Neuromuscular rehabilitation by treadmill running or electrical stimulation after peripheral nerve injury and repair

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Marqueste, Tanguy, Jean-Roch Alliez, Olivier Alluin, Yves Jammes, and Patrick Decherchi. Neuromuscular rehabilitation by treadmill running or electrical stimulation after peripheral nerve injury and repair. J Appl Physiol 96: 1988–1995, 2004. First published November 21, 2003; 10.1152/japplphysiol.00775.2003.—Numerous studies have been devoted to the regeneration of the motor pathway toward a denervated muscle after nerve injury. However, the regeneration of sensory muscle endings after repair by self-anastomosis are little studied. In previous electrophysiological studies, our laboratory showed that the functional characteristics of tibialis anterior muscle afferents are differentially affected after injury and repair of the peroneal nerve with and without chronic electrostimulation. The present study focuses on the axonal regeneration of mechano- (fibers I and II) and metabosensitive (fibers III and IV) muscle afferents by evaluating the recovery of their response to different test agents after nerve injury and repair by self-anastomosis during 10 wk of treadmill running (LSR). Data were compared with control animals (C), animals with nerve lesion and suture (LS), and animals with lesion, suture, and chronic muscle rehabilitation by electrostimulation (LSE) with a biphasic current modulated in pulse duration and frequency, eliciting a pattern mimicking the activity delivered by the nerve to the muscle. Compared with the C group, results indicated that肌肉的重量在LS和LSR组中较小, 2) the fatigue index was greater in the LS group and smaller in the LSE group, 3) metabosensitivity remained altered in the LS and LSE groups, and 4) mechanosensitivity presented a large increase of the activation pattern in the LS and LSE groups. Our data indicated that chronic muscle electrostimulation partially favors the recovery of muscle properties (i.e., muscle weight and twitch response were close to the C group) and that rehabilitation by treadmill running also efficiently induced a better functional muscle afferent recovery (i.e., the discharge pattern was similar to the C group). The effectiveness of the chronic electromyostimulation and the treadmill exercise on afferent recovery is discussed with regard to parameters listed above.

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tabosensitive muscle afferents by evaluating the recovery of their response after nerve injury and repairs by self-anastomosis during 10 wk of treadmill running. In a previous rat study (8), our laboratory reported the responses of tibialis anterior muscle afferents to different test agents [KCl, lactic acid, electrically induced fatigue (EIF), and tendon vibrations] in control (C) conditions, after nerve repair using an epineural suture technique (11), and after chronic electromyostimulation (34).

METHODS

Animals

Experiments were conducted in 48 female, 4-mo-old, Sprague-Dawley rats, obtained from Ifla Credo (Les Oncins, France). Housing, surgical procedures, and assessment of analgesia were performed according to the French law on Animal Care Guidelines, and the protocols were approved by the Animal Care Committee of our University. Efforts were made to minimize animal suffering and to only use the number of animals necessary to produce reliable scientific data. Animals were housed in smooth-bottomed plastic cages at 22°C with a 12:12-h light-dark cycle. Food (Purina rat Chow) and water were available ad libitum. Animals were randomly placed in four groups.

In the first group (C group, n = 10), animals were left intact. In the others groups [animals with nerve lesion and suture (LS), n = 14; animals with lesion, suture, and chronic muscle rehabilitation by electromyostimulation (LSE), n = 12; and animals with 10 wk of treadmill running (LSR), n = 12], the left peroneal nerve was sectioned then immediately self-anastomosed. In the LS group, the left peroneal nerve was sectioned and the animals were housed for the next 10 wk. In the LSE group, the denervated muscle was stimulated daily by a biphasic current, whereas in the LSR group animals ran on the treadmill daily. Electromyostimulation and exercise were performed 5 days/wk for 10 wk. All animals were housed for the next 10 wk in smooth-bottomed plastic cages at 22°C with a 12:12-h light-dark cycle. In this study, we did not include a group with only electrodes placed because we considered that there is no effect of the electrode placement as an isolated variable. Indeed, data reported in the literature indicate that in sham-operated animals (animal with only electrode implanted) no differences were found with the C group (4, 51).

Common Surgical Procedures

As previously described (11, 34), surgery was carried out on animals under deep pentobarbital sodium anesthesia (Nembutal, 60 mg/kg ip, Sanofi, Libourne, France), and atropine (1 mg/kg) was administered intraperitoneally to reduce airway secretions. Briefly, with the use of an operating microscope (×40, OPM 11 Zeiss, Oberkochen, Germany), and under aseptic conditions, the common peroneal nerve was exposed in the popliteal fossa on one left side. This procedure was performed by exposing the left peroneal nerve in the posterior thigh, thereby freeing the nerve from the popliteal vessels. Muscles innervated by this nerve are particularly suitable for reinnervation studies, because this nerve lies very superficially at the knee, thereby minimizing the requisite surgery. The distance from the transection nerve entry point to the muscle under study, i.e., the tibialis anterior, was ~10 mm. To obtain best results, the two stumps of the sectioned nerve were immediately (50) stitched without tension (52) by five epineural sutures (Ethilon, 10-0; Omnium, Médical, Neuilly, France). No further surgery was performed until the electrophysiological studies. Muscles and skin were closed with 3-0 suture (Triny, Omnium Médical) and locally disinfected.

Functional Electromyostimulation

In LSE groups, two electrodes were intramuscularly fixed for stimulation (diameter: 500 μm). They were connected to silver cables (PTFE ET 32.07; Draka Fileca, Saint-Geneviève, France) tunneled subcutaneously from the left muscle to the skull. The distal end of cables were attached (Ethilon, 8-0; Omnium Médical) to the muscular epinurium, whereas the other end was connected to a microconnector (Radiospares, Beauvais, France) fixed on the skull with stainless steel screws and dental cement (Ivoclar, Liechtenstein). Animals were stimulated for 10 consecutive wk (5 h/day, 5 days/wk). The stimulation pattern used was an alternative biphasic stimulation (10 V) modulated in frequency at 4–7.5–4 Hz and shock duration at 200–150 ms. This stimulation pattern lasted 6.5 s and was delivered once a minute by a clinical stimulator (Multiprocess 16+. Physitec, Electronique Médicale, Marseille, France) used in electrophysiotherapy and physiotherapy. One week after surgery, chronic stimulation was performed 5 h/day, 5 days/wk for 10 consecutive wk.

Exercising Protocol

The running session was performed on the LSE group 1 wk after self-anastomosis on a treadmill (Medical Développement, Saint-Étienne, France). All animals ran in the same condition (90) at 10 m/min continuously for 1.5 h, twice a day, 5 days/wk. This daily protocol, based on a previous study (53), was chosen to activate the denervated/reinnervated muscle. The exercising and functional electrical stimulation protocols were performed during 10 consecutive wk.

Nerve Recordings

After a 10-wk rehabilitation period, rats were anesthetized by an intraperitoneal injection (1.0 ml solution/100 g body wt) of solution containing pentobarbital sodium and 0.9% sodium chloride in 1:10 volume proportion. The trachea was cannulated for artificial ventilation (Harvard volumetric pump: rate 40–60 breaths/min, tidal volume 2–4 ml; Southmatic, MA). A catheter was also inserted into the right femoral artery and retrogradely advanced as far as the fork of the abdominal aorta to retrogradely inject bolus of KCl and lactate (Lac) solutions to the contralateral studied muscle. The catheter was positioned so that the blood flow to the left tibialis anterior muscle was not interrupted. Animals were paralyzed by an intra-arterial injection of pancuronium bromide (Pavulon, 10 mg/kg; Sanofi, Fresne, France). The left tibialis anterior muscle was dissected and freed from surrounding tissues, but tendons were left intact. Knee and ankle were firmly held by clamps on a horizontal support to avoid limb motion. Dissection at the midheight level was carried out to expose the common peroneal nerve. Then, it was dissected free from surrounding tissues at a length of 5–6 cm, and its proximal portion was cut.

To record the compound nerve action potential (CNAP), the whole peroneal nerve was placed on bipolar electrodes and was electrically stimulated 1 mm from its insertion point in the muscle with single shocks (0.1–1 ms, long rectangular pulses, supramaximal) delivered by a S8800 stimulator (Grass, Quincy, MA) through an isolation unit. CNAPs were recorded by using bipolar electrodes placed 15 mm from the stimulating electrodes. Raw afferent activity was displayed on a storage oscilloscope (DSO 400 Gould, Madison, WI) to average CNAPs evoked by the stimulation of the distal nerve. The conduction velocities, at the peak of the waves, were calculated by using an interelectrode distance of 15 mm.

To record activity from the muscle afferents, the free end of the distal nerve portion was divided into several filament bundles on a nerve support with warm paraffin oil by using an operating microscope (×40, OPM 11 Zeiss). Each bundle was placed sequentially on a monopolar tungsten electrode. The nerve activity was referred to a nearby ground electrode, amplified (50–100 kHz), and filtered (30–10 kHz) by a differential amplifier (MP2 SARL, Marseille, France). The afferent discharge was displayed on a chart recorder (TA 4000 Gould, Balanvilliers, France), and the potentials were fed into a pulse window discriminators built in our laboratory, which simultaneously analyzed afferent populations. The output of these discriminators provided noise-free tracings (discriminated units), which were counted by two
frequency meters at 1-s intervals (F_{impulse/s}) and then displayed on the chart recorder. The discriminated units were counted and recorded on separate tracings. Due to the small sizes of action potentials of the thin afferent fibers in each bundle, the window discriminators allowed us to select two to three units in each afferent population.

**Mechanical Muscle Properties**

In all groups, muscular responses to nerve and muscle stimulation were studied. Comparison between nerve and muscle stimulation permitted evaluation of the nerve reconnections (end-plate efficiency). To measure the strength of muscle contraction elicited by nerve or muscle stimulation, a steel hook was implanted in the distal tendon and connected to an isometric strain gauge (Microdynamometer S60, Ugo Basile Narco Biosystem, Houston, TX). The output signal of the strain gauge was fed to a chart recorder (model TA 4000 Gould, Madison, WI). The whole cut peroneal nerve was desheathed and connected to an isometric strain gauge (Microdynamometer S60, Herts, UK). Vibrations were applied for 5-s periods, and the vibration frequency was increased from 10 to 90 Hz. Mechanical vibrations allowed evaluation of the nerve reconnections (end-plate efficiency). The maximal relaxation rate (MRR) was determined, i.e., to ensure that the recorded activity was initiated from the muscle, the belly of tibialis anterior muscle was touched with a blunt rod. 2) The compound waves were recorded. The CMAP evoked by electrical stimulation provides a measure of the number and also the properties of the fibers in the nerve; i.e., the calculation of the area under the curve of each peak wave gave an indication of the proportion of the different fibers in each filament bundle. 3) The response of mechanosensitive afferent fibers to mechanical vibrations delivered to the distal tibialis anterior tendon by a vibrator (Ling Dynamic Systems, Baldrock RD, Royston, Herts, UK). Vibrations were applied for 5-s periods, and the vibration frequency was increased from 10 to 90 Hz. Mechanical vibrations were used to identify muscle mechanoreceptors (spindle and Golgi tendon organs) (8, 11). And 4) the response of metabosensitive afferent fibers to intra-articular bolus injection of KCl (1–20 mM in 0.5 ml of saline) and Lac solution (0.5–3 mM in 0.1 ml of saline) and to a 3-min period of electrically induced muscle fatigue (3-min EIF) was recorded. The duration of each stimulating train was 500 ms, and trains were repeated each second to produce a series of contractions. Pulse durations of 0.1 ms were delivered in each 500-ms train (10 Hz). Muscle fatigue was assessed from the decay of force throughout each 3-min EIF period. The fatigue index, defined conventionally as the percentage of the force lost at the end of the 3-min EIF trial, was then calculated (32, 33).

In all cases, the intensity of muscle stimulation was adjusted to elicit the same maximal increase in muscle force at the beginning of the electrical muscle stimulation trial. Injected chemicals and EIF mimicking the fatigue are used to activate the group III-IV muscle afferents (7, 8, 11).

To avoid interactive effects of the multitude of tests performed on each animal, 30 min of recovery were left between each test. The tests were always conducted randomly.

At the end of the experiment, animals were killed by an overdose of anesthetic, and right and left tibialis anterior muscles were removed, trimmed of excess fat and connective tissues, and weighed.

**Statistical Analysis**

Statistical analyses were performed with a software program (InStat, GraphPad). Prestimulus (PS) values of frequency of impulses per second of afferent fibers were averaged 2 min before the application of each stimulus. Then, significant changes in the afferent activity induced by each tested agent were determined with respect to the corresponding averaged PS values (100%). Mean values of firing rate (F_{impulse/s}) are given as means ± SE, and their variations are expressed in percentage of the corresponding control discharge rate (baseline activity). We used nonparametric one-sample tests (Wilcoxon test) to compare the afferent discharge frequency before and after drug injections, EIF, or tendon vibrations. A one-way ANOVA (with P coefficient being significant at <0.05) was also used to test the intergroup differences. This test was used to compare muscle mass, MRR/A, and the effect of KCl, Lac, tendon vibration, EIF, and fatigue index. We used a Student-Newman-Keuls (with P being significant at <0.05) post hoc test to compare all pairs of groups. The x^2 test was applied to compare the percentage of fibers responding to mechanical vibrations.

**RESULTS**

**Muscle Weight**

In LS and LSR groups, the weight of operated tibialis anterior muscles, expressed as the ratio of the muscle weight to the animal weight, was significantly lower than that of the nonoperated muscle in the C group (61 ± 7%, P < 0.01 and 70 ± 2%, P > 0.05, respectively). By contrast, in the LSE group, the muscle mass did not differ from the C group (93 ± 5%). Results are reported in Table 1.

**Twitch Characteristics**

Data were compared with those measured in the C group. Two months after nerve injury and repair, nerve and muscle stimulation in the LS, LSE, and LSR groups always elicited muscle contractions, indicating that muscles were reinnervated by motor axons. Table 2 indicates that A significantly decreased in the LS group but not in the LSE and LSR groups, regardless of whether the stimulation was applied to the nerve or directly to the muscle. MRR/A never varied. Only when twitches were induced by muscle stimulation did CT significantly (P < 0.01 and P < 0.001) decrease in the LS and LSE groups, but it must be pointed out that a tendency for shortened twitch duration was observed in the LSE group.

**Table 1. Animal and muscle weights**

<table>
<thead>
<tr>
<th>Animal</th>
<th>C (n = 10)</th>
<th>LSE (n = 12)</th>
<th>LSR (n = 12)</th>
<th>LS (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal weight, g</td>
<td>341 ± 5</td>
<td>336 ± 4</td>
<td>327 ± 3</td>
<td>344 ± 4</td>
</tr>
<tr>
<td>Muscle weight, mg</td>
<td>0.77 ± 0.03</td>
<td>0.71 ± 0.02</td>
<td>0.52 ± 0.02*</td>
<td>0.48 ± 0.03†</td>
</tr>
<tr>
<td>Ratio</td>
<td>2.25 ± 0.03</td>
<td>2.09 ± 0.04</td>
<td>1.58 ± 0.08*</td>
<td>1.38 ± 0.09†</td>
</tr>
</tbody>
</table>

Values are means ± SE. C, control; LSE, denervated muscle stimulated daily by a biphasic current; LSR, exercised on a treadmill daily; LS, left peroneal nerve was sectioned. Muscle weight was significantly different from that measured in C rats: *P < 0.05; †P < 0.01.
Table 2. Twitch properties of tibialis anterior muscle from C, LSE, LSR, and LS rats

<table>
<thead>
<tr>
<th></th>
<th>C (n = 10)</th>
<th>LSE (n = 12)</th>
<th>LSR (n = 12)</th>
<th>LS (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A, mg</strong></td>
<td>1.700 ± 0.001</td>
<td>1.338 ± 0.001</td>
<td>1.074 ± 0.001</td>
<td>0.947 ± 0.001</td>
</tr>
<tr>
<td><strong>MRR/A</strong></td>
<td>0.046 ± 0.0006</td>
<td>0.047 ± 0.0006</td>
<td>0.042 ± 0.0013</td>
<td>0.056 ± 0.0015</td>
</tr>
<tr>
<td><strong>CT, ms</strong></td>
<td>34.7 ± 5.5</td>
<td>22.8 ± 2.0</td>
<td>24.9 ± 2.9</td>
<td>25.9 ± 2.4</td>
</tr>
</tbody>
</table>

Table 3. Conduction velocity and proportion of each group of nerves calculated from areas of CNAP waves

<table>
<thead>
<tr>
<th></th>
<th>C (n = 10)</th>
<th>LSE (n = 12)</th>
<th>LSR (n = 12)</th>
<th>LS (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proportion of nerve fibers, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I/II like</td>
<td>9.6 ± 2.2</td>
<td>15.9 ± 2.1</td>
<td>36.4 ± 3.3</td>
<td>13.3 ± 5.4</td>
</tr>
<tr>
<td>Group III like</td>
<td>46.8 ± 2.7</td>
<td>49.4 ± 8.8</td>
<td>33.8 ± 5.8</td>
<td>39.1 ± 5.6</td>
</tr>
<tr>
<td>Group IV like</td>
<td>43.6 ± 2.5</td>
<td>34.6 ± 8.9</td>
<td>29.8 ± 5.4</td>
<td>47.5 ± 6.2</td>
</tr>
<tr>
<td><strong>Conduction velocity, m/s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I/II like</td>
<td>10.8 ± 1.0</td>
<td>10.5 ± 1.0</td>
<td>46.5 ± 6.8</td>
<td>9.1 ± 1.0</td>
</tr>
<tr>
<td>Group III like</td>
<td>2.9 ± 0.6</td>
<td>3.7 ± 0.3</td>
<td>5.3 ± 0.4</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>Group IV like</td>
<td>0.5 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. CNAP, compound nerve action potential. Data significantly differed from that measured in C rats: *P < 0.01; †P < 0.001. Significant variation compared with the LS group: ‡P < 0.05; §P < 0.01.

CT values was also present in the three operated groups in response to nerve stimulation.

**Fatigue Index During EIF**

Compared with the C group, rats in the LS group showed an increase in fatigue index, whereas we measured a better tolerance to fatigue in the LSE group. No difference was observed between the LSR and C groups (Fig. 1).

**CNAP**

The different peaks of CNAP reflect the proportion of group fibers within the whole nerve. A measure of the surface gives an idea of each proportion. Furthermore, because electrical stimulation was applied at the distal peroneal nerve, the first peak (groups I–II) also contained a contribution of an antidromically activated α-motor efferent fiber, the second one (group III) may also include γ-motor fibers, and the activation of sympathic fibers may also contaminate the group III and IV waves. Thus we prefer to use the terms of group I/II-like, III-like, or IV-like fibers and discuss the diameter of fibers and quality of each. Table 3 shows an increase in conduction velocities of the slowest fibers between the LSE and C groups.

![Fig. 1](image1.png)

Fig. 1. Fatigue index represents the force developed after a 3-min muscle stimulation, as a percentage of that measured at the beginning of electrically induced fatigue (EIF). LS, animals with nerve lesion and suture; LSE, animals with lesion, suture, and chronic muscle rehabilitation by electrostimulation; LSR, animals with 10 wk of treadmill running; C, control animals. Fatigue index significantly differed from that measured in C rats (*P < 0.05; ***P < 0.001).

![Fig. 2](image2.png)

Fig. 2. Response [frequency of impulses (Fimpulse/s)/%control level] of tonically active muscle afferents during 3 min of EIF. EIF is known to induce release of metabolites in the muscle interstitium that activate muscle-free afferent endings. EIF elicited a marked activation of peroneal nerve afferents in C rats. Effects were weaker in LS vs. C. **Significant decrease in activity (P < 0.001) compared with the C group value.**
Afferent Responses to Tendon Vibrations

In the C group, two populations of muscles mechanosensitive afferents were identified with respect to the frequency of vibrations giving an optimal activation: 40–50 Hz (63%) and 70–80 Hz (37%). The afferent response corresponds to an abrupt increase in the discharge frequency that persisted throughout the vibration period. Compared with the proportion of afferents activated by the different vibration frequencies within the C group, this proportion was not different in the LSE (45 and 55%) group but was significantly different in the LS (28 and 72%) and LSR (33 and 67%) groups. Thus, in the LS and LSR groups, the majority of muscle mechanoreceptors responded to the highest vibration frequencies. Figure 6 represents the changes in afferent discharge (% frequency of impulses per second compared with the baseline activity) elicited by the various frequency tendon vibrations. In LS rats, a significant increase of the nerve response was measured between 60 and 80 Hz compared with C values. In the LSE group, the response increased for 10 and 20 Hz, and from 60 to 80 Hz compared with C values. In the LSR group, the response increased for 10 and 20 Hz, and from 60 to 80 Hz compared with C values. In the LS rats, a significant increase of the nerve response was measured between 60 and 80 Hz compared with C values. In the LSE group, the response increased for 10 and 20 Hz, and from 60 to 80 Hz compared with C values. In the LSR group, the response increased for 10 and 20 Hz, and from 60 to 80 Hz compared with C values.

DISCUSSION

Except for the mechanosensitivity that remained partially altered, data from the present study reveal that a running exercise during the rehabilitation period improves the recovery of the neuromuscular response (LSR group), i.e., the responses of III and IV afferent fibers, the fatigue index, and the twitch characteristics. Data also revealed that the maintenance of muscle activity by a running exercise induces better recovery than the maintenance of muscle activity by a chronic electrical muscle stimulation protocol (LSE), although electromyostimulation with biphasic current modulated in frequency and pulse duration avoids muscle atrophy and conserves the proportion of fibers responding to tendon vibrations. The present study also confirms that the functional characteristics of muscle afferents are affected after nerve section followed by self-anastomosis when the muscle was not provided with activity during the postoperative period (13). Namely, the proportion and the response to tendon vibration shift toward the highest mechanical frequencies, whereas the post-EIF activation is lowered.

Muscle Properties

Compared with animals without rehabilitation, the postoperative running protocol avoids atrophy. However, the muscle weight recovery is less important than those obtained by chronic electrical stimulation. Several hypotheses could be found to explain these observations. 1) The intensity of direct muscle stimulation, which was adapted daily to induce near-maximal contraction of muscle, was such that it activated a great proportion of muscle fibers. During running exercise, physiological cortical activation never recruited the entire muscle as intensively as the external stimulator did. And 2) with the use of electromyostimulation, muscle fibers were activated before the regenerating nerve fibers completely reached the muscle. Then, in the LSR group, the muscle fibers were reactivated progressively by motor fibers reconnecting the muscle. We could suggest that, in the LSE group, muscles were maintained both for a greater duration and intensity.

Contrary to the LSE and LSR groups, the reduced force-generating capacity (A) was significantly and markedly reduced in the LS group. Reduced force production could be explained by the muscle atrophy, which was marked in LS rats. The better tolerance to fatigue observed in the LSE group could be explained by the marked reduction of the Lac dehydrogenase enzymatic activity in chronically stimulated muscles and thus the reduced lactic acid production during EIF.

The shorter CT values measured in the LS and LSR groups could result from a higher muscular excitability (45) associated with the transformation of the muscle fiber phenotype. Our laboratory already reported that, in a nondenervated rat chronically stimulated with a modulated sine wave current, a marked shortening of the CT and an increase in the proportion of oxidative enzymes concomitant to changes in the contractile properties of muscle fibers toward those of fast twitch ones was
afferents to tibialis anterior tendon vibrations (10–90Hz difference compared with C group (*P < 0.05; **P < 0.001). The general pattern of response was similar in the C and LSR groups, except for the value measured at 50 Hz. Response was altered in LSE and LS groups. Significance difference compared with C group (**P < 0.01; ***P < 0.001).

Fig. 5. Examples of metabosensitive fiber activation after KCl (10 mM) injections. Note an increase in the discharge pattern and the number of impulses per second after injection and return to baseline level several minutes after injection. AP, action potentials.

Fig. 6. Response (F impulses/s in %baseline activity) of mechanical muscle afferents to tibialis anterior tendon vibrations (10–90 Hz). Tendons vibrations elicited for each frequency a significant stable increase in the afferent nerve discharge from the resting discharge, the peak activity varying with the vibration frequency. The general pattern of response was similar in the C and LSR groups, except for the value measured at 50 Hz. Response was altered in the LSE and LS groups. Significant difference compared with C group (**P < 0.05; ***P < 0.001).

present (54). Cole and Gardiner (6) also reported that muscle denervation was effective in attenuating the strength-related but not the speed-related changes in neuromuscular function.

Nerve Recordings

CNAP analysis reveals an increase in proportion and conduction velocity of fast I/II-like nerve fibers in the LSR group indicating that the regenerating nerve contains a greater proportion of large nerve fibers innervating fast motor units. In the LSE and LSR groups, fibers from groups III and IV also present an increase in conduction velocity, indicating that the maintenance of muscle activity act on the regenerating motoneurons. Choi et al. (5) reported an increase in conduction velocity in the regenerating fibers. However, Horch and Lisney (23) reported a reduction in axon diameter (and conduction velocity) that should normalize with recovery with a long period after reinnervation. The fact that the size of the myelinated fibers remains low for a long time after nerve injury, whereas the size of the unmyelinated fibers is less affected, and the fact that the number of regenerated fibers changes over time, with a sustained increase in the number of sprouts supported by myelinated fibers and a decrease of unmyelinated fibers (10), should be taken into account in the interpretation of our results. The maintenance of muscle activity (electromyostimulation and running exercise) over time may affect the proportion of fibers in each group that could be observed several weeks after self-anastomosis in a greater proportion of large-diameter fibers. Furthermore, during reinnervation, trophic factors from muscle and nerve are involved in the communication between growth cones and the target (37). Exercise is known to increase the trophic factors (brain-derived neurotrophic factor, NT-3 and NT-4) release from muscle (17).

Then, we can hypothesize, as suggested by Rasband et al. (42), that maintenance of denervated muscle activity increases and modifies the trophic factor release and the potassium channel distribution on regenerating fibers.

The most significant finding in these experiments concerns the better functional recovery of the sensitive afferent fibers from the tibialis anterior muscle after nerve repair and rehabilitation by treadmill running (LSR group). Our observations point out that increasing motoneuron inputs during the regeneration/reinnervation period may influence the functional outcome. Previous studies evidenced that axons of slow motor units appeared to regenerate at a faster rate (12) and that motoneuron sprouting was enhanced in regularly exercising and exercise-trained rats (14, 15). Noah et al. (39), using morphometrical analysis, recently reported a positive effect of exercise on muscle reinnervation. However, Guttmann and Jakoubek (20) indicated a detrimental effect of daily prolonged swimming on regenerated peripheral axon diameter. Herbison et al. (22) suggested that overwork may damage partially denervated muscle that brief contractions may be more beneficial for muscle than a program of exhausting activities.

In humans (2) and cats (36), previous studies have already demonstrated, in noncontracting muscles, that all mechanosensitive endings responded to vibrations, with a wide range of responsiveness attributed to the different type of endings. For example, primary endings of spindles could generally be driven by the highest rates of vibration and the secondary ending by the lowest ones (2). Our laboratory has already suggested (8, 11) that the two populations of mechanosensitive units identified in the rat peroneal nerve, with optimal discharges phase locked to vibration cycles at 70 and 50 Hz, corresponded to primary and secondary spindle endings, respectively. The present experiment also reveals that the pattern
of response for the LS and LSR group afferent changed markedly with an increased proportion of afferents having an optimal activation at a vibration frequency of 70–80 Hz. The LS and LSR groups are significantly different from the C and LSE groups, with the LSR group presenting a diminished response at 50 Hz. However, it must be pointed out that, although the proportion of mechanosensitive fiber is inversely in the LS and LSR groups compared with the C and LSE groups, the kinetic of the response curve resembles the C group. In the LSE group, it seems that the fiber discharge rate to vibration is increased for each stimulating frequency. This increase in mechanosensitivity response could be enhanced by high and intensive chronic stimulation placed directly on muscle. This confirms previous observations by White and Devor (55), who found that changing the components of physical activity during skeletal muscle reinnervation can alter several attributes of the muscle phenotype. The consensus of several experiments supports the interpretation that proper muscle fiber recruitment and development of force are essential variables. As previously described (34), our results could indicate that after denervation, regeneration, and reconnection with the target muscle, the mechanosensitivity remains partially altered even if a slight beneficial effect is observed when muscles were electrostimulated during the rehabilitation period.

Finally, the present study shows that regenerated thin fibers from all experimental groups are operational to detect the changes in extracellular fluid composition. This signifies that fibers from groups III and IV not only have regenerated toward the muscle target but are able to detect metabolic changes occurring in the interstitium. However, in the LS and LSE groups, even if these metabosensitive fibers were able to respond to muscle metabolic changes, they present an altered response that could disorganize the sensorimotor control in exercising muscle. The absence of adaptive feedback mechanisms (muscle wisdom) and the diminished responses of III and IV afferent fibers after the 3-min EIF could be implicated in the exercise capacity (9). Because our experimental groups’ measurements of the fatigue index were obtained after the muscle was disconnected to the central nervous system, the disorganization in the sensorimotor control cannot explain the variability in muscle resistance to fatigue observed in the LS and LSE groups. One explanation could be found by changes in the muscle fiber histological and enzymatic properties. Total absence of significant differences between the C and LSR groups would signify that a running exercise during the rehabilitation period induces more benefits than the chronic electromyostimulation. The chronic electrical muscle stimulation could activate, to a greater extent, the denervated muscle and promote a deleterious effect on muscle fibers (48).

In conclusion, the findings indicate that direct muscle electrostimulation is efficient for maintaining the muscle weight, but other parameters, such as twitch characteristics, fatigue index, mechanosensitivity, and metabosensitivity, are totally restored when an animal performs a running exercise during the rehabilitation period. In the future, it would be interesting to compare different exercise intensities and types and analyze the muscle histochemical properties during the reinnervated process. Clinical applications could be found for patients undergoing nerve injury and repair.

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