Muscle stimulation in a rodent model: electrode design, implantation and assessment

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Abstract

We present initial development of a paraplegic rodent model for FNS-assisted locomotor therapy. Stimulating electrodes were fabricated and implanted in flexors and extensors of the hip, knee, and ankle joints using new surgical implant procedures. The electrodes remain well anchored. The strength-duration curves were assessed periodically over eight weeks and indicated stable thresholds. The thresholds were also assessed after a daily stimulation paradigm. Graded torque can be generated by varying pulse width or current amplitude. Such a FNS rodent model could be utilized for assessing the ability to promote functional recovery by providing a mechanism to generate very repeatable movements alone or in combination with other therapies.

1 Introduction

Functional electrical stimulation techniques have been used in motor retraining paradigms for people with incomplete spinal cord injury (SCI) and found to enhance locomotor recovery [1][2]. An animal model in which the mechanisms underlying the beneficial effects of Functional neuromuscular stimulation (FNS) therapy could be investigated would be very useful but are not available. The rodent model of spinal neurotrauma is extensively being utilized to investigate the consequences of the trauma and development of therapeutic strategies.

Our goal is to develop a rodent model of FNS locomotor therapy after SCI. To develop such a model it is necessary to map the muscle electrode implantation sites that generate isolated muscle contractions and assess stability of electrode implantation over time. Here, we present electrode fabrication techniques, surgical implant procedures and results assessing viability of chronically implanted electrodes in paraplegic rodents.

2 Methods

2.1 Electrode fabrication

Stimulation electrodes were custom made using Teflon coated stainless steel wire (AS632-50-µ multistranded, Cooner Co.), non-absorbable suture (Mersilene, braided Polyester Suture; diameter 0.7 mm, Ethicon Inc.) and end plates (2 mm diameter circle of Mylar® MO21-750, thickness 0.81mm, Dupont Co. Hopewell, VA). 1 mm of the wire tip was de-insulated, tied into a knot, and then connected to the suture. This suture material has high tensile strength (knot-pull strength; 0.2kgf), minimal tissue reactivity, and high tissue drag. The suture was used for positioning the electrode near the motor-point (see below). The end plates were used to anchor the electrodes to the muscles. The free end of the electrode was soldered to a connector (Connector Term 26-30AWG male, Digi-key Co.) for in-line connection to the wires leading to a head connector.

2.2 Electrode implantation and Spinal transection

In female Long Evans adult (age: 2-3 mo; 220-250g) rats with complete spinal transection at T8, electrodes were implanted in the ankle dorsiflexor (tibialis anterieor, TA n=5) and plantarflexor (extensor) (gastrocnemius medialis, GM n=5), knee flexor (semitendonosus, ST n=2) and extensor (vastus lateralis, VL n=2), and hip flexor (iliacs, n=1) and extensor (biceps femoris, BF n=1) under Sodium Pentobarbital anaesthesia (40mg/kg ip). The approximate location of the motor point of each muscle was determined by stimulating the skin/muscle surface using a 30 G straight needle using a handheld stimulator (DigiStim 3 Plus). After exposing the muscle through the incision site, a 30 G needle was inserted into the muscle to find the precise location of the motor point which would provide the maximal contraction with 0.1-0.2 mA, 200µsec cathodic pulses at 100Hz). Once the position was
located, the electrode-suture assembly was connected to a curved eye needle. The needle was inserted adjacent to the 30 G straight needle. The latter was removed and the curved needle used to thread the suture with the electrode tip through the muscle. The location of the de-insulated portion of the electrode wire was set by pulling the two ends of the suture outside the muscle. The de-insulated portion of the electrode wire was anchored in place using the end plates such that the muscle was sandwiched by the two end plates. Both end plates were sutured to the surface of the muscle membrane. The electrode wires were routed sub-cutaneously to the back where they were connected via the in-line connectors to a custom head connector (Omnetics Inc.).

A T8-T9 laminectomy was performed and a 2-5 mm section of the spinal cord transected and removed at spinal level T8 to produce a complete paraplegia. The animals were allowed to recover from anaesthesia, treated with an analgesic, and with antibiotics for 7 days. Manual bladder expression and hydration with sub-cutaneous 0.9% sterile saline were performed as part of regular animal care.

2.3 Assessment of Electrode Stability

Assessment of mechanical and electrical stability of the electrodes over time was performed using strength-duration (SD) and torque recruitment curves. SD curves are obtained by determining the current strength required to achieve muscle twitch recruitment (visible twitch verified to be about 0.01N-0.35N force) in response to stimulation with different pulse width durations (500, 300, 100, 70, 40, 20, 10 usec) with 5-10 second interval between stimulation. SD curves were obtained over 6-8 weeks at approximately one week intervals. Isometric torque recruitment curves were also obtained for some electrodes 3 weeks and 6 weeks post implantation.

The SD curves and torque measurements were made with the rat placed on a horizontal base plate, mounted on a custom frame placed on an air table. The rat’s pelvis was gently clamped in place using a pelvic compressor. The unloaded hindlimb with the muscle of interest was held in place using self-designed custom braces and stoppers. When needed, the brace was connected to a six degree-of-freedom force transducer (model 20E12A, JR3 Inc. Woodland, CA). During hip muscle activation/joint torque data collection, the rat’s shank was secured to a shank brace and the rat’s thigh was held in a fixed position using a plastic stopper. During ankle muscle activation/joint data collection, the rat’s foot was secured to a foot brace with the ankle at 90° to the shank and the thigh and shank held in fixed positions using plastic stoppers. (e.g. Fig. 1). Braces were not used for visual SD curve assessment.

The effect of rhythmic long duration daily stimulation on the SD curves was also assessed. Eight weeks post implantation and spinal transection, repeated rhythmic daily stimulation was performed in 3 muscles. In 6 other muscles, the repeated stimulation regimen was initiated one week after implantation/SCI. Control SD curves were obtained prior to initiation of the daily stimulation regimen and 1 week later. The rhythmic stimulation was at 1 Hz and was provided with a train of biphasic pulses at 75 Hz, with pulse amplitude current of 1.5 x twitch threshold current of a 40 usec pulse width for 15 minutes/day for 5 days. The pulse widths and duration were 400 µsec and 90 msec for TA, 500 µsec and 70 msec for ST, 200 µsec and 220 msec for VL, 100 µsec and 130 msec for BF, and 400 µsec and 200 msec for BF. The total duration of stimulation was determined from prior EMG data collection during treadmill walking and the pulse widths chosen from prior torque recruitment curves in anesthetized rodents with similar implanted electrodes [3].

3 Results

The typical SD curve was non-linear with larger current required to elicit muscle twitch at smaller pulse widths. Figure 2 shows the SD curves for a GM muscle over 8 weeks post implantation. In general, the SD curves
remained stable over several weeks. For any given pulse width the coefficient of variation (CV=standard deviation/mean) was higher at lower pulse widths than at higher pulse widths. The range of CV for the ankle extensor GM (n = 4) was 0.18 to 0.31, for the ankle flexor TA (n=4) was 0.21 to 0.39. The knee extensor ST had a CV of 0.20 and the knee flexor VL (n=2) had a range of 0.09 to 0.43.

**Figure 2: SD curves for GM over 8 weeks.**

Figure 3 illustrates the torque generated at the ankle on stimulation of GM at different pulse widths and current amplitudes in an anesthetized rat.

**Figure 3: Steady-state isometric torque generated during the last half second of a 2.5 sec stimulation of GM**

Figure 4 illustrates the SD curves obtained from a GM muscle before and 7 days after repeated daily stimulation begun 7 days post implantation (dpi). Similar, overlapping SD curves were obtained for the other ankle, knee and hip muscles that were subjected to daily stimulation beginning 1 week or 8 weeks post implantation.

4 Discussion and Conclusions

In this work we present initial development of a rodent model for FNS-assisted locomotor therapy. Such a model could be utilized for assessing the ability to promote functional recovery by providing a mechanism to generate very repeatable movements and to gradually allow the voluntary motor command to take over control of the movement.

We were able to successfully implant electrodes in flexor and extensor muscles of the hip, knee, and ankle in the hindlimbs. The electrodes are stable over several weeks. Initial assessment after daily repeated stimulation, akin to that which will be necessary for therapy, shows promising outcomes. Further assessment over longitudinal studies are required. In anesthetized rodent preparations sufficient torque can be generated using these electrodes.

**References**


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