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Muscle Plasticity in Rat Following Spinal Transection and Chronic Intraspinal Microstimulation

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Abstract

Intraspinal microstimulation (ISMS) employs electrical stimulation of the ventral grey matter to reactivate paralyzed skeletal muscle. This work evaluated the transformations in the quadriceps muscle that occurred following complete transection and chronic stimulation with ISMS or a standard nerve cuff (NCS). Stimulation was applied for 30 days, 4 hours/day. Both methods induced significant increases in time-to-peak tension (ISMS 35%, NCS 25%) and $\frac{1}{2}$ rise-time (ISMS 39%, NCS 25%) compared to intact controls (IC). Corresponding increases in type-IIA myosin heavy chain (MHC) and decreases in type-IID MHC were noted compared to IC. These results were unexpected because ISMS recruits motor units in a near-normal physiological order while NCS recruits motor units in a reversed order. Spinal cord transection and 30 days of stimulation did not alter either recruitment profile. The slope of the force recruitment curves obtained through ISMS following transection and 30 days of stimulation was similar to that obtained in intact animals, and 3.4-fold shallower than that obtained through NCS. The transformations observed in the current work are best explained by the near maximal level of motor unit recruitment, the total daily time of activity and the tonic nature of the stimulation paradigm.

Index Terms

Functional electrical stimulation; muscle plasticity; spinal motoneurons

I. Introduction

Intraspinal microstimulation (ISMS) is a new model of functional electrical stimulation (FES) that has been investigated for its ability to produce weight-bearing standing [1] and stepping [2] in cats. Previous reports showed that, in contrast to various peripheral FES approaches, ISMS generates graded force recruitment and produces coordinated multi-joint synergies [2,3]. We have attributed the gradual force recruitment characteristics of ISMS to its ability to activate motoneurons in a near normal physiological order based upon their size

[3]. Presumably, this ordered recruitment is due to the preferential activation of fibers-in-passage, and the resultant trans-synaptic activation of motoneurons [4,5].

The purpose of this work was to evaluate the muscle transformations that occur following complete spinal cord transection (ST) and the potential for ISMS to rescue a normal muscle fiber phenotype. In the rectus femoris muscle, this would mean the maintenance of a mixed concentration of myosin heavy-chain (MHC) type-I and type-IIA fibers in the deepest portion of the muscle and a large concentration of type-IID/X and type-IIB fibers in the intermediate and superficial regions. Accordingly, this mixed muscle would maintain the isometric twitch properties of an intact muscle.

We have shown previously that acute ISMS produces near normal motor unit recruitment and preferential activation of slow, fatigue-resistant muscle fibers while peripheral stimulation through a nerve cuff produces reversed recruitment and activation of fast, fatigable fibers [3]. Given these results, we hypothesized that chronic ISMS in spinal cord transected rats would rescue many of the functional properties of an intact muscle and its corresponding contractile proteins. We further hypothesized that chronic nerve cuff stimulation (NCS) in spinal cord transected rats would cause a dramatic fast-to-slow transformation in MHC-based fiber content and corresponding slowing of the isometric muscle twitch. We tested these hypotheses by transecting the spinal cords of rats at the eighth thoracic (T8) level, and stimulating one leg of each rat with either ISMS or NCS for thirty days while the contralateral leg served as an unstimulated control. We found that chronic ISMS in rat reverses the slow-to-fast transformation following ST and, surprisingly, that the nature of the transformation was similar to that produced by chronic stimulation through a nerve cuff. This work is the first to evaluate the muscle transformations induced by chronic ISMS, an electrical stimulation method that produces near-normal recruitment of motoneurons.

II. Muscle transformation

A. Stimulation caused slowing of the muscle twitch

Aseptic transection of the spinal cord at T8 was carried out on 12 adult, female Sprague Dawley rats (250g–300g) according to previously established methods [2]. After a two-week recovery period, transected animals were implanted with a nerve cuff over the femoral nerve (n=6), or a 4-wire ISMS array (n=6) targeting the quadriceps motoneuron pool bilaterally [3]. In all animals, one leg was used for stimulation while the other was used as a contralateral control (i.e. nerve cuff control leg, NCC, or intraspinal microwire control leg, ISC). Beginning one-week later, animals received 30 days of stimulation for 4 hours each day at an aggregate frequency of 50 pulses per second (pps): monopolar ISMS was interleaved between two wires (25 pps each) while bipolar NCS was delivered at 50 pps through the 2-contact cuff electrode. Stimuli were composed of biphasic, cathodic-first, 200 μ s, charge balanced pulses. In addition to the NCS and ISMS stimulated groups, a total of 6 animals were used as intact controls (IC).

Stimulation through the nerve cuff proceeded at supramaximal levels of 4.0 ± 0.0 times the activation threshold (the lowest stimulus amplitude that produced a muscle twitch). The mean activation threshold through the nerve cuff was $145.9 \pm 57.0 \mu$ A. The use of supramaximal stimulation was necessary to produce stable contractions in the quadriceps muscle. In all cases, NCS produced strong extension of the leg at the knee throughout the thirty day stimulation period. Stimulation through the ISMS microwires produced a more complex response. The mean activation threshold through the ISMS microwires was $88.9 \pm 22.1 \mu$ A. Stimulation caused weak quadriceps contractions at activation threshold, and muscle synergies at higher stimulus amplitudes. Out of 12 microwires that were used for

stimulation 8 produced extension synergies of the whole leg involving hip and knee extension while the other 4 microwires produced a forward movement involving knee extension and hip flexion. In order to standardize the muscle responses across all nerve cuff and ISMS animals, the experimental ISMS legs were stimulated at the maximal amplitude that produced knee extension but not muscle synergies. When expressed as a function of activation threshold these stimulus amplitudes were a mean of 2.55 ± 0.87 times the activation threshold. This level of stimulation produced strong activation of the quadriceps muscle in each animal throughout the thirty day stimulation period.

Thirty days of stimulation caused a significant lengthening in the duration of supramaximal isometric twitches. Both NCS and ISMS legs showed lengthening of time-to-peak tension (TTP), one-half rise time (1/2RT) and one-half fall time (1/2FT) compared to their respective NCC and ISC control legs (Figure 1). Furthermore, the NCS and ISMS legs also exhibited significant lengthening of both TTP and 1/2RT when compared to the IC legs.

Only the ISMS group showed significant lengthening of 1/2FT in comparison to the IC legs. This difference suggests that the intraspinal stimulation paradigm caused a stronger transformation in the components of muscle relaxation; however, the exact nature of these changes is unclear. Contralateral control ISC and NCC legs were not significantly different from the IC legs for any measurements of isometric twitch length.

B. Stimulation caused a fast-to-slow fibre-type transformation

Immunohistochemical identification of MHC isoforms in rectus femoris muscle was used to confirm the fast-to-slow transformation detected by the lengthening of the isometric twitch. Primary monoclonal antibodies were applied overnight at 4° C at the following dilutions in blocking solution: anti-MHC type-I clone BA-D5 (1:400); anti-MHC type-IIA clone SC-71 (1:100); anti-MHC type-IIB clone BF-F3 (1:400). Secondary antibodies were applied for one hour at room temperature at the following dilutions: biotinylated horse anti-mouse IgG (Vector Laboratories, for BA-D5 and SC-71) diluted 1:400 in blocking solution or with biotinylated goat anti-mouse IgM (Vector Laboratories, for BF-F3) diluted 1:400 in blocking solution. The analysis of fiber-type transformation focused on the slowest, deepest region of rectus femoris as this area contained a mixture of fiber types. This allowed for the detection of fast-to-slow as well as slow-to-fast transformations

The slowing of muscle twitch in stimulated legs was matched by a fast-to-slow transformation of MHC content in the slowest region of the rectus femoris muscle in these legs (see Figures 2 and 3). This finding was verified with whole muscle analysis which showed an increase in the area fraction for MHC IIA fibers in stimulated NCS and ISMS legs compared to the IC control legs (Figure 4). In contrast to the stimulated legs, a mild slow-to-fast transformation was observed in the NCC and ISC legs compared to IC legs. Despite the increase in fast MHC isoforms in the slowest region of unstimulated rectus femoris muscles (Figures 2 and 3), there were no indications from isometric twitch measures that these legs were functionally faster than the IC legs (Figure 1). This is presumably because the content of slower type-I and -IIA fibers in the rectus femoris muscle of the IC legs is small and the isometric twitch measures from the whole quadriceps muscle are dominated by the far greater content of the faster type-IID/X and -IIB fibers. The slow-to-fast transformations in unstimulated NCC and ISC muscles did not play a large role in altering isometric functional measures since they took place in a relatively small population of slower fibers.

III. Recruitment order

A. Force recruitment through NCS and ISMS is unchanged following chronic spinal cord transection

Stimulation for 30 days through intraspinal wires (ISMS group) or through the nerve cuff (NCS group) resulted in nearly identical slowing of isometric twitches and corresponding fast-to-slow transformations in rectus femoris muscle. This suggests that the nature of the transformations produced by these different stimulation paradigms was similar in many respects, a result we did not anticipate given that findings in acute experiments showed that NCS recruits greater force than ISMS and that the recruitment of force is more gradual with ISMS than NCS [3]. A possible explanation for these results was that the nature of the recruitment order was altered after ST. This could occur either because of plastic alterations in neuronal properties, or because of damage to the fibers-in-passage in the grey matter of the spinal cord due to microwire implantation and chronic ISMS. In order to rule out this possibility we obtained force recruitment curves through ISMS and NCS in the chronically implanted and stimulated animals and compared these to previous results from intact animals.

The mean slope of recruitment curves generated through ISMS microwires in the ISMS legs was 0.0048 ± 0.0027 normalized force/ μA , while that generated through nerve cuffs in NCS legs was significantly sharper at 0.016 ± 0.010 normalized force/ μA (t-test, $p = 0.036$). This represents a 3.4 fold sharper recruitment of force through the nerve cuff compared to recruitment through ISMS microwires (see Figure 5). While this is less than the 4.9 fold difference previously found for intact animals [3], it represents a significantly more gradual recruitment of force through the ISMS microwires. Furthermore, the mean slope of the recruitment curves generated through ISMS microwires after transection and thirty days of stimulation (0.0048 ± 0.0027) was nearly identical to the previously reported mean slope generated through ISMS microwires in intact animals (0.0041 ± 0.0021) [3]. This suggests that the recruitment order of motor units through ISMS and NCS was not altered following spinal cord transection; either by neuroplastic adaptations or damage of spinal networks. Note that the mean maximal force evoked by stimulation with a nerve cuff in the anesthetized animals during the terminal experiments was $6.29 \text{ N} \pm 4.02 \text{ N}$ while the mean maximal force evoked through ISMS wires was $1.64 \text{ N} \pm 0.39 \text{ N}$ (Figure 5). The maximal force level evoked by ISMS differed from our observation of strong quadriceps activation by ISMS during daily stimulation with the animals awake. This discrepancy is a result of the depressant effect of isofluorane on the neural circuitry of the rat spinal cord resulting in a lower maximal evoked force by ISMS during the terminal experiments [6].

IV. Conclusion

A. Overview

Skeletal muscle is a highly adaptive tissue that responds to imposed demands by altering its functional properties including fatigue resistance, sag profile and the speed of isometric twitches. Tests for these properties reveal the functional outcomes of the muscle transformation. Key contractile proteins such as the MHCs can also reveal the direction of transformation as they are tightly correlated with these functional properties [7].

The overall goal of this work was to assess the long-term effects of ISMS on the functional characteristics and phenotypic make-up of muscles that had been paralyzed due to a complete ST. We found that chronic ST resulted in a mild slow-to-fast transformation in contralateral, unstimulated ISC and NCC legs. The extent of this transformation was limited by the fact that rat quadriceps muscles already demonstrate a fast phenotypic profile which cannot be made substantially faster [3]. Both ISMS and NCS legs showed nearly identical

slowing of quadriceps function and corresponding fast-to-slow transformations in rectus femoris muscle. This suggests that the nature of the transformations produced by these different stimulation paradigms was similar in many respects. This was unexpected as our previous findings in acute experiments showed that ISMS recruited force more gradually than NCS, although NCS did recruit greater force [3].

B. Pattern of quadriceps activation through ISMS and NCS

According to Kernell et al. the impetus for the transformation of motor units arises from the daily amount of activity imposed upon the muscle [8]. Given that both ISMS and NCS underwent similar fast-to-slow transformations it is possible that both stimulation paradigms were activating a similar proportion of their motor units, both large and small. In the case of NCS, the reversed recruitment order makes this almost certain since the whole population of motor units are recruited over a very small range of stimulus amplitudes. Furthermore, the instability of force evoked by the bipolar nerve cuff at submaximal stimulation levels makes stimulation at supramaximal levels, and the concomitant activation of all motor units, the only option for achieving stable force. In the case of ISMS, stimulation levels were chosen to provide muscle contractions that resembled those obtained by NCS as closely as possible. Therefore, it seems very likely that a larger proportion of motor units in the quadriceps muscle was recruited by ISMS, thereby generating the same transformation seen with NCS. Previous studies have shown that large forces can be evoked by trains of ISMS delivered through individual microwires [9–11].

While full recruitment of motor units in the ISMS animals is likely a main contributor to the dramatic fast-to-slow transformations in the quadriceps muscle, these effects are further compounded by the duration of daily activation. Four hours of continuous stimulation is, in retrospect, inappropriate for maintaining a mixed muscle phenotype. This duration of activation is akin to an endurance training paradigm in marathon runners, a population with proportionally higher representation of type-I and IIA fiber types in their leg muscles [12].

C. Summary and future directions

Thirty days of stimulation through either intraspinal microwires or a nerve cuff induced similar fast-to-slow transformations as measured by isometric twitch speed measures, sag profile and MHC content in rectus femoris muscles. This was surprising in light of a number of studies in intact and spinalized animals showing that ISMS recruits gradual, fatigue resistant force suggesting a more normal recruitment order. We verified directly the near-normal recruitment order of ISMS in an acute rat model and showed that recruitment order did not change in the ISMS animals used in the current work. Although it remains possible that there was a transient alteration of recruitment properties following transection, the most likely explanation for the current results is that the populations of motor units recruited by both NCS and ISMS were nearly identical. This led to the dramatic and similar fast-to-slow transformations in both stimulated legs. In the future, ISMS should be used to produce submaximal contractions. Stable contraction levels at forces below 50% of maximal voluntary activation are readily achievable with ISMS, in contrast to peripheral FES approaches (e.g. Figure 5). Such contraction levels are more representative of the natural recruitment order and activation paradigms utilized for achieving the majority of daily activities [13].

Acknowledgments

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Figure 1. Summary of twitch width measurements

A) TTP lengthened in both NCS and ISMS legs compared to NCC and ISC control legs as well as IC legs. B) 1/2RT lengthened in both NCS and ISMS legs compared to NCC, ISC and IC legs. C) 1/2FT lengthened in NCS and ISMS legs as compared to the NCC and ISMS legs. Only the ISMS legs showed significant lengthening of 1/2FT when compared to the IC leg. (a) different from intact control, (b) different from contralateral control.

Figure 2. Myosin heavy chain (MHC) labelling with immunohistochemistry

Representative examples of MHC staining from rectus femoris muscles at 5X magnification.

A) Intact control legs show a mixed phenotype with a preponderance of MHC type-IIB and unstained -IID fibres. B, D) Following spinal cord transection, unstimulated nerve cuff control (B) and intraspinal microwire control (D) legs show increases in MHC type-IIB fibre content and decreases in MHC type-IIA fibre content. C, E) Thirty days of stimulation through a nerve cuff (C) or intraspinal microwires (E) causes an increase in MHC type-IIA fibre content and a decrease in MHC type-IIB content. A mean of 432.3 ± 96.1 rectus femoris muscle fibres were analyzed in each leg for a total of 12970 fibres.

Figure 3. Summary of MHC immunohistochemistry results from the deep portion of the muscle Stimulation with either NCS or ISMS caused a fast-to-slow conversion as demonstrated by increases in type-IIA fibre content and corresponding decreases in type-IID fibre content as compared to the IC leg. Control NCC and ISC legs showed a slow-to-fast conversion as demonstrated by increases in type-IIB fibre content and decreases in type-IIA fibre content. (a) different from intact control, (b) different from contralateral control

Figure 4. Whole muscle analysis of the MHC IIA content

Whole muscle images immunolabeled for MHC IIA (A) were binarized so that stained pixels were rendered completely black and all other pixels completely white (B). Image analysis software was used to quantify black pixels and express them as a fraction of all pixels within the muscle boundary. (C) The area of the muscle containing MHC IIA increased in stimulated ISMS and NCS legs compared to control legs ISC and NCC and to the IC leg. (a) different from intact control, (b) different from contralateral control.

Figure 5. Pattern of force recruitment after chronic ISMS and NCS

Normalized force recruitment curves obtained through A) 6 nerve cuff electrodes and B) 5 ISMS microwires, after 30 days of stimulation. Graphs show force recruited by one second long 50 pps trains of randomly assigned amplitudes. The mean slope of the force recruited from 10% to 90% of peak force was 3.4 fold sharper in the nerve cuff group. Maximal force evoked was $6.29 \text{ N} \pm 4.02 \text{ N}$ through the nerve cuff and $1.64 \text{ N} \pm 0.39 \text{ N}$ through the ISMS microwires.