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Research Article

Force Characteristics of the Rat Sternomastoid Muscle Reinnervated with End-to-End Nerve Repair

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The goal of this study was to establish force data for the rat sternomastoid (SM) muscle after reinnervation with nerve end-to-end anastomosis (EEA), which could be used as a baseline for evaluating the efficacy of new reinnervation techniques. The SM muscle on one side was paralyzed by transecting its nerve and then EEA was performed at different time points: immediate EEA, 1-month and 3-month delay EEA. At the end of 3-month recovery period, the magnitude of functional recovery of the reinnervated SM muscle was evaluated by measuring muscle force and comparing with the force of the contralateral control muscle. Our results demonstrated that the immediately reinnervated SM produced approximately 60% of the maximal tetanic force of the control. The SM with delayed nerve repair yielded approximately 40% of the maximal force. Suboptimal recovery of muscle force after EEA demonstrates the importance of developing alternative surgical techniques to treat muscle paralysis.

1. Introduction

The sternocleidomastoid (SCM) is a long neck muscle with two bellies, a medially located sternomastoid (SM) and laterally positioned cleidomastoid (CM). Innervation of the SM originates from the accessory nerve (eleventh cranial pair) [1]. The SM is involved in the control of head movements and causes cranial displacement of the sternum and ribcage during conscious respiratory efforts [2–6]. It has been widely used as the muscle and myocutaneous flap for the reconstruction of oral cavity and facial defects [7, 8] and as a candidate for reinnervation studies [9].

The SM muscle is not active during resting ventilation but is recruited only during higher ventilatory demands [10]. Damage to the supplying nerve leads to significant atrophy in the muscle. The SM muscle is a good target for development of new reinnervation techniques due to its superficial location in the neck, easy surgical access, and a single nerve supply [9]. In addition, several neighboring cervical strap muscles (i.e., sternohyoid, sternothyroid, and omohyoid) and their innervating nerves could be potentially used as donors to reinnervate the paralyzed SM muscle [9].

End-to-end nerve repair has gained popularity in the use for restoring paralyzed muscles [11]. Many other techniques of reinnervation like end-to-side neuroraphy, autologous nerve grafting, tubulization with a nerve guide tube, direct nerve implantation, and nerve-muscle pedicle transfer were also developed [9, 12]. However, despite advances in microsurgery and extensive studies on nerve repair, the presently used reinnervation methods result in poor functional recovery [13]. As reported, results of nerve repair to date have been no better than fair, with only about 50% of patients regaining useful function [14, 15]. Hall [16] reviewed the degree of functional recovery after traumatic injury to a peripheral nerve in humans and stated that such recovery is rarely satisfactory.

Poor motor recovery after nerve end-to-end anastomosis (EEA) could be attributed to the inability of denervated muscles to accept reinnervation and to recover from denervation atrophy. It could also result from a reduced ability of injured motoneurons to regenerate their axons after prolonged axotomy [16, 17].

A new reinnervation technique developed in this laboratory called nerve-muscle-endplate band grafting (NMEG)

has been described [9]. This method could be more effective as compared to the end-to-end nerve repair technique. In the NMEG experiments, the experimentally paralyzed SM muscle was reinnervated by transplanting an NMEG harvested from a sternohyoid (SH) muscle. The NMEG contained a muscle block, a nerve branch with nerve terminals, and a motor endplate (MEP) band with numerous neuromuscular junctions.

Muscle force measurement is an objective method used to evaluate the functional outcome of reinnervation. However, to evaluate the success of a new reinnervation technique, the reference data assessing muscle force in an intact SM and an SM repaired with the standard technique are needed. We recently described the results of muscle force studies in the intact SM muscle of the rat [18]. However, in literature there are no data available on force produced by SM muscle reinnervated with the EEA method.

In the present study, we described the force characteristics of the rat SM muscle reinnervated with the classical EEA method. Muscle force was studied 3 months after nerve anastomosis with different delays (from 0 to 3 months) between nerve transection and repair. The muscle force characteristics obtained in the present study using the classical EEA nerve repair can be used as a reference for evaluating the extent of functional recovery produced by new reinnervation techniques.

2. Materials and Methods

2.1. Subjects. The experiments were performed on 25 adult (3.5 months old) Sprague-Dawley female rats (Charles River Laboratories, Mass), weighing 350–450 grams. We used female rats because their body weights become stable in adulthood (starting at about 3 months) [19]. Studies by Cantillon and Bradford [20] showed that there is no difference between the male and female rat in the contractile properties of upper airway muscles, both in young and old animals. The rats were provided with *ad libitum* access to food and water and housed in standard cages in a 22°C environment with a 12 : 12-h light-dark cycle. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee prior to the onset of our experiments. The experiments were performed in accordance with the *Guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996). All efforts were made to minimize the number of animals and their suffering in the experiments.

2.2. Description of Nerve Transection and Repair with EEA. Particulars regarding the surgical procedures and postoperative assessment have been described in our previous papers [9, 18]. Briefly, during each of the two stages of the operation, rats underwent general anesthesia with a mixture of ketamine (80 mg/kg body wt) and xylazine (5 mg/kg body wt) administered intraperitoneally. Supplementary doses were administered to maintain a constant depth of anesthesia. Under an Olympus SZX12 Stereo zoom surgical microscope (Olympus America Inc., Center Valley, Pa), a midline cervical incision was made extending from the hyoid bone to the sternum to expose the right SM muscle and its innervating nerve. During the first operation stage, the SM nerve was sharply transected approximately 3 mm proximal to the SM muscle. After surgery, the wound was closed in layers with interrupted simple sutures of 4-0 prolene. During the second operation stage, the transected right SM nerve was repaired in three different groups of rats (7 rats in each group) with two 10-0 interrupted epineurial sutures at different delay time points after nerve transection: immediately, 1-month delay, and 3-month delay. An additional group of 4 rats with SM nerve transection without nerve repair served as a denervation control for muscle weight evaluation. After the final operation, the rats were housed individually during a 3-month recovery period. Figure 1 illustrates the SM nerve 3 months after transection and repair with EEA.

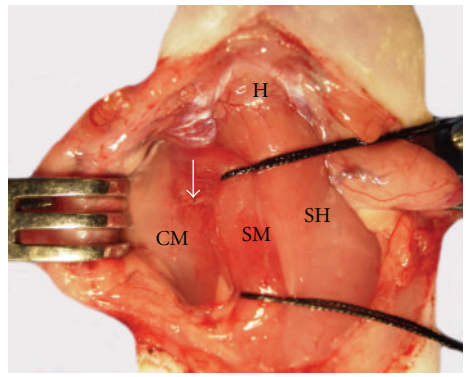

Figure 1: The SM nerve 3 months after transection and repair with EEA. Photograph from an open neck of a rat who underwent EEA procedure following transection of the SM nerve on the right side. After the SM was retracted medially by two sutures, its innervating nerve was visualized between the SM and cleidomastoid (CM) belies. The SM nerve was transected and both nerve cut ends were united with two 10-0 nylon sutures (white arrow). H: hyoid bone; SH: sternohyoid muscle.

2.3. Recording Setup. We built the acquisition system that provided user-controlled output signals (electrical stimulation to the nerve or muscle and the signal that controlled the length of the muscle) as well as recorded muscle force data. The system was assembled from a National Instruments multipurpose board (NI USB 6251, National Instruments, Austin, Tex) and an Aurora system (305-LR, Aurora Scientific, Aurora, Calif). It was controlled by a Dell laptop with the user written LabView (National Instruments) program. Data were analyzed offline with DIAdem software (National Instruments). The scheme of components and connections of the system is shown in Figure 2.

2.4. Muscle Attachment for Force Measurement. The details regarding the force measurement of the rat SM muscle have been provided in our recent publications [9, 18]. Briefly, the SM muscles on both sides were exposed under aseptic conditions. Each of the SM muscles was dissected free from the surrounding tissue. Care was taken so that the nerve branch

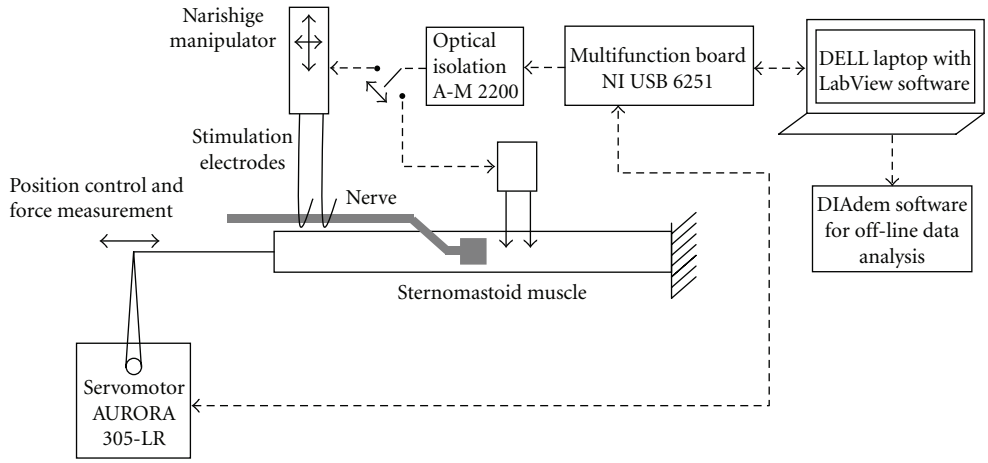

Figure 2: The schematic for the stimulation and recording system. The SM muscle is illustrated in the center. An Aurora Servomotor unit (305-LR) was used to stretch the SM muscle to optimal length and record muscle force. Electrical stimulation (200 ms trains of 200 Hz biphasic pulses) was generated by a National Instruments multifunction board (NI USB 6251). The stimulation trains were optically isolated from ground with an Optical Isolation Unit (A-M 2200) and directed to the SM nerve or directly to SM muscle. A Dell laptop with user-written LabView software was used to control the output signals (intensity of electrical stimulation and position of the servomotor lever) and to record the input signal (muscle force). Data were analyzed offline with National Instruments DIAdem software.

and blood vessels supplying the muscle remained intact. The rostral tendon of the SM was dissected free, cut close to the insertion, tied together with a 2-0 suture, and connected to a servomotor lever arm (Model 305B Dual-Mode Lever Arm System, Aurora Scientific Inc., Aurora, Ontario, Canada). The right SM nerve with EEA was identified, isolated from surrounding tissues, and placed on a bipolar stimulating electrode for nerve stimulation. On the control side, the muscle force of the left normal SM was measured by stimulating the intact SM nerve with the same bipolar electrode. In addition to nerve stimulation, direct muscle stimulation was also performed. The muscle was stimulated with a pair of needle electrodes inserted into the muscle at the level where the nerve enters the muscle. During the experiment, the rat was placed supine on a heating pad (homoeothermic blanket system, Stoelting, Wood Dale, Ill) and the core body temperature was monitored with a rectal thermistor and maintained at 36°C. The muscle and nerve examined were regularly bathed with warmed mineral oil throughout the testing to maintain muscle temperature between 35 and 36°C.

2.5. Stimulation Procedure. Functional reinnervation was evaluated using muscle force response to electrical stimulation at different intensities (0–1 mA). Optimal parameters of electrical stimulation of the SM nerve and muscle were established in our preliminary study on 10 control rats [18]. The stimulation signal used in this study consisted of a 200 ms train of biphasic pulses of constant current at a frequency of 200 pulses/s. Stimulation with a train duration of 200 ms was used to ensure that during stimulation, tetanic force reached a plateau in the fast-twitch SM muscle [21]. Each pulse was made of two opposite rectangular phases, with each phase lasting 0.2 ms. The stimulation was optically isolated from ground (optical isolation unit A-M 2200, A-M Systems, Sequim, Wash) and sent to two silver hook electrodes separated by a 5 mm distance on which the stimulated nerve was placed. Alternately, the muscle was stimulated directly with two silver needle electrodes inserted in the muscle 10 mm rostral to the nerve entrance to the muscle. The separation distance between electrodes was 5 mm.

2.6. Statistical Analysis. Muscle force was analyzed with a two-factor repeated measure ANOVA followed by Tukey's test for post-hoc comparisons. Muscle weight was analyzed with a one-way ANOVA followed by Tukey's test. SAS/STAT 9.2 software (SAS Institute Inc., Cary, NC) was used in statistical comparisons.

3. Results

3.1. Stimulation of SM Muscle at Optimal Length. Muscle force characteristics were studied when the SM was stretched at an optimal length with a passive tension of 0.08 N. The selection of this tension level was based on the results of our previous study on 10 control rats [18]. That study showed that the SM muscle, when stretched with 0.08 N tension, is able to produce maximal muscle force (active muscle tension to electrical stimulation). The SM force declined to 60% at tensions of 0.02 N and 0.2 N. The same relationship between muscle force and muscle tension was confirmed in the present study in the reinnervated muscles. Figure 3 illustrates the group average of active muscle force produced by reinnervated SM muscles stretched at different passive tensions in rats which had EEA delayed for 3 months. Rats from this group were subjected to muscle denervation by a transection of the SM nerve. After a 3-month delay, the transected SM nerve was repaired with EEA. Three months after nerve repair, muscle force-tension characteristics were analyzed. The muscle force results from each subject were normalized by dividing muscle force data

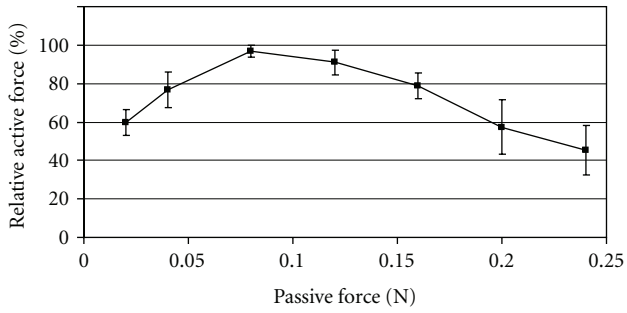

Figure 3: The active force generated by the SM muscle as a function of passive tension, with which the muscle was stretched just before stimulation. The active force was produced by the SM muscle in response to electrical stimulation of the SM nerve. A 200 ms train of 1 mA biphasic pulses at 200 pulses/s was used. Each phase of biphasic pulse lasted 0.2 ms. Data shown in this graph represent average active force, normalized by maximal force (observed at 0.08 N tension). Vertical bars represent standard error. The SM muscle was denervated for 3 months, repaired by end-to-end anastomosis, and left to recover for additional 3 months. The same muscle force-tension characteristic was observed in the noninjured control muscles [18].

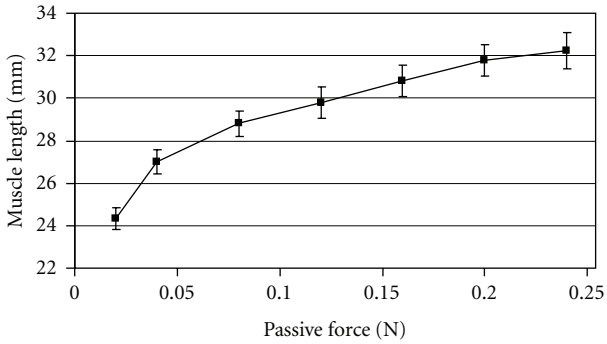

Figure 4: Length of the SM muscle as a function of the passive tension.

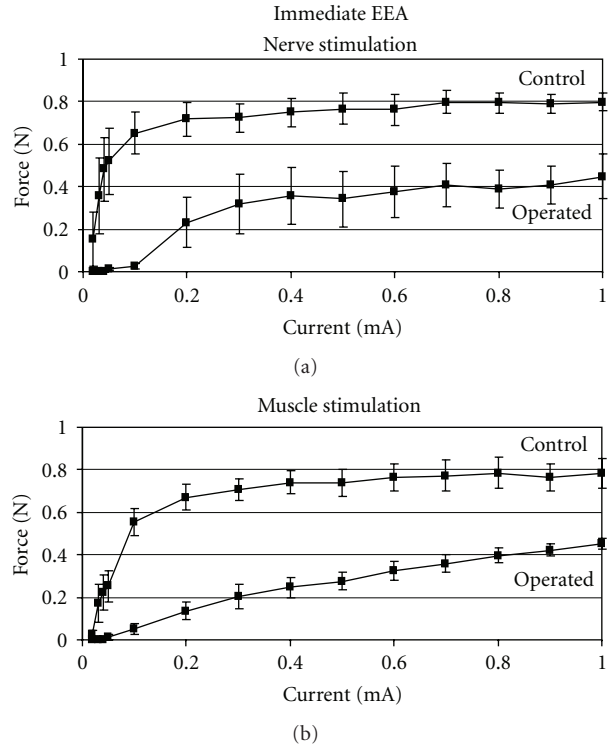

Figure 5: Force-current characteristics of the SM muscle in the immediate EEA group evoked by nerve stimulation or by direct muscle stimulation. (a) Illustrates superimposed force-current characteristics of the control and operated SM muscles evoked by stimulation provided to the SM nerve. In the control SM muscle the stimulation threshold, which produced noticeable muscle contraction, was 0.02 mA. Muscle force grew with increased stimulation current until it reached a plateau at 0.2 mA. The operated muscle had a larger stimulation threshold (with detectable muscle contraction at 0.1 mA) and produced a smaller force than the control muscle. (b) Shows superimposed force-current characteristics from the operated and control SM muscles evoked by direct SM muscle stimulation.

recorded at different tensions by the maximal muscle force observed at optimal tension (0.08 N). A 200 ms train of biphasic pulses (with a 0.2 ms pulse width) with a repetition frequency of 200 Hz was used to stimulate the SM nerve while the muscle was stretched with tensions ranging from 0.02 to 0.24 N. The stimulation current was set at 1 mA.

The length-tension relationship of the SM muscle is shown in Figure 4. The average length of the SM was 24.4 mm at a tension of 0.02 N, 28.8 mm at the optimal tension of 0.08 N and 31.8 mm at a tension of 0.2 N.

3.2. Force-Current Characteristics in Immediate EEA Group. The averaged SM muscle force-current relationships observed in animals that underwent nerve transection and repair with EEA are shown in Figure 5 (immediate nerve repair group), Figure 6 (1-month delayed repair group), and Figure 7 (3-month delayed repair group). The contralateral intact SM muscle served as a control. Vertical bars illustrate standard error.

Averaged maximal muscle forces produced by the SM muscles using a 0–1 mA range of stimulation currents in different studied groups are shown in Figure 8. There is no significant difference in maximal muscle force between nerve and muscle stimulations on the control side (average data from control sides in all three EEA-operated groups are shown in Figure 8(b)). The maximal muscle force evoked by direct muscle stimulation was 97% of the maximal muscle force evoked by nerve stimulation in the group with immediate nerve repair, 91% in the group with 1 month delayed nerve repair, and 94% in the group with 3 month delayed nerve repair.

3.2.1. Control Muscle Force to Nerve Stimulation. For the control SM muscle, the force-current function (Figure 5) had a threshold response at 0.02 mA. Muscle force grew with an increase of stimulation current until it reached a plateau at about 0.2 mA. Maximal muscle force produced by the SM muscle was about 0.8 N.

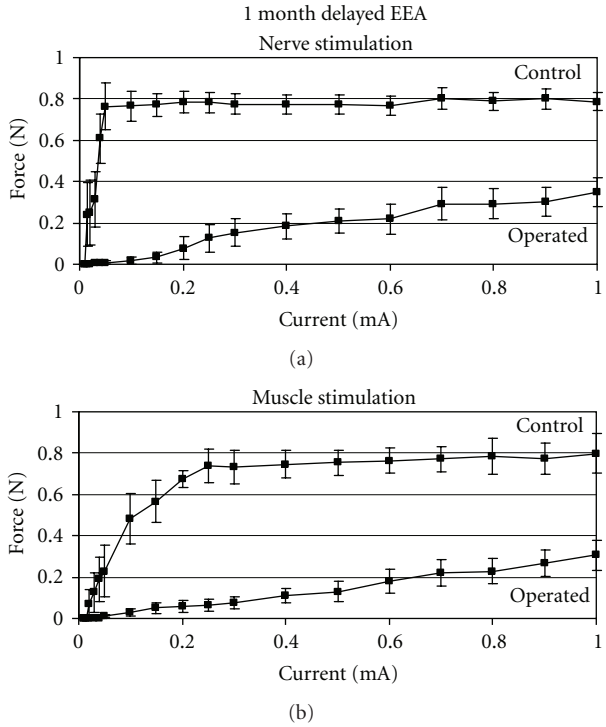

Figure 6: Force-current characteristics of the SM muscle in the 1-month delay EEA group. Muscle force in the operated and control SM muscles was produced by nerve stimulation (a) or by direct muscle stimulation (b). Note that the delayed reinnervation of the SM muscle produced a smaller muscle force as compared with immediate reinnervation (illustrated in Figure 5).

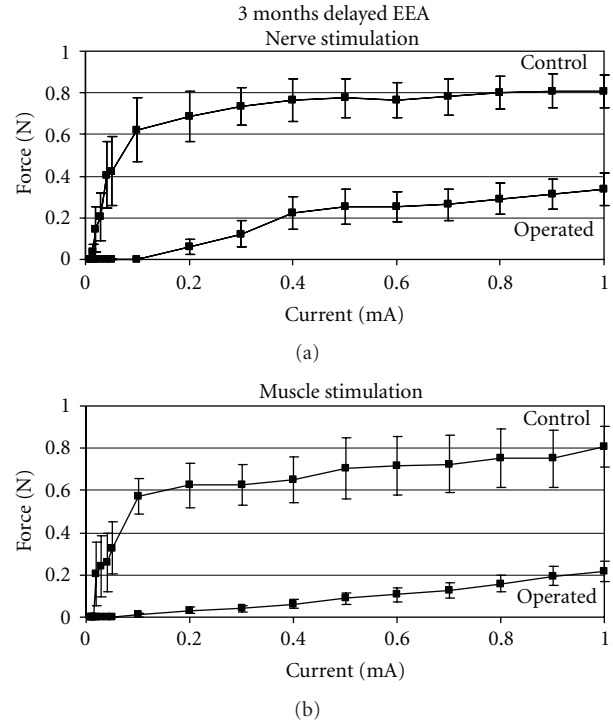

Figure 7: Force-current characteristics of the SM muscle in the 3-month delay EEA group. Muscle force in the operated and control SM muscles was produced by nerve stimulation (a) or by direct muscle stimulation (b).

3.2.2. Operated Muscle Force to Nerve Stimulation. For the dennervated and immediately repaired SM muscle, the nerve stimulation threshold to produce detectable muscle contraction was 0.1 mA (Figure 5). The maximal force of the operated muscle was 60.7% of the maximal force produced by the control, the nonoperated muscle on the contralateral side). A two-factor repeated ANOVA (group and intensity of stimulation as main factors) showed a statistically significant difference in muscle force between groups (operated and control muscles, $F = 17.94$, $P < 0.0005$) as well as the interaction of group with the intensity of stimulation ($F = 5.26$, $P < 0.005$ with a Greenhouse-Geisser correction).

3.2.3. Control Muscle Force to Muscle Stimulation. In addition to muscle force evoked by nerve stimulation, we also recorded muscle force produced in response to direct muscle stimulation. In the control muscles, similar maximal force-current relations were observed during nerve stimulation and direct muscle stimulation (Figure 5). Direct stimulation of the control muscle produced 97% of the maximal force observed during nerve stimulation. However, the stimulation threshold was smaller and the force-intensity curve reached a plateau earlier during nerve stimulation (as compared to direct muscle stimulation). A two-factor repeated ANOVA (group and intensity of stimulation as the main factors) showed no statistically significant difference between groups (force produced by muscle and nerve stimulation) but a statistically significant interaction of group with intensity of stimulation (14.08, $P < 0.0001$ with G-G correction).

3.2.4. Operated Muscle Force to Muscle Stimulation. Similar force-current relationships during nerve stimulation and during direct muscle stimulation were observed in the operated muscles (Figure 5). During muscle stimulation of the operated muscle, the threshold current of 0.1 mA produced visible muscle contractions. The maximal force of the operated muscle was 54.6% of the control (maximal force produced by the control, the nonoperated muscle on the contralateral side). Interestingly, variability of the muscle-force data to direct muscle stimulation was about 2-3 times smaller than that to nerve stimulation.

3.3. Force-Current Characteristics with Delayed Reinnervation. The SM muscles with a one-month as well as a three-month delay between nerve transection and repair showed a smaller muscle force as compared with the SM muscles, which were repaired immediately after denervation (Figures 5, 6, 7, and 8). The stimulation threshold was 0.1–0.2 mA. The maximal force of the operated muscle to nerve stimulation was reduced to about 39% of the maximal force produced by the control, the nonoperated muscle (39.8% in the 1-month delay EEA group and 38.0% in the 3-month

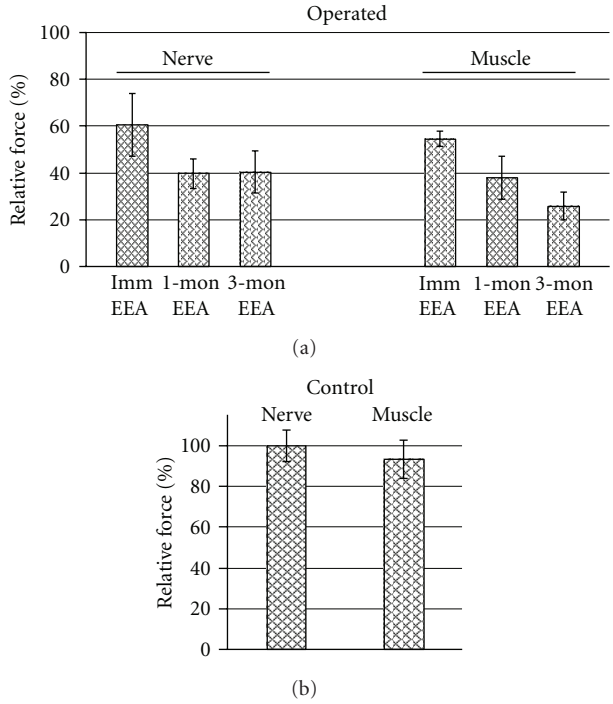

Figure 8: Average maximal muscle force of the SM muscle in different experimental conditions. (a) Shows maximal muscle force recorded from the operated SM muscles with immediate nerve repair after nerve transection (Imm EEA), with 1-month delay nerve repair (1-mon EEA) and with 3-month delay nerve repair (3-mon EEA). Data were normalized through dividing the maximal muscle force from the operated SM muscle by the maximal muscle force from the control SM muscle. Maximal muscle force produced by nerve stimulation is shown at the left side of the graph and by direct muscle stimulation at the right side of the graph. (b) Illustrates a comparison between the maximal muscle force evoked through nerve stimulation and through direct muscle stimulation of the control muscle (the intact muscle on the contralateral side). Muscle force (average from all three EEA groups) is presented as a percentage of maximal muscle force produced by nerve stimulation.

delay EEA group). A two-factor repeated ANOVA (group and intensity of stimulation as main factors) showed a statistically significant difference in muscle force between groups (control, immediately operated, and operated after 3-month delay, $F = 22.82$, $P < 0.0001$) as well as an interaction of group with intensity of stimulation ($F = 4.06$, $P < 0.002$ with a Greenhouse-Geisser correction). With direct muscle stimulation the maximal force of reinnervated muscle was reduced to 40.2% in 1-month delay EEA group and 25.8% in 3-month delay EEA group.

3.4. Muscle Weight. Figure 9 illustrates the effects of reinnervation on muscle mass. A normal SM muscle (on the control side) was compared to the operated SM muscle. The operated muscle was repaired with an immediate EEA (at the left), with a 3-month delayed EEA (in the middle) and was left denervated (at the right). The weight of the operated muscles with immediate nerve repair was 78% of the weight of the muscles on control side (Figures 9 and 10).

Delayed EEA resulted in smaller muscle weight as compared with immediate EEA (Figures 9 and 10). Averaged muscle weight was 68% of the control for 1-month delay EEA and 64% of the control for 3-month delay EEA. The mean muscle weight of the EEA reinnervated muscles was greater than that of the denervated SM muscles (36% of the control). An ANOVA showed a significant difference between experimental groups ($F = 39.2$, df $= 3/21$, $P < 0.001$). Tukey's test showed that the immediate nerve repair group has a significantly larger weight than any other group. There was no significant difference in muscle weight between 1-month and 3-month delay EEA groups.

4. Discussion

4.1. Data Summary. Our present study provides the force characteristics of the rat SM muscle, which was denervated through an SM nerve transection and reinnervated at different time points with the classic EEA method.

The results showed only a partial recovery of muscle force at 3 months after reinnervation surgery. The recovery level of muscle force decreased when additional delay periods (1 or 3 months) were introduced between nerve transection and EEA nerve repair.

4.2. The Level of Force Recovery in the SM Muscle Reinnervated with EEA Immediately after Nerve Transection. The partial recovery of force generated by the reinnervated SM muscle was reflected in a higher stimulation threshold to produce any muscle contraction and smaller maximal muscle force. On the operated side, the threshold current needed to produce detectable SM muscle contraction was five times larger than that on the intact side. The maximal muscle force produced by a 0–1 mA nerve stimulation of the reinnervated SM muscle was reduced to about 60% of the force produced by the intact SM muscle.

Similar reduction of maximal muscle force after EEA repair was reported previously in other rat muscles. Meyer et al. [13] analyzed force produced by the soleus muscle following transection and traditional end-to-end epineurial repair of the sciatic nerve in the rat. By 32 weeks, the maximum isometric muscle force recovered to about 70% of the force of an intact muscle. Interestingly, recovery was much better (90% of normal) when the nerve went through a crash injury instead of transection. The authors concluded that poor recovery after nerve transection could be caused by the innervation of a muscle by inappropriate axons. Master et al. [22] studied the force produced by the gastrocenemius muscle in rats that had tibial nerve transected (4–5 mm proximal to the neuromuscular junction) followed by immediate neurorraphy. At 3 months postoperatively, the mean force of the operated gastrocenemius returned to 68% of the force produced by nonoperated one.

The mechanisms responsible for the reduced force produced by denervated and reinnervated muscles are not completely understood but may include muscle atrophy, reduced

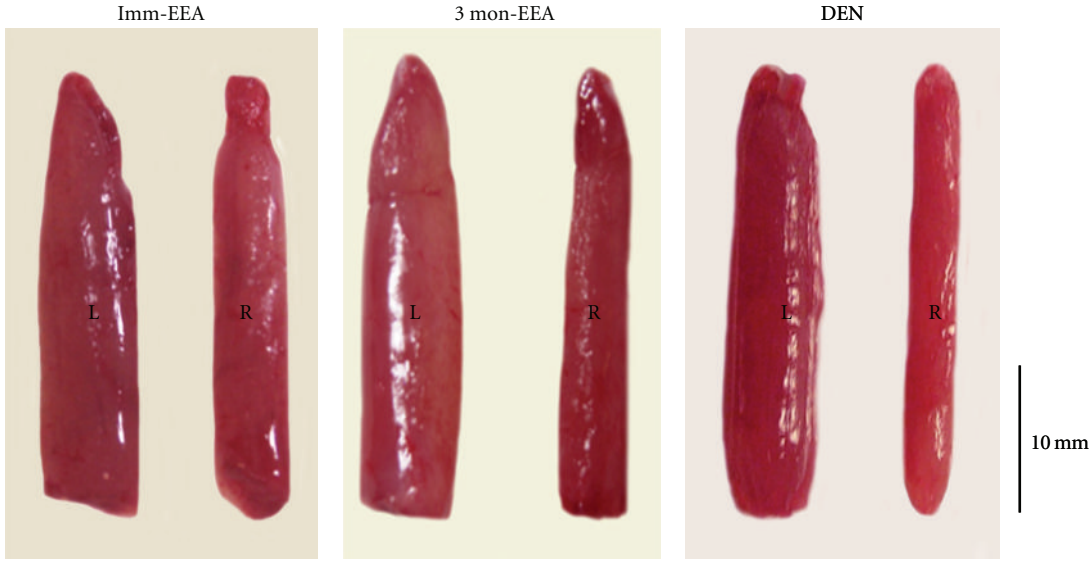

Figure 9: Photographs of the removed SM muscles showing the extent of muscle atrophy in three experimental groups. (a) A pair of SM muscles from a rat in the immediate EEA (Imm-EEA) group. (b) A pair of SM muscles from a rat in the 3-month delay EEA (3 mon-EEA) group. (c) A pair of SM muscles from a rat with denervation (DEN) of the right SM caused by nerve transection. In each rat, the left (L) SM was normal, whereas the right (R) SM was reinnervated or denervated.

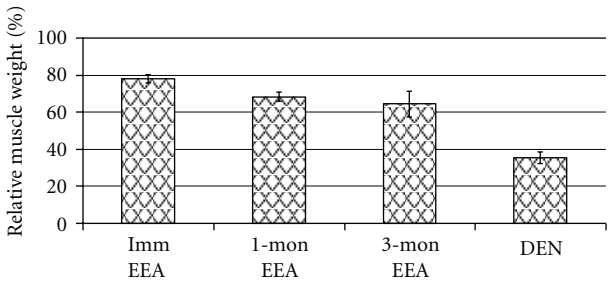

Figure 10: Average relative SM muscle weight in the denervation (DEN) and reinnervation experimental groups with immediate (Imm EEA), 1-month delay (1-mon EEA), and 3-month delay (3-mon EEA) nerve repair. SM muscle weight on the operated side was presented as a percentage of the SM muscle weight on the control side.

axonal numbers, altered axonal spatial organization, diminished muscle oxidative capacity, motor unit remodeling, and alternations in muscle fibers [23].

4.3. Decreased Level of Force Recovery in the SM Muscle with Delayed EEA Reinnervation. Delaying the process of reinnervation leads to an even larger decline in the ability of the SM muscle to generate force. As compared to immediate nerve repair, the 3-month delay between operation and reinnervation increased 2 times the threshold of current needed to produce muscle contraction. The maximal force produced by 0–1 mA nerve stimulation of SM muscle was reduced to about 40% of the force produced by the intact SM muscle.

Fu and Gordon [24] also reported poor functional recovery when nerve repair was delayed. They used a nerve cross-anastomosis paradigm in the rat. The tibial nerve was axotomized up to 12 months before it was cross-sutured to the distal stump of the freshly cut common peroneal nerve to innervate the freshly denervated tibialis anterior muscle. The authors concluded that prolonged axotomy significantly reduces the number of motor axons that regenerate and make functional connections with denervated muscle fibers.

Aydin et al. [25] denervated rat's gastrocnemius muscle via tibial nerve transection and delayed reconstruction of the nerve for periods ranging from 2 weeks to 1 year. They showed large deficits in muscle mass and maximum tetanic force caused by delayed reinnervation. The deficits were directly proportional to the denervation interval.

Swanson et al. [26] denervated the rat tibialis anterior by transecting the common peroneal nerve and after varying delays (ranging from 0 to 5 months) repaired the nerves with EEA. As in the present study, during muscle force testing, the muscle was stretched to the optimal tension needed to elicit a maximal force. Immediate reinnervation provided for very good maximal muscle force equal 94% of control (although this result was not very reliable because of large (30) standard error). After 3 months, muscle force fell to only 38%.

4.4. No Difference in Maximal Muscle Force Evoked by Muscle and Nerve Stimulations. One of physiological measures of muscle innervation is the ratio of tetanic muscle force to indirect and direct muscle stimulation. In normal muscles, this ratio is 100% and might be reduced in partially denervated muscles due to axonal damage, loss to neuromuscular junctions (NMJs), or severe defects in NMJ

synaptic transmission [27]. No difference in average maximal tetanic muscle force was observed in the present study when EEA reinnervated muscle contraction was evoked indirectly—through SM nerve stimulation, and with direct muscle stimulation. A similar observation that there is no difference between the maximal tetanic force evoked by nerve stimulation and by direct muscle stimulations, we reported previously in the SM muscle, which was repaired using the recently developed "nerve-muscle-endplate band grafting" (NMEG) technique [9]. Lack of power of the indirect/direct force ratio might be caused by "safety factor," the excess of released transmitter during transmission of activity from nerve to muscle (the amount of transmitter released per nerve impulse is greater than that required to trigger an action potential in the muscle fiber). The safety factor allows neuromuscular transmission to remain effective under various difficult physiological conditions and stresses [28].

Interestingly, in the EEA reinnervated groups, the between-subject variability of maximal muscle force was larger during indirect (nerve) then during direct (muscle) stimulation. Gruner and Mason [29] showed considerable variation in recruitment of different muscle compartments during muscle force generation. The recruitment of different muscle compartments might be more variable in EEA group during indirect muscle stimulation (as compared to direct muscle stimulation), causing larger variability of the total tetanic muscle force.

4.5. Substantial Loss of Muscle Weight after Denervation. Large SM muscle atrophy (36% of the control muscle) caused by a 3-month denervation was illustrated in Figure 9. Similarly, strong denervation atrophy was observed in the rabbit tibialis anterior (TA) muscle by Ashley et al. [30]. After 2.5 months of denervation, muscle weight was reduced to 44% of weight observed in the innervated contralateral control TA. To prevent or at least limit the atrophy of the muscle during the denervation period (when final reinnervation had to be postponed) some authors used a two-stage operation process. In this process, an early temporary intermediate reinnervation (so called motor "baby sitting") was followed by a final reinnervation [23]. However, the results of such double-stage operations produce worse outcomes than reinnervation made without intermediate reinnervation. Interestingly, muscle mass did not significantly differ between the normal and operated (dennervated and repaired) groups which might suggest that denervation atrophy does not play a primary role in the reduction of tetanic force. Larger recovery of muscle mass (70% and 83%) than maximal tetanic force (43% and 70%) was observed in the rat soleus muscle, with its sciatic nerve transected, repaired with anastomosis, and studied 2 and 8 months after the repair [13]. However, a significant increase in muscle weight recovery was observed only in rats with long recovery time after reinnervation (between 4 and 8 months). There was no significant increase of muscle weight between 2 and 4 months after nerve repair. Swanson et al. [26] analyzed the weight of the rat tibialis anterior muscle and found that muscle weight after denervation and repair with EEA is reduced to 75% (of the weight on the unoperated, contralateral side) for immediate EEA and to 59% after a 3-month delayed denervation. We observed similar levels of weight reduction in the present study in reinnervated SM muscles.

4.6. Need for the Search of Other Alternating Reinnervation Techniques. In general, nerve repair is accomplished by conventional end-to-end anastomosis when the two stumps can be approximated without tension [11, 31, 32]. A moderate functional recovery after nerve anastomosis points to the importance of developing new alternative surgical techniques, which may lead to the optimal reinnervation of paralyzed muscles. We hope that our new NMEG grafting method [9] has the potential to generate a better outcome than the commonly used EEA, especially with further refinement of the surgical procedure, the better placement of the donor NMEG graft at the recipient muscle, and by adding other factors facilitating the innervation process (e.g., electrical stimulation, neurotrophic factors, stem cells). Hall [16] in his review paper postulated that the next advances in nerve repair will depend upon manipulating the injury response around the injury site using cells and/or exogenous peptides rather than fine-tuning microsurgical techniques. Our recent studies [9] suggest that advances in muscle innervation techniques might be still critical component of a reinnervation strategy leading to optimal muscle reinnervation.

5. Conclusions

The present experiments describe characteristics of the force produced by the SM muscle of the rat with its supplying nerve severed and subsequently repaired with end-to-end anastomosis. Our study shows that a reinnervated SM muscle needs more than a 5-time larger nerve stimulation current to generate muscle contraction and is able to produce about 60% of tetanic force observed in the intact SM muscle.

The drop in the muscle force is more severe when there is an additional delay between nerve damage and repair. With a 3-month delayed reinnervation, the maximal tetanic muscle force drops to about 40% of the force observed in an intact SM muscle.

The results of this study provide reference levels for the tetanic force generated by the SM muscle in rats reinnervated with classic EEA. They can be used to assess the success of functional reinnervation produced by new reinnervation techniques.

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