4.4 Fused contractions at 75Hz and 100Hz

In our experiments, fused contractions were observed at 75Hz and 100Hz stimulus frequency. The force response obtained during 25Hz and 50Hz stimulation frequency was not fused (Figure 6A). Twitches generated by the slow motor units can reach a tetanic state at 25–30Hz while the fast motor units may achieve fusion at 80–100Hz (Burke, 1981; McPhedran et al., 1965). Our observations agree with these observations and reflect the presence of predominantly fast twitch muscle fiber types in rat hindlimb with the exception of the soleus muscle (Armstrong and Phelps, 1984; Gramsbergen et al., 1996). The fusion frequencies also agree with the reported range of discharge rates of fast muscles (60 to 100 Hz) versus the rates from slow muscles (about 30 Hz) recorded during locomotion in the conscious rat (Gorassini et al., 2000).

4.5 Sufficient and selective angular excursion

To generate locomotor type movement patterns it is necessary to obtain sufficient angular excursion during electrically stimulated movements. Using NMES we were able to achieve a total excursion of 70° at the ankle joint (35° for flexion and 35° of extension, Figures 8A, 8B) and a total excursion of 51° at the knee joint (30° flexion and 21° of extension Figures 8C, 8D). These values are very close to the ranges of motion (flexion plus extension) at the ankle joint (approx. 65°) and knee (approx. 51°) that we previously observed in 3D-kinematic analysis during treadmill walking for Long-Evans rats (Thota et al., 2005). The hip extensor angle values during NMES were lower than those obtained during treadmill walking, since in

the unloaded position utilized during NMES the hip was already in an extended position thereby allowing for a very narrow extension range (data not shown). We also observed that there was very little movement of the hip and knee during ankle activation and hip and ankle during knee activation suggesting selective activation of muscles can generate appropriate movements. Additionally, for the knee and ankle, the excursion in joint angle was achieved rapidly and hence short bursts of stimulation may be sufficient to obtain typical movements.

4.6 Additional studies and use of 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. It is important to note, however, that in this study, the responses were not affected by normal or altered reflexes that may be active in awake, incomplete or complete spinal cord injured rodents or other models of awake, CNS impaired rodents. Additionally, this study does not address the stability and performance of chronically implanted electrodes repeatedly used for muscle stimulation that would be needed for several weeks of NMES-assisted movement therapy in awake rodents. In other work, we have evaluated the effectiveness and stability of these electrodes and recruitment patterns in awake, spinal cord transected rodents over several weeks (Venkatasubramanian et al., 2005) and the ability to provide movement therapy using NMES of a limited muscle set in an awake incomplete thoracic contusion injury rodent model (Jung et al., 2006). Collectively, our prior work and the current study demonstrate electrode stability, sufficient muscle recruitment, the long-term administration of NMES therapy in a limited muscle set, and the activation of a muscle set that may be sufficient for locomotion. It should be noted, however, that NMES is likely to use only a subset of the muscles typically activated during overground locomotion.

It has been suggested that passive movement therapy can prevent degradation in the dendritic arbor of motoneurons below the injury site and restore motoneuronal properties (Beaumont et al., 2004; Gazula et al., 2004). Active movement therapy can be provided by patterned NMES, during which there is rhythmic limb activity and active contraction of muscles results in activation of sensory afferents of receptors in the muscles, tendons, and joint capsules usually present during natural movement (Lynskey et al., 2008). With the development of an NMES rodent model, future studies could be conducted to compare different types of movement therapy, for example very repeatable cyclic movement, movements with cycle-to-cycle variability in movement, movements with different types of afferent inputs and load bearing. For cutaneous afferent inputs, it may suffice to have a bristle bush across which the plantar surface of the foot brushes as the limb goes through a flexion-extension cycle mimicking previous approaches for providing non load-bearing cutaneous inputs (Muir and Steeves, 1995). To generate stepping patterns, NMES therapy may have to be administered in the context of a partial body weight supported therapy paradigm, as is being done for people with spinal cord injuries (Field-Fote, 2001; Sheffler and Chae, 2007). Some of these paradigms for more complicated stepping patterns will require sophisticated multi-joint adaptive control algorithms. We have recently initiated work on such adaptive control algorithms (Kim et al., 2007).

This study lays the initial groundwork for the development of a neuromuscular electrical stimulation-assisted movement therapy paradigm that could be used to study recovery of locomotor function in rodent models of spinal cord injury, traumatic brain injury or stroke. Such rodent models could be utilized to investigate mechanisms of action, identify key windows of opportunity for maximizing therapeutic efficacy, explore combination therapies, and maximize the impact of neural plasticity on functional abilities.

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Figure 1.

Intramuscular electrode design, motor points, and implant procedure. (A) Intramuscular stimulating electrode consisting of a suture string (blue) and retaining disc (green circular disc) on the stimulating side to allow for easy and stable anchoring. Coiled electrode wire provides strain relief, and pin connector crimped to the other end allows inline connection to another lead connected to a head connector. (B) Target hindlimb muscles and their motor points (indicated by ★) along with the implant procedure. Primary function of each muscle is indicated in parenthesis. TA-Tibialis anterioralis, GM-Gastrocnemius medialis, VL-Vastus lateralis, ST-Semitendinosus, BF_h- Biceps femoralis, IL- Iliacus. (1) Approximate motor point location is found by stimulating skin surface using a 30G needle connected to the neurostimulator. (2) After skin incision, a curved needle holding the electrode is inserted beside the 30G needle. (3) The curved needle holding the electrode-suture assembly is pulled out and the de-insulated area of the electrode is positioned near the motor point. (4) The most appropriate location at which maximal muscle contraction was obtained on stimulation with 200μsec cathodic pulses at 100Hz with the least amount of current (usually 0.1–0.2 mA)) is detected and the electrode is anchored using retaining discs and non-absorbable suture.

Figure 2.

Isometric torque recruitment measurement and experimental set up. Experimental set up to measure the isometric torque recruitment curve for (A) Hip joint (IL, BF_h). (B) Knee joint (VL, ST). (C) Ankle joint (TA, GM). The rat was held on a platform and custom-made braces were attached to the transducer for each joint. The limb was attached to the brace using porous tape. Stoppers and holders were used to position each limb at a specific angle. Also shown is the force sensor axis orientation and parameters for isometric torque calculation.

Figure 3.

Detailed anatomy of the targeted hindlimb muscles highlighted in Figure 1B. (A) TA lateral view: BF_k was removed and proximal side of TA was elevated off the tibia. (B) GM medial view: GR and GM were elevated. (C) VL medial view: GR was removed. VL lateral view: GluS and BF_k were removed. (D) ST lateral view: BF_k was removed. ST posterior view: BF_k was removed. (E) IL medial view: EO was cut and IL was elevated. (F) BF_k lateral view: BF_k was separated from BF_h and detached from the tibial bone and elevated. The notations and symbols used in the figure are detailed in Table 1. ★ - motor point; ▲ - landmark; ─ - 5mm.

Figure 4. Strength-duration curves for the different hindlimb muscles. A) Tibialis anterioris (TA). B) Gastrocnemius medialis (GM). C) Vastus lateralis (VL). D) Semitendinosus (ST). E) Biceps femoralis (BF). F) Iliacus (IL). The symbols (○, Δ) indicate a different rat for the same muscle.

Figure 5.

Isometric torque recruitment curves obtained by modulating the pulse amplitude during single pulse stimulation (pulse amplitude modulation) at different pulse width levels (..... 500 μ s, ... 300 μ s, Δ 100 μ s, $\times$ 70 μ s, $\Diamond$ 40 μ s, $\blacklozenge$ 20 μ s). A) Ankle joint torque obtained during stimulation of TA. B) Ankle joint torque obtained during stimulation of GM. C) Knee joint torque obtained during stimulation ST. D) Knee joint torque obtained during stimulation of VL and E) Hip joint torque obtained during the stimulation of BF_h.

Figure 6.

Effects of stimulus frequency and pulse width changes on force and torque generation during stimulation of the gastrocnemius muscle. A) Force pattern at varying stimulus frequencies shows fused contractions at 75Hz and 100Hz stimulation (pulse width=500 μ sec, current amplitude=1.5 times twitch threshold for 40 μ s pulse) B) Steady-state torque generated at different pulse widths during multiple pulse stimulation (frequency=75Hz, current amplitude=1.5 times twitch threshold for 40 μ s pulse). At longer pulse widths maximal torque generated is higher.

Figure 7.

Isometric torque recruitment curves obtained during multiple pulse stimulation. A) The steady state torque obtained at the ankle joint during stimulation of TA. B) The steady state torque obtained at the ankle joint during stimulation of GM. C) The steady state torque obtained at the knee joint during stimulation of ST. D) The steady state torque obtained at the knee joint during stimulation of VL. E) The steady state torque obtained at the hip joint during stimulation of BF_h. The steady state torque was calculated at four different frequencies – 25Hz (x), 50Hz (▲), 75Hz (■) and 100Hz (●) at 1.5T where T is the twitch threshold current obtained with a 40μs pulse width.

Figure 8.

Stick figures and joint angles curves generated during muscle stimulation with multiple pulses. Steady-state change in angle during activation of A) TA ($\Delta=35^\circ$), B) GM ($\Delta=35^\circ$), C) VL ($\Delta=21^\circ$) and D) ST ($\Delta=30^\circ$). The corresponding stick figures show multiple frames of videos (60 frames/sec) during tetanic stimulation.

Table 1

Description of abbreviations used to label Figure 3. Function of muscles stimulated is described in Figure 1 and explained in the text.

Abbreviations	Description
BFh	Biceps femoralis, hip extension
BFk	Biceps femoralis, knee flexion
EO	External abdominal oblique
FC	Femoral condyle, lateral
FH	Fibular head
GL	Gastrocnemius lateralis
GluS	Gluteus superficialis
GM	Gastrocnemius medialis
GT	Greater trochanter
GR	Gracilis
IL	Iliacus
RF	Rectus femoralis
ST	Semitendinosus
TA	Tibialis anterior
TH	Tibial head
TT	Tibial tuberosity
VL	Vastus lateralis