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NEUROMUSCULAR ELECTRICAL STIMULATION INDUCED FORELIMB MOVEMENT IN A RODENT MODEL

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Abstract

Upper extremity neuromuscular electrical stimulation (FNS) has long been utilized as a neuroprosthesis to restore hand-grasp function in individuals with neurological disorders and injuries. More recently, electrical stimulation is being used as a rehabilitative therapy to tap into central nervous system plasticity. Here, we present initial development of a rodent model for neuromuscular stimulation induced forelimb movement that can be used as a platform to investigate stimulation-induced plasticity. The motor points for flexors and extensors of the shoulder, elbow, and digits were identified and implanted with custom-built stimulation electrodes. The strength-duration curves were determined and from these curves the appropriate stimulation parameters required to produce consistent isolated contraction of each muscle with adequate joint movement were determined. Using these parameters and previous locomotor EMG data, stimulation was performed on each joint muscle pair to produce reciprocal flexion/extension movements in the shoulder, elbow, and digits, while 3D joint kinematics were assessed. Additionally, co-stimulation of multiple muscles across multiple forelimb joints was performed to produce stable multi-joint movements similar to those observed during reach-grasp-release movements. Future work will utilize this model to investigate the efficacy and underlying mechanisms of forelimb neuromuscular stimulation therapy to promote recovery and plasticity after neural injury in rodents.

Keywords

neuromuscular electrical stimulation; forelimb; rodent; reach; grasp; kinematics

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1. Introduction

Approximately 250,000 people live with spinal cord injury (SCI) in the United States, with 11,000 new cases annually. Cervical spinal cord injuries affect more than half of those individuals and produce significant and lasting impairments in sensory and motor function in all four extremities (tetraplegia), as well as autonomic function, frequently resulting in severe and permanent disabilities (Center, 2003). Among the most devastating effects of injuries to the cervical spine is the loss of hand function. When asked to prioritize lost functions that they would most like to regain, the majority of individuals living with cervical spinal cord injury list hand function at or near the top of their list (Anderson, 2004). The loss of arm and hand function severely limits independence and employment opportunities, which can greatly increase the extent, duration, and costs of care. Thus, improving hand function is an important rehabilitation objective for people with tetraplegia and new approaches to providing therapy to enhance recovery of upper limb function are needed.

Electrical stimulation has been used to activate paralyzed muscles in human subjects for a variety of applications in both the research laboratory and the clinic. Functional neuromuscular stimulation (FNS) is a rehabilitation strategy that applies sequences of low-level electrical current pulses to nerves that control muscles in order to generate functional movements. The technique has been used successfully as a neuroprosthesis for restoration of motor function in individuals with neurological disorders such as spinal cord injury and stroke (Bajd et al., 1985; Barbeau et al., 1999; Field-Fote, 2001; Peckham and Keith, 1992; Ring and Rosenthal, 2005; Stein et al., 1993; Yarkony et al., 1992). For example, an implanted electrical stimulation device, the “Freehand system” that can restore hand grasp function to people with tetraplegia secondary to spinal cord injury and hemiparesis secondary to stroke has FDA approval (Keith, 2001; Peckham et al., 2001), and has been implanted in more than 230 individuals. A non-invasive electrical stimulation device, the “Ness-H200”, has been recently developed for the restoration of hand grasp and release for people with impaired hand function after spinal cord injury or stroke and is now available in the clinic (Hendricks et al., 2001; Ring and Rosenthal, 2005; Snoek et al., 2000). Both the Freehand Systems and Ness-H200 are being used with success as neuroprostheses, clearly demonstrating that neuromuscular stimulation can be effectively used to activate paralyzed muscles for performing motor activities of daily living. In addition, evidence is emerging that suggests that neuromuscular stimulation used as a therapeutic intervention can improve recovery of upper extremity function in individuals with either complete or incomplete cervical SCI (Beekhuizen and Field-Fote, 2005; Daly et al., 1996; Popovic et al., 2006; Rushton, 2003). While the mechanisms mediating FNS induced neural plasticity and recovery are unclear, there are studies suggesting that peripheral electrical stimulation 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). Thus, the observed improvements in functional behavior promoted by FNS therapy could be mediated by molecular plasticity in the central nervous system.

The variability in functional capacity and motor recovery that is observed across individuals makes it difficult to quantitatively assess the therapeutic benefits of FNS-assisted retraining. An animal model would be very useful in examining the different levels of plasticity induced by this mode of therapy. However, there are no published animal models of FNS induced upper extremity (forelimb) movement that could enable controlled studies to assess the efficacy and the mechanisms involved in FNS-assisted reach-grasp-release. Initial development of such a model requires suitable electrodes and identification of appropriate implantation sites for stimulation electrodes. It is also essential that the implanted electrodes provide sufficient recruitment of motor units to achieve appropriate contraction of the muscles. Previously, we have successfully developed a rodent model for neuromuscular stimulation controlled

movement of the hindlimbs (Ichihara et al., 2004). Here, we have successfully developed this technique to produce FNS-controlled forelimb movement (reach-grasp-release) in rodents. This rodent model of FNS-controlled forelimb movement could be used to assess mechanisms of neuromotor plasticity for this therapeutic rehabilitation technique.

2. Materials and methods

2.1. Animals

Six adult female Long Evans rats (Charles River, 250-300g) were individually housed in an AALAC accredited university animal care facility with a 12-hour light/dark cycle, with access to food and water *ad libitum*. Detailed anatomical dissection was performed in two animals to identify and locate anatomical landmarks for the motor points of several forelimb muscles guided by previously a published albino rat anatomical reference (Greene, 1955). An additional four animals were used to determine the stimulus parameters that provide selective activation and sufficient recruitment of motor units to achieve appropriate contraction of each targeted forelimb muscle. Two of those four were also used to assess the ability to produce rhythmic reach-grasp-release movements, consisting of shoulder forward flexion with abduction, elbow extension, and digit extension followed by simultaneous digit flexion, elbow flexion and shoulder extension followed by passive digit extension, elbow extension, and shoulder flexion to a neutral position

All procedures listed were approved by the Arizona State University Institutional Animal Care and Use Committee.

2.2. Electrode implantation

Custom monopolar intramuscular stimulating electrodes were implanted in muscles acting at the shoulder (extensor/external rotator, spinodeltoideus, n=4; flexor/abductor, supraspinatus, n=4), elbow (extensor, triceps brachii, n=4; flexor, biceps brachii, n=4) and digit (extensor, extensor digitorum communis, n=4; flexor, flexor digitorum profundus, n=4) joints. Both the custom stimulation electrodes and implant procedure were similar to those developed in a previous study in our laboratory (Ichihara et al., 2004). Similar intramuscular and epimysial electrodes have been approved by the Federal Drug Administration, USA and been implanted for functional electrical stimulation in people with spinal cord injuries for long durations (Agarwal et al., 2003; Kobetic et al., 1999; Peckham et al., 2001; Sharma et al., 1998).

Prior to surgery, animals were anesthetized with Isoflurane gas (1.5- 2%, inhaled). Toe pinch and visual monitoring of respiration were used as indicators of adequate anesthesia. The rectal temperature of the rat was monitored and maintained within a range of 36-38°C using warming pads and an infrared heating lamp during experiment.

The monopolar stimulating electrodes were implanted at the motor point of the unilateral spinodeltoideus, supraspinatus, triceps brachii, biceps brachii, extensor digitorum communis and flexor digitorum profundus muscles. After exposing the muscle through the appropriate incision site, the approximate location of the motor point of each muscle was determined by stimulating the muscle surface using a 30 G straight needle (diameter; 0.63mm) using a handheld stimulator (DigiStim 3 Plus, Neuro Technology Inc. Kerrville, TX). The needle was then inserted into the muscle to find the 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). Once the position was located, the electrode-suture assembly was connected to a curved eye needle (MANI Co., Ophthalmic Taper point 5/16 circle, Spring eye, Size 0, Length 13mm). The needle was inserted adjacent to the 30 G straight needle. The latter was removed and the curved needle was used to thread the suture-electrode lead assembly

through the muscle. The location of the de-insulated portion of the electrode lead was set adjacent to the appropriate motor point while the suture entered and exited the muscles in an arc. The free ends of the suture were threaded through 1.5 mm wide circular endplates. The location of the de-insulated portion of the electrode lead was verified using stimulation of the free end and the endplates were then sutured to the surface of the muscle membrane using 5-0 non-absorbable suture (Dexon II ; United States Surgical, Tyco Healthcare, Mansfield, MA) to anchor the electrode-suture assembly. A 1 cm circular ground connector made from 0.003" stainless steel shim stock was sutured to the muscles on the back.

2.3. Strength duration (SD) curves and stimulation kinematics

After the electrode implantation surgery, anesthetized animals were placed on an underbelly platform such that the head and torso were supported. The platform was designed to allow the unloaded forelimbs to move freely.

In order to assess the suitability of electrode implantation at the motor point, strength duration curves, a graphical representation of the threshold current required to activate the tissue, were determined for each muscle implanted. The curves are generated by plotting twitch threshold currents (strength levels) versus the pulse widths (duration of the stimulating pulse). The twitch threshold current was defined as the minimum current required for producing a visually discernible twitch in the muscle. Threshold currents were obtained for 210 msec duration stimulation at 75 Hz using biphasic (cathodic first) pulses with pulse widths of 10, 20, 40, 70, 100, 300, or 500 μ sec/phase. The frequency for stimulation was fixed at 75 Hz, since fused contractions were observed at this stimulus frequency in a previous study (Ichihara et al., 2004).

From the curves, the rheobase and a chronaxie were defined. Rheobase is defined as the twitch threshold current at very long pulse duration (here 500 μ sec). Chronaxie is defined as the stimulus duration at the point where the twitch threshold current is twice the rheobase. These measures allowed quantitative measures to assess the excitability of the muscle. Threshold data are represented as average $\pm$ standard error (SE).

Electrical stimulation of the muscles in the freely hanging unloaded forelimb caused movement. This movement was monitored and the joint angle determined using a reflective marker based kinematic analysis system (Peak Motus v. 8.5, Vicon, Centennial, CO) as described in detail previously (Thota et al., 2005). Briefly, cone shaped three dimensional reflective markers (3M infrared reflective tape) were placed on the scapula (lateral aspect), shoulder (greater tubercle), elbow (lateral epicondyle), wrist and digit (fourth digit) of the forelimb respectively. The infrared cameras of the Peak Motus system were focused to capture the reflective markers during limb movement on electrical stimulation. A computer controlled custom stimulator (FNS-16, CWE, Inc.) provided electrically isolated constant current pulses for neuromuscular stimulation of the forelimb muscles using the implanted FNS electrodes. The pulse widths used for stimulation were 40, 70, 100 or 300 μ sec at a current amplitude of 1.5 times the twitch threshold current at 40 μ sec or 3 times the twitch threshold current at 40 μ sec. The captured video data was analyzed offline to determine the recruitment curves. The appropriate stimulation parameters required to produce consistent isolated contraction of each muscle with adequate joint movement were determined using these joint angle data.

Using these parameters and previous locomotor EMG data (Thota et al., 2005), pre-specified (open-loop) stimulation patterns were developed and the pulse trains were delivered to each joint muscle pair to produce reciprocal flexion/extension movements in the shoulder, elbow, and digits, while 3D joint kinematics were assessed. Additionally, open-loop co-stimulation of multiple muscles across multiple forelimb joints was performed to produce stable multi-joint movements similar to those observed during reach-grasp-release movements. In one

animal, an extended fatigue trial was performed with stimulation mediated reach-grasp-release movements repeated at an interval of 1.5 seconds for 14 minutes using the predetermined stimulation protocol. The stimulation was provided using a computer controlled custom stimulator (FNS16 - CWE, Inc., Ardmore, PA).

3. Results

3.1. Anatomy of target muscles and motor point location

Figure 1 illustrates the detailed anatomy of the muscles and their innervation. The spinodeltoideus (SD) was targeted for implantation to achieve shoulder extension/external rotation. It arises from the infraspinatus fascia and anterior two-thirds of the scapular spine (ScS) and converges toward its insertion into the deltoid ridge of the humerus. The muscular branch of the axillary nerve innervates SD and the motor point is located distally (Figure 1A). The supraspinatus (SS) was targeted for implantation to achieve shoulder flexion/abduction. This muscle is situated beneath the cervical trapezius (CT). It arises from the anterior margin of the scapula, from its vertebral border as far as the spine, from the superior surface of the spine, and from the whole supraspinous fossa. It is inserted by a tendon into the anterior margin of the head of the humerus. The suprascapular nerve innervates SS and the motor point is located distally (Figure 1B).

The triceps brachii (TB) and biceps brachii (BB) were targeted for elbow extension and flexion, respectively. TB is made up of three heads, the long, medial and lateral. We targeted the long head which arises from the ventral third of the axillary border of the scapula by a broad tendinous attachment and inserts into the olecranon of the ulna. The radial nerve innervates TB and the motor point is located proximally (Figure 1C). BB is made up of two heads, the long and short. We targeted the long head which arises from the anterior edge of the glenoid cavity and inserts at the radial tuberosity. The musculocutaneous nerve innervates BB and the motor point is located in the middle (Figure 1D).

The extensor digitorum communis (EDC) and flexor digitorum profundus (FDP) were targeted for digit extension and flexion, respectively. EDC arises from the lateral epicondyle of the humerus. The muscle divides into four slips which pass over into tendons in the carpal region. These tendons pass under the annular ligament to their insertion at the base of the distal phalanx of digits two to five. The radial nerve innervates EDC and the motor point is located proximally (Figure 1E). FDP arises by four heads; (a) superficial head from the medial epicondyle of the humerus; (b) ulnar head from the medial epicondyle of the humerus; (c) radial head from the proximal part of the flexor surface of the radius; (d) middle head from the flexor surface of the ulna. Its tendon expands into a broad sheath lying immediately dorsal to the tendon of flexor digitorum sublimis; from this sheath four tendons are given off one to each of the digits from two to five, each tendon passing along the ventral face of the digit and inserting into its distal phalanx. The ulnar and volar interosseous branch of the median nerve innervate FDP and the motor point is located proximally (Figure 1F).

3.2. Strength duration curves

The rheobase currents of all of the implanted electrodes were determined from the strength duration curves, which are shown in Figure 2. The strength duration curves for all four implanted animals show a typical nonlinear hyperbolic relationship in which stimulation pulses with longer pulse widths require lower current amplitudes to produce muscle twitch. Typical rheobase values ranged from 0.11mA to 0.37mA. They differed slightly across animals and muscles (SD = $0.27\text{mA} \pm 0.07$, SS $0.19\text{mA} \pm 0.06$, TB = $0.15\text{mA} \pm 0.04$, BB = $0.22\text{mA} \pm 0.06$, EDC = $0.17\text{mA} \pm 0.1$, FDP = $0.19\text{mA} \pm 0.09$, avg. $\pm$ SD). The chronaxie values varied across animals and muscles and were typically in the range of 70-100 μsec with no pattern for a

particular muscle type. The consistency of shape of the strength duration curves and low rheobase indicate the ability to consistently implant and stimulate the motor points of multiple forelimb muscles across multiple animals.

3.3. Recruitment curves and 3D-kinematics for single muscle stimulation

Single-joint movements during single muscle stimulation using different pulse widths and current amplitudes were evaluated in three dimensions to determine the stimulation parameters that produce the largest amount of movement while delivering the lowest charge. The joint angle excursions (range) obtained for each animal were normalized with respect to the maximum angle range obtained at a 300 μ sec pulse width and a current of 3 times the threshold at 40 μ sec (100%). The resultant normalized recruitment curves for 3 implanted animals are plotted in Figure 3. Analysis of these curves indicates that the forelimb muscles stimulated in this model respond differently to electrical stimulation. The muscles differ regarding 1) the amount of movement produced by each level of stimulation, 2) the level of stimulation that produces maximal joint movement, and 3) the level of stimulation that produces “spillover” into adjacent musculature.

Using multiple 75 Hz, 210msec burst width, 70 μ sec per phase pulse width stimulation trains at 3x the twitch threshold delivered every 60 seconds, we assessed the quality and repeatability of joint movement during single muscle stimulation within each animal implanted. Figure 4 illustrates multiple single-joint angle plots from a representative animal, demonstrating smooth single-joint stimulation induced movement at the shoulder, elbow and digits. The considerable similarity and overlap between traces within each graph indicate high within animal repeatability and consistency of response obtained on stimulation of each muscle implanted.

3.4. 3D-kinematics for multi-muscle stimulation

Once the parameters for single muscle stimulation were identified, coordinated stimulation of multiple muscles was used to produce rhythmic flexion/extension movements at 1 and 2 joints, as well as, reach-grasp-release movements. Moving from simple single-joint movements produced by single muscle stimulation to coordinated single and multi-joint movements produced by multi-muscle stimulation requires the implementation of precisely timed stimulation protocols for multiple channels of stimulation.

Rhythmic single-joint movements—Rhythmic alternating movements at a single-joint were produced by alternate stimulation of flexor and extensor muscles of that joint. Figure 5A depicts shoulder movement mediated by alternating stimulation of the spinodeltoideus (3.0mA, 70 μ sec pulse width at 75 Hz for 600msec) and supraspinatus (2.42mA, 70 μ sec pulse width at 75 Hz for 400msec) muscles. Figure 5B depicts elbow movement mediated by alternating stimulation of the triceps brachii (1.11mA, 70 μ sec pulse width at 75 Hz frequency for 400msec) and biceps brachii (1.26mA, 70 μ sec pulse width at 75 Hz for 200msec at a 200msec delay from the termination of triceps brachii stimulation) muscles. Figure 5C depicts digit movements mediated by alternating stimulation of the extensor digitorum communis (1.25mA, 70 μ sec pulse width at 75 Hz for 500msec) and flexor digitorum profundus (1.33mA, 70 μ sec pulse width at 75 Hz for 500msec) muscles.

Rhythmic multi-joint movements—Synchronous rhythmic alternating elbow and digit movements (1Hz frequency) were produced by simultaneous stimulation of the biceps brachii (2.0mA, 70 μ sec pulse width at 75 Hz for 250ms) and flexor digitorum profundus (3.45mA, 70 μ sec pulse width at 75 Hz for 250ms) alternating with simultaneous stimulation of the triceps brachii (0.82mA, 70 μ sec pulse width at 75 Hz for 250ms) and extensor digitorum communis (1.2mA, 70 μ sec pulse width at 75 Hz for 250ms) (Figure 6 A-C). Analysis of the angle plots and captured video revealed consistent, smooth, coordinated elbow and digit flexion alternating

with elbow and digit extension (Figure 6A). The total joint excursions for the elbow and digits were 35 degrees and 73 degrees, respectively with very little variation between repetitions.

Reach-grasp-release movements—Using both EMG and kinematic data from previous studies we developed a forelimb stimulation protocol that produced a reach-grasp-release movement sequence task (Hyland and Jordan, 1997; Whishaw, 1996). In the current protocol, the reach-grasp-release movement sequence can be broken down into three phases: the forward reach phase followed by the grasp-retraction phase, followed by the release-relaxation phase (Figure 7). In the forward reach phase, the sequence of movements produced was: shoulder forward flexion with abduction, elbow extension, and digit extension (paw opening) (Figure 7A, panels 1, 2 and 3). In the grasp-retraction phase, the movements produced were simultaneous digit flexion (paw grasping), elbow flexion and shoulder extension (Figure 7A, panel 4). In the release-relaxation phase, passive digit extension, elbow extension, and shoulder flexion occur after the termination of stimulation, bringing the limb back to a neutral position (Figure 7A, panel 5). In order to achieve forward flexion and abduction of the shoulder, the supraspinatus was stimulated for 500 ms coupled with a 400 ms stimulation of the biceps brachii, which was initiated after a 100ms delay (Figure 7B). Elbow extension during forward shoulder flexion was achieved by stimulation of the triceps brachii for 350 ms started 150ms after initiating stimulation of the supraspinatus and continuation of the biceps brachii stimulation (Figure 7B). Digit extension (paw opening) during shoulder flexion/abduction and elbow extension was then achieved by stimulation of the extensor digitorum communis for 300 ms started 200ms after initiation of supraspinatus stimulation (Figure 7B). Digit flexion (paw grasping), elbow flexion, and shoulder extension were achieved by costimulation of the flexor digitorum (500ms duration started 500 ms after initiation of supraspinatus stimulation), biceps brachii (stimulation continued for 500ms) and spinodeltoideus (400 ms duration started 600 ms after initiation of supraspinatus stimulation) (Figure 7B). Qualitative observations and 3D kinematic data revealed that this stimulation protocol produced consistent reach-grasp-release movements of with appropriate range of motion and little variation (Figure 7A, C). Multiphasic linear velocity peaks were observed during the reaching phase (0-0.5sec), grasp-retraction phase (0.5-1.0sec), and release-relaxation phase (1.0-1.5sec) of stimulated movement (Figure 8). The velocity peaks observed during the reaching phase represent a complex pattern of forepaw movement away from the body. The pause between the reach and grasp-retraction phases represents the period of the movement pattern in which the forepaw is at its furthest point away from the body with digits extended. This pause also corresponds to the angle plateau observed around video frame 2 in Figure 7C. The peaks observed during the grasp-retraction phase represent a complex pattern of forepaw movement towards the body. The pause between the grasp-retraction and release-relaxation phases represents the period of the movement pattern in which the forepaw is at its closest point to the body with digits closed. This pause also corresponds to the angle plateau observed around video frame 4 in Figure 7C. The peaks observed during the release-relaxation phase represent gravity mediated forepaw movement towards the resting position as stimulation is absent; note that these peaks are smaller than those that occurred during stimulation.

In addition to being highly reproducible, the forelimb movements produced by this stimulation protocol were fairly fatigue resistant. The range of motion produced at all three joints (shoulder, elbow, and digits) decreased less than 50% at 7 and 14 minutes of stimulation (1.5 second movement cycles, 560 repetitions over 14 minutes) (Figure 9)

4. Discussion

While human and primate studies have historically been the preferred choices to evaluate reach and grasp movements (Castiello, 2005; Sabes, 2000), research conducted by multiple groups over the last 15 years has eloquently elucidated the ability of rodents to perform skilled forelimb

and paw movements, such as target reaching, grasping, and fractionated digit movements (Whishaw, 1996; Whishaw and Gorny, 1994; Whishaw et al., 1998). Given the ability of rodents to perform skilled forelimb movement, the clinically relevant rodent models for cervical spinal cord injury (Gensel et al., 2006; Lynskey et al., 2006), stroke (Carmichael, 2005), and traumatic brain injury (Morales et al., 2005), and the clinical interest in neuromuscular stimulation as both an upper extremity prosthesis (Peckham et al., 2001) and as a therapeutic device after neurological injury (Popovic et al., 2006), we believe that development of a clinically relevant rodent model of forelimb neuromuscular stimulation that could be used to assess molecular, synaptic and circuit level neural plasticity mechanisms underlying neuromuscular stimulation therapy is timely. Such investigations are currently difficult to carry out in people. As per our review of the literature, the presented work is the first such model development.

In this study we have provided detailed anatomical description of forelimb musculature motor point location and electrode implantation procedures in the rodent. The data demonstrate that neuromuscular electrical stimulation using implanted electrodes can consistently produce both isolated single-joint movement and coordinated multi-joint movements in the rodent forelimb. Additionally, the experiments performed in this study characterized the effects of altering the pulse stimulation parameters (pulse width, amplitude and frequency) on the generation of graded muscular recruitment as reflected in both the strength duration and recruitment curves. The recruitment curves provide guidelines for the choice of appropriate range of stimulation parameters that can be used to produce the joint range of motion and velocity required for a variety of forelimb movements, including reach-grasp, and release. The kinematic data during both single-muscle and the reach-grasp and release stimulation protocols clearly demonstrates that paw opening and grasping can be elicited by stimulation of the extensor digitorum communis and flexor digitorum profundus, respectively. Significant elbow flexion and extension were also elicited by stimulation of the biceps brachii and triceps brachii respectively. In addition, the velocity profile produced during stimulation mediated reach-grasp-release movements is quite similar to that previously reported for target reaching movements in neurologically intact adult Long-Evans rats, albeit at approximately half speed (Whishaw, 1996).

With the particular parameters we have chosen we were able to achieve fairly long-duration repeated movements without elicitation of observable fatigue in the muscles. While the current experiments were on anesthetized intact rodents these results bode well for the use of this particular stimulation paradigm to be used for evaluating daily movement therapy in awake rodent models of incomplete cervical spinal cord injuries or unilateral middle cerebral artery occlusions. We have been successful in transitioning hindlimb electrode implant studies in anesthetized rodents to awake chronically complete and incomplete spinal cord injured rodents. In these studies we were able to maintain electrodes for long durations and also provide daily stimulation of the hindlimbs (Ichihara et al., 2004; Jung et al., 2006; Venkatasubramanian et al., 2005). Studies on rodents with injuries that prevent limb use will be needed to indicate whether muscle fiber type changes affect this apparent fatigue resistance to neuromuscular stimulation in the forelimb.

The numbers of muscles stimulated in this model (two per joint for 6 total) are small compared to the numbers of muscles that are known to be active during forelimb movements such as reach and grasp (Hyland and Jordan, 1997). We were limited in our choice because of the practical difficulty of implanting multiple muscles in the forelimb at a given time, because of their size. Despite this limitation, we were able to achieve fairly complex coordinated movement (Figure 7). If the desire were to do a targeted reach, it is likely that we will have to stimulate additional muscles. This will be especially true for the movements occurring at the shoulder joint, since the shoulder is a ball and socket joint, rather than a hinge or saddle. The

degrees of freedom and mobility of a ball and socket joint require the co-contraction of multiple muscles around that joint to produce straight flexion or extension. It may be possible to develop advanced dynamic stimulation paradigms that sequence the different muscles in a more complex temporal pattern to accomplish targeted reach with lower numbers of muscles. However, the accuracy of the targeted reach may not be critical for the therapeutic paradigm, which is the primary intended application of this work. The ability to make repeatable (albeit relatively inaccurate) reach-grasp-release movements in a chronic rodent model will allow investigations of efficacy and mechanisms of this mode of rehabilitative therapy for promoting functional recovery of movement after central nervous system injury.

Another aspect of this work that could be further explored is whether the digit stimulation provides sufficient grip force. While no force data was generated to determine if this movement constituted a functional grasp, 3D kinematic data indicates that stimulation of the flexor digitorum profundus produces a large amount of coordinated flexion in all 4 digits and a grasping movement. In addition, repeated stimulation demonstrated that the grasping movements were consistently reproducible and resistant to fatigue. Future versions of this model could quantify the strength of the stimulated grasp using force transducers.

In summary, we have developed a rodent model based movement therapy in which we can reliably implant electrodes in multiple muscles for neuromuscular stimulation to achieve a complete reach-grasp-release movement. The development of this model will now allow for the investigation of mechanisms by which functional neuromuscular stimulation could promote plasticity at neuroanatomical, cellular, and molecular levels both at spinal and supra-spinal levels that were previously not feasible in humans and non-human primates. In combination with a cervical spinal cord injury or cortical injury rodent model, this forelimb stimulation model could also be utilized as a test-bed alternative to non-human primate and human studies for exploring advanced adaptive algorithmic and control approaches for developing co-adaptive brain-machine interfaces to control paralyzed limbs using a neuroprosthesis.

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Figure 1.

Detailed anatomy of the targeted forelimb muscles identifying motor points. (A) Spinodeltoid (SD) lateral view: Cervical trapezius (CT) is detached from the scapular spine and elevated cranially. SD is detached from the scapular spine and elevated rostrally. Red star shows the entry point of the muscular branch of the axillary nerve. (B) Supraspinatus (SS) lateral view: CT and SS are detached from the scapular spine and elevated cranially. Red star shows the entry point of the suprascapular nerve. (C) Triceps brachii (TB) medial view: Acromiodeltoid (AD) and pectoralis minor (PMI) are detached from the deltoid ridge of humerus and elevated cranially. Pectoralis major (PMA) is detached from the deltoid ridge of humerus and retracted rostrally. Red star shows the entry point of the radial nerve. (D) Biceps brachii (BB) medial view: AD is detached from the deltoid ridge of humerus and elevated cranially. PMA is detached from the deltoid ridge of humerus and retracted rostrally. BB is rotated medially. Red star shows the entry point of the musculocutaneous nerve. (E) Extensor digitorum communis (EDC) lateral view: The lateral head of TB is elevated cranially. Extensor carpi radialis (ECR) and EDC are detached from the lateral epicondyle of the humerus and elevated distally. Red star shows the entry point of the radial nerve. (F) Flexor digitorum profundus (FDP) medial view: Flexor carpi radialis is retracted medially. Pronator teres (PT) is detached from the medial surface of the shaft of the radius and elevated cranially. Red star shows the entry point of the ulnar and volar interosseous branch of the median nerve. The notations and symbols used in the figure are detailed in Table 1. ★ - motor point; ▲ - landmark

Figure 2.

Strength duration curves plotting the minimum stimulation current amplitude (cathodic first, biphasic pulses applied for 210msec duration at 75Hz) that produced a visible muscle twitch in the Spinodeltoideus (A, SD), Supraspinatus (B, SS), Triceps Brachii (C, TB), Biceps Brachii (D, BB), Extensor Digitorum Communis (E, EDC), and Flexor Digitorum Profundus (F, FDP) across multiple pulse widths. Each line indicates a separate animal (n = 4).

Figure 3. Recruitment curves plotting the average normalized angular excursion (% range) produced during stimulation of the Spinodeltoideus (A, SD), Supraspinatus (B, SS), Triceps Brachii (C, TB), Biceps Brachii (D, BB), Extensor Digitorum Communis (E, EDC), and Flexor Digitorum Profundus (F, FDP) at pulse amplitudes of 1.5 (gray) and 3x (black) the threshold at a pulse width of 40μsec across multiple pulse widths. (n = 3, Avg.±SEM)

Figure 4. Single-joint movement produced by open-loop single muscle stimulation (75Hz, 210msec burst width, 70μsec/phase pulse width, 3x twitch current threshold at 40msec/phase) of the Spinodeltoideus (A, SD), Supraspinatus (B, SS), Triceps Brachii (C, TB), Biceps Brachii (D, BB), Extensor Digitorum Communis (E, EDC), and Flexor Digitorum Profundus (F, FDP). Two consecutive stimulation trials (separated by 60 seconds) from a representative animal are plotted in each graph (black and gray curves). The average maximal range of motion achieved during those two trials is indicated. Note that in each trial the joint angle recovers to pre-stimulation levels passively when stimulation is stopped. Panels A-D are from one animal, while panel E is from a separate animal. The stimulation period is identified by a horizontal bar ■ flexion ■ extension.

Figure 5.

Single-joint movements produced by alternating open-loop stimulation of muscle pairs (75Hz, 210msec burst width, 70 μ sec/phase pulse width, 3x twitch current threshold at 40 μ sec/phase) starting with flexor stimulation. (A) Shoulder movement produced by stimulation of the Spinodeltoideus followed by stimulation of the Supraspinatus. (B) Elbow movements produced by stimulation of the Triceps Brachii followed by stimulation of the Biceps Brachii. (C) Digit movement produced by stimulation of the Extensor Digitorum Communis followed by stimulation of the Flexor Digitorum Profundus. Average range from a representative animal (thick black lines) $\pm$ standard deviation (thin gray lines) calculated from 3 trials per animal. The joint angle recovers to pre-stimulation levels passively when stimulation is stopped. The

stimulation period is identified by horizontal bars flexion extension. The 200msec delay between the extension and flexion in panel B is identified by hatched bar .

Figure 6.

Forelimb neuromuscular stimulation mediated multi-joint flexion-extension movements. (A) Coordinated movements at the elbow and digit joints produced by four consecutive cycles of open-loop programmed stimulation of the flexor muscles alternating with extensors (75Hz, 210msec burst width, 40 or 70 μ sec/phase pulse width, 3x twitch current threshold at 40 μ sec/phase, 250msec duration) with 275msec between cycles. Stick figures of forelimb from one cycle of stimulation inset. Note that a small amount of passive shoulder motion occurs during stimulation, and that the joint angles return to neutral between stimulation cycles. (B) Video still of forelimb with elbow and digits flexed. (C) Video still of forelimb with elbow and digits extended. The stimulation period is identified by horizontal bars flexion extension. Stimulation off period is identified by hatched bars .

Figure 7. Forelimb neuromuscular stimulation mediated reach-grasp-release movement sequences. (A) Multiple video frames (1-5) illustrating coordinated movements at multiple joints (reach-grasp-release) produced by open-loop programmed stimulation of multiple forelimb muscles; (B) Open-loop coordinated stimulation paradigm. Stimulation at 75Hz, for 210msec using 40 or 70µsec/ phase pulses, with current amplitude at 3x twitch current threshold at 40µsec/phase. (C) Joint angle trajectories for the shoulder, elbow and digits during three repeated movement sequences. Each movement sequence lasted 1 sec. Location of video frames 1-5 (A) are indicated on the angular trajectories. Dashed vertical lines indicate beginning of cycles two and three.

Figure 8.

Linear wrist velocity during forelimb neuromuscular stimulation mediated reach-grasp-release movements. Average velocity over 10 repetitions is indicated by the thick solid black line. Standard deviations are indicated by thin solid gray line. Dashed lines separate the three stages of movement: reach followed by grasp followed by release and relaxation.

Figure 9. Muscle fatigue during prolonged stimulation. Normalized average angles and standard errors (10 cycles) plotted for the shoulder, elbow and digits at times 0, 7 minutes and 14 minutes of the reach-grasp-release stimulation protocol in a single animal.

Table 1

Summary of Abbreviations and Symbols

AD	Acromiodeltoid
AP	Acromion process
BC	Brachialis
BB	Biceps brachii
CT	Cervical trapezius
DR	Deltoid ridge of humerus
ECR	Extensor carpi radialis
ECU	Extensor carpi ulnaris
EDC	Extensor digitorum communis
FCR	Flexor carpi radialis
FCU	Flexor carpi ulnaris
FDP	Flexor digitorum profundus
IS	Infraspinatus
LE	Lateral epicondyle of humerus
PMA	Pectorallis major
PMI	Pectorallis minor
PT	Pronator teres
ScS	Scapular spine
SD	Spinodeltoid
SS	Supraspinatus
TB	Triceps brachii
▲	Landmark
★	Motor point