Effects of Treadmill Training on Functional Recovery Following Peripheral Nerve Injury in Rats

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Abstract

Exercise, in the form of moderate daily treadmill training following nerve transection and repair leads to enhanced axon regeneration, but its effect on functional recovery is less well known. Female rats were exercised by walking continuously, at a slow speed (10 M/min), for one hour a day on a level treadmill, beginning three days after unilateral transection and surgical repair of the sciatic nerve, and conducted five days per week for two weeks. In Trained rats both direct muscle (M) responses to tibial nerve stimulation and H reflexes in soleus re-appeared earlier and increased in amplitude more rapidly over time than in Untrained rats. The efficacy of the restored H reflex was greater in Trained rats than in Untrained controls. The reinnervated tibialis anterior and soleus were co-activated during treadmill locomotion in Untrained rats. In Trained animals, the pattern of activation of soleus, but not tibialis anterior, was not significantly different from that found in Intact rats. The overall length of the hindlimb during level and up and down slope locomotion was conserved after nerve injury in both groups. This conservation was achieved by changes in limb orientation. Limb length was conserved effectively in all rats during downslope walking but only in Trained rats during level and upslope walking. Moderate daily exercise applied immediately after sciatic nerve transection is sufficient to promote axon regeneration, to restore muscle reflexes, and to improve the ability of rats to cope with different biomechanical demands of slope walking.

Key Words: exercise, axon regeneration, peripheral nerve
Introduction

Traumatic peripheral nerve injuries occur commonly and even though axons in peripheral nerves are capable of considerable regeneration, only about 10% of these patients ever recover full function (Frostick et al., 1998; Scholz et al., 2009). A common reason given for the poor functional outcomes is the slow process of axon regeneration (Gordon, 2009). Thus, one potential target for therapeutic intervention in treating peripheral nerve injuries might be to enhance the process of axon regeneration. Indeed, a number of rehabilitative approaches have been advocated to promote functional recovery following injury to peripheral nerves (reviewed in Udina et al., 2011). In studies from our lab in mice (Sabatier et al., 2008), exercise in the form of moderate daily treadmill training results in a marked enhancement of axon regeneration in cut nerves. Others have reported a variety of different training paradigms in different injury models (crush or transection) in rats that result in enhanced axon regeneration (Asensio-Pinilla et al., 2009; Ilha et al., 2008; Seo et al., 2006; Udina et al., 2011). We have used a combination of transgenic and knockout mice to show that the enhancement of axon regeneration produced by this moderate exercise is the result of an autocrine neurotrophin stimulation of regeneration of injured axons (Wilhelm et al., 2012).

Whether the enhancement of axon regeneration produced by exercise leads to improved functional recovery is less clear. Using nerve conduction studies, Navarro and his colleagues (Asensio-Pinilla et al., 2009) studied the effects of treatment of rats with four weeks of twice daily exercise after sciatic nerve transection and repair. For both the tibialis anterior and plantar foot muscles, training produced only very modest increases in the amplitude of direct muscle (M) responses to stimulation of the sciatic nerve above the transection until two months following injury, when the responses were significantly larger. A similar effect was observed using passive cycling of the limbs (Udina et al., 2011). Only when treadmill exercise was
combined with brief electrical stimulation, was the magnitude of this functional outcome measure greater at earlier survival times (Asensio-Pinilla et al., 2009).

Interpretations of the effectiveness of any treatment in improving functional recovery following peripheral nerve injury also should include behavioral analyses. The repertoire of such assays applied to laboratory rodents has been reviewed recently (Brushart, 2011; Wood et al., 2011). A complication of the use of many of these assays is the behavioral compensation made in response to the initial lesions. We have argued that analysis of slope walking is a behavioral assay in which those compensations are less complicating (Sabatier et al., 2011b).

The biomechanical demands of walking up and down slopes are quite different (Carlson-Kuhta et al., 1998; Maas et al., 2009) and intact animals and humans adapt to those demands by altering muscle activation patterns and overall limb movements (Lay et al., 2006; Sabatier et al., 2011b). We have shown that in Untrained rats recovering from sciatic nerve injuries, their ability to change either the pattern or intensity of activation of reinnervated muscles or the movements of the affected hindlimb when walking on different slopes is lost (Hamilton et al., 2011; Sabatier et al., 2011a; Sabatier et al., 2011b). Thus, we propose to evaluate the efficacy of exercise as a treatment following nerve injury by measuring the extent of restoration of the ability of rats to adapt to walking on different slopes. The overall goal of this paper is to report the results of nerve conduction studies and studies of slope walking on the effects of exercise on functional recovery following sciatic nerve injury in rats. A preliminary report of these findings has been made (Boeltz et al., 2010).
Materials and Methods

General methods - All methods used were approved by the institutional animal care and use committee of Emory University. Experiments were conducted using 12 female rats, BW ~250 gm. Females were chosen because they autotomize (chew toes) less following peripheral nerve injuries than male rats. Our original experiments were conducted using eight Sprague-Dawley rats, but because several of these rats did autotomize and had to be removed from the study prematurely, we added four Lewis rats to the study. None of these rats had to be euthanized prematurely. Thus, data from the longest survival times studied include recordings made in smaller numbers of animals, as indicated throughout the text. Originally, six rats were assigned to a Trained group and six to an Untrained group. Two Lewis rats were assigned to each group. Pre-transection measurements from both groups were pooled to form a third, Intact group for some analyses. All rats in the Untrained and Trained groups tolerated the nerve repair surgery and were studied throughout the post-injury period. At the conclusion of the study period, all experimental animals were euthanized using an IP injection of 100mg/Kg of pentobarbital. Some of the data from some (N=4) of the Sprague-Dawley rats in the Untrained group has been reported previously (Sabatier et al., 2011b). They are included here for comparative purposes.

Recording hardware was implanted into ketamine/xylazine anesthetized animals for chronic use. All implanted electrodes were constructed as described previously (English et al., 2007a). Bipolar fine wire EMG electrodes were implanted into the soleus (SOL) and tibialis anterior (TA) muscles to record electrical potentials produced by these muscles. The wires were secured in place with fine (6-0 nylon) sutures to minimize their movement while the muscle is contracting. Wire electrodes were implanted into the muscle mass, in an anatomically consistent location in each muscle, where, based on preliminary experiments, the atrophy of the muscles following denervation would be unlikely to result in the exposure of de-insulated tips of
the wires outside of the muscle. Wire locations were visualized post-mor tem and judged to be in place in all animals studied. A bipolar stimulating cuff electrode, made of silastic tubing and the same fine wire (Stein et al., 1977), was implanted around the exposed tibial nerve below its branching from the sciatic nerve. The design of these cuffs is for them to fit rather loosely on the nerve so that any swelling of the nerve after implantation did not induce a strangulation of the tissues. At post-mortem, there was no visible effect of the cuff on the nerve in any of the rats studied, except for an anticipated increase in connective tissue surrounding the cuff. All wires, including those attached to the stimulating cuff were led subcutaneously to a small plug (Plastics One, Roanoke VA, part # MS363), approximately 1 cm in diameter, mounted on the head of the animal with stainless steel bone screws implanted into the skull and secured with dental acrylic.

Before transecting the sciatic nerve, the tibial nerve was stimulated through the cuff and evoked EMG activity was recorded from SOL and TA in the anesthetized animals. The sciatic nerve was then cut just above its branching into tibial, common fibular, and sural trunks and proximal to the implanted cuff, using sharp scissors, and the proximal and distal stumps of the cut nerve were aligned and secured in place using fibrin glue. The glue was manufactured at the time of use from equal parts of thrombin and a 1:1 mixture of fibrinogen and fibronectin (MacGillivray, 2003). Once the sciatic nerve was repaired and the fibrin glue was cured (1-2 min) to form a clot about the repaired nerve, the surgical site was closed in layers with appropriate sutures.

**Exercise** - Treadmill training began on the third day following nerve repair. Trained rats walked on a custom treadmill on a level surface at 10 m/min for one hour, five days per week. This is a very slow speed and rats, even those with sciatic nerve injuries, manage this training paradigm without any adverse effects. Animals were selected for inclusion into this study if they are able to run on the treadmill in pre-surgical screenings, at a speed of up to 20 m/min without
the need for reinforcement. To avoid any complications of pre-injury exercise, rats were not exposed to the treadmill for at least two weeks prior to nerve repair surgery. Rats assigned to the Untrained group were not exposed to the treadmill during the study period, except during locomotion studies done at two, four, and 10 weeks after nerve transection and repair, when the rats walked on the treadmill for a total of ten minutes over a period of at least thirty minutes.

*Nerve conduction studies* - Muscle reinnervation was assessed by stimulating the tibial nerve just below the nerve transection and recording EMG activity from muscles (SOL and TA) innervated approximately 15 mm below the nerve transection. We chose to stimulate below the injury site primarily so that we could stimulate only axons that had regenerated, not all axons. Stimulation of the tibial nerve, rather than the entire sciatic nerve, also enabled us to avoid any volume conducted EMG artifact that might result from activating the large adjacent hamstring muscles. In intact rats, tibial nerve stimulation resulted in two compound muscle action potentials in SOL: a direct muscle response, also known as the M-response, and the H-reflex (Fig. 1A, Intact). The latter reflects the synaptic activation of motoneurons in response to stimulation of afferent axons in the tibial nerve. Rats were free to move about their cages during the nerve conduction studies, but mostly they did not move and curled up in a corner of the cage. We did not control for the animal’s movements, but instead opted to control for resting EMG activity levels prior to the delivery of each stimulus. Ongoing EMG activity in the SOL was monitored and when such activity was maintained for 10 sec within a user-defined voltage window, a very short (0.1 msec) constant voltage stimulus pulse was delivered to the tibial nerve via the implanted nerve cuff electrode, under computer control, and the EMG activity in SOL and TA was recorded. Stimuli were delivered no more frequently than once every three seconds, as that has been shown to produce consistent responses without muscle fatigue (English et al., 2007a). At each recording session, ramps of successively increasing stimulus voltages, ranging from subthreshold to supramaximal, were applied to generate a full H reflex.
recruitment curve. Maximum M-responses and the maximum H-reflexes were recorded separately. Final responses for each animal at each recording session were the result of averages of multiple stimulus presentations, corrected for background EMG activity, as we have described previously (English et al., 2007a). Recordings were made at multiple times over a period of 10-15 weeks following sciatic nerve transection and repair.

We measured the latency from stimulus application to the initiation of the M-response and the H-reflex, as well as the average rectified amplitudes in time windows adjusted to be appropriate to these responses in each muscle (see below). Before nerve transection, a baseline recording of the M-response and H-reflex was determined in each rat. These measurements were repeated on the third day following nerve transection and repair, when treadmill training began in the Trained group. After the two week treadmill training program ended, recordings of evoked EMG activity were made five days a week until the first H reflex could be recorded. Recordings of evoked EMG activity were made at weekly intervals thereafter. The amplitudes of maximal M responses and H reflexes were expressed as a percentage of the corresponding peak amplitudes obtained in pre-transection recordings. Average intensities were computed for rats in the Untrained and Trained groups during weekly intervals after nerve transection and repair and the data (post-transection time vs. amplitude) were fit with a least squares linear regression method. Significance of differences in slopes between groups was made using Multiple Linear Regression Analysis (Statistica, StatSoft, Inc.).

Locomotor EMG activity - In intact rats, SOL and TA are reciprocally activated during locomotion (Sabatier et al., 2011b; Thota et al., 2005). However, following transection and repair of the sciatic nerve in Untrained rats, co-activation of the TA and SOL is common (Gramsbergen et al., 2000; Sabatier et al., 2011b). Activity in these muscles during treadmill locomotion was recorded from implanted EMG electrodes at four and 10 weeks after nerve transection and repair in Trained rats and compared both to data from Untrained rats at similar
times after nerve repair and to data obtained from rats prior to nerve transection (Intact rats).

Details of the recording conditions are given in our previous publications (Sabatier et al., 2011b; Sabatier et al., 2012). Muscle activity and video records were synchronized to an LED pulse in the video field whose timing was recorded with the EMG activity.

Factor Analysis with Principal Components was used as a classification scheme to investigate whether the timing of EMG activity in SOL and TA during treadmill locomotion was different in Intact, Untrained, and Trained rats. From single frame analysis of video records, the timing of paw contact and paw off during step cycles in which rats walked at constant speed on the treadmill was determined and used to extract the EMG activity during individual step cycles. The extracted EMG activity in SOL and TA during these selected step cycles was rectified, low pass filtered at 10 Hz, and normalized to 100 samples, representing percentiles of the step cycle, using a cubic spline interpolation function. Activity was then averaged within slopes over several step cycles in the same experiment and subjected to Principal Components Analysis (Statistica, StatSoft, Inc.). The results of PCA are a set of new correlated variables, or principal components. Since in our data set, the first three Principal Components accounted for more than 60% of the variance in the data, and because no significant differences were found in factor loadings (see below) for factors 4-6, we restricted our analysis to the first three Principal Components. For each of these three factors, we determined factor loadings, the correlations between the principal components and the original data set. For each of the three treatment groups defined above, the pattern of locomotor EMG activity in SOL and TA at different times after denervation and on different slopes was characterized by the factor loadings for the first three principal components associated with that activity. Means of these three factor loadings for different animals at the different times and speeds/slopes were determined and significance of differences in their means was evaluated using analysis of variance (ANOVA), with post-hoc paired (Fisher least significant difference (LSD)) testing, where appropriate. Any two vectors
that differed significantly ($p<0.05$) in one or more factor loading were considered to be derived from different patterns of locomotor EMG activity.

*Kinematics* – Following recorded locomotion sessions, videos of the selected step cycles were subjected to single frame analysis. Three previously marked reflective spots located over the greater trochanter, lateral malleolus, and fifth metatarsophalangeal (MP) joint were assigned Cartesian coordinates. A position vector between the markers at the greater trochanter and the fifth MP joint was determined from each frame. The magnitude of this vector was defined as the length of the entire extensible hind limb. The direction of the vector is the angle of the entire limb to the treadmill belt. This vector was used as a global measure of limb movement. In a recent study, the measurement of the position of the hip joint during treadmill locomotion using x-ray video recordings and was compared to that obtained with markers placed as described above (Bauman and Chang, 2010). These authors described a significant difference between the methods and attributed the differences to skin movements over the pelvis during walking. Acknowledging the importance of these observations, we want to make very clear that, when we refer to measurements of limb length in this paper, we mean the *distance between the applied skin markers*, and not necessarily the actual distance between the hip and MP joints. We are very likely not really measuring limb length accurately but regard our measure of limb length as a reasonable facsimile. We determined three parametric measures, each, for limb length and limb angle (minimum, maximum, and average) in selected step cycles in the different treatment groups. Significance of differences in these measures between Intact, Untrained, and Trained rats was determined using ANOVA with appropriate post-hoc paired testing, as described above.

*Terminal experiments* - The extent of misdirection of regenerating axons was evaluated in terminal acute physiology experiments. Under ketamine/xylocaine anesthesia, the sciatic nerve was exposed at least 1 cm above the transection site. The epineurium was incised with
sharp iridectomy scissors and the tibial and common fibular fascicles were separated, as
described by (Evans et al., 1991). A stimulating cuff electrode was placed around the exposed
nerves, one at a time, and used to stimulate muscle fibers innervated by axons coursing in that
branch. Evoked activity in SOL and TA muscles was recorded using patch-type EMG
electrodes (Loeb and Gans, 1986) placed on the surfaces of the muscles. Within the defined M
response windows, mean rectified and integrated EMG activity was measured for each muscle
in response to stimulating each of the nerves. The proportion of the total activity evoked in
reinnervated muscles produced by tibial or common fibular nerve stimulation was determined
and compared to that recorded from contralateral, intact muscles using an unpaired t-test
(α=0.05).
Results

Effects of exercise on restoration of evoked SOL EMG activity – Tibial nerve stimulation evoked a direct muscle (M) response and an H reflex in the SOL muscle of intact rats (Fig1A: Intact). Both evoked potentials disappear in denervated muscles (Fig. 1A: 2 wks) but later re-appear as muscle fibers are reinnervated. They are first observed at a longer latency than found in intact SOL muscles, probably a reflection of the small size of the regenerating axons at that time and their diminished axonal conduction velocity, as has been observed by others (Foehring et al., 1986). In addition, these early restored potentials are found not as a single compound action potential but as a temporally dispersed series of small potentials (Fig. 1A:3.5 wks). Over time, the latencies to the two evoked potentials shorten and the distinction between M responses and H reflexes becomes more clear (Fig. 1A: 5 wks, 7 wks). For this reason, we considered the earliest potentials as putative M responses and putative H-reflexes. The distinction between the two early responses in each muscle thus is somewhat arbitrary (English et al., 2007a).

In Trained rats the putative M response appeared significantly (unpaired t-test, p<0.002) earlier (on average, 20.00 days vs. 27.80 days) than in Untrained rats (Fig 1B). Following transection and repair of the rat sciatic nerve, a putative soleus H reflex is found to appear slightly later than the earliest observation of a restored M response in Untrained rats (English et al., 2007a). As noted above for the reappearance of the M response, the putative soleus H reflex is first seen significantly earlier (unpaired t-test, p<0.009) in Trained rats compared to Untrained rats (on average, 20.00 days vs. 29.25 days) (Fig. 1B).

The amplitude of the largest restored M response (M_{Max}) increased linearly over time in both Trained and Untrained animals (Fig. 2A). These data were subjected to multiple linear regression analysis, using survival time, treatment group (Trained=1 or Untrained=0), and their
product as independent variables and M response amplitude as the single dependent variable. Correlations of the data to the regression lines were very high, accounting for 82 and 97 % of the variance in the respective data sets (Fig. 2A). Regression coefficients were significantly different from zero for all three independent variables, including the product of survival time and treatment (p<0.00009). Two inferences are drawn from this analysis. The slopes of the individual regression lines for the two treatment groups both are significantly different from zero and the slopes of these lines for Trained and Untrained rats are significantly different from one another. Based on the magnitude of these differences in slopes of the regression lines in the two groups, we conclude that, over the entire period of study, the amplitude of the evoked M response increased faster with time in Trained rats than in Untrained rats. When the data are scaled as a function of the M response amplitude found prior to nerve transection, M responses that were significantly greater than 1.0 (greater than pre-transection, Figure 2A, horizontal dashed line) were found in Trained rats only.

In both Untrained and Trained rats, the initial restored M response was found at a longer latency than found in Intact rats. This latency decreased rather rapidly over time in both groups until it was no longer different from that found in intact rats, but not until approximately 10 weeks post-operative in both groups (Fig. 2B). The inter-animal variability in mean M response latency was quite large at first, and we believe that this is accounted for by the difficulty in producing accurate measurements of latency when very small potentials are encountered. This large variability was found only at the first two measurement times. By six weeks after nerve repair surgery, it was very small. At all times studied, data from the two Lewis rats in each group in this series were in the same range as the data from the four Sprague-Dawley rats in each group.

The amplitude of the restored SOL H reflex also increased linearly over time in both Untrained and Trained rats (Fig. 3A). Using multiple linear regression analysis described
above, the slopes of the lines fitted to the data for both Untrained and Trained rats were found to be significantly different from zero and that of the Trained group is greater than the slope of the line fitted to the data from the Untrained group. Over the entire period of study, the amplitude of the SOL H reflex increased faster in Trained rats than in Untrained rats. Based on the same multiple linear regression analysis, the slopes of the lines for the M responses and H reflexes were compared within each treatment group. Slopes of regression lines were significantly different for Untrained rats but not Trained rats, suggesting that the ability of afferent axon stimulation to produce an EMG response was greater in Trained rats.

A way of comparing the efficacy of the restored H reflex in reinnervated muscles to that found in Intact rats is to compute the ratio of the maximum H response amplitude to the maximum M response amplitude (Navarro et al., 2007). The time course of recovery of this \(\frac{H_{\text{Max}}}{M_{\text{Max}}}\) ratio for Trained and Untrained rats following sciatic nerve transection and repair is shown in Figure 3B. Values of \(\frac{H_{\text{Max}}}{M_{\text{Max}}}\) are expressed as a proportion of the \(\frac{H_{\text{Max}}}{M_{\text{Max}}}\) ratio measured prior to transection (\(\frac{H_{\text{Max}}}{M_{\text{Max}}}/\text{Intact}\)). In both Trained and Untrained rats, the \(\frac{H_{\text{Max}}}{M_{\text{Max}}}\) ratio at the earliest times of recovery is nearly three times greater than found prior to transection. In Untrained rats, this large response was maintained or increased slightly over a three week period and then declined rapidly until a stable ratio was established at a level significantly lower than pre-transection levels (Fig. 2B, and (English et al., 2007a). In Trained rats, the initial response declined more gradually, and stabilized at approximately nine weeks after nerve repair surgery. The \(\frac{H_{\text{Max}}}{M_{\text{Max}}}\) ratio over the time period from 10-13 weeks after nerve transection and repair was significantly larger in Trained rats than found in Untrained rats (ANOVA: \(F_{1,6}=5.987, p<0.038\)). In Trained rats, the mean \(\frac{H_{\text{Max}}}{M_{\text{Max}}}\) ratio over this time period, when scaled to the same ratio prior to nerve transection and repair, (1.02, SD 0.24) is not significantly different from 1.0. Over the same time period, the ratio for Untrained rats (0.52, SD 0.20) remains significantly less than 1.0. Thus, one effect of exercise is a restoration of the
efficacy of this spinal reflex to its pre-lesion magnitude.

*Effects of exercise on the specificity of muscle reinnervation* - After injury to nerves as large as the sciatic nerve, many regenerating motor axons are directed into terminal nerve branches that lead either to the same muscle targets they innervated prior to injury or to anatomical synergists. Other motor axons are misdirected to inappropriate nerve branches, such as those supplying anatomical antagonists, and yet other regenerating motor axons are known to branch to both appropriate and inappropriate nerve branches (English, 2005b; English et al., 2009). We evaluated the extent to which exercise might contribute to these two different forms of axon misdirection in two sets of parallel experiments.

The two main terminal branches of the sciatic nerve, the common fibular and tibial nerves, supply muscles that are anatomical antagonists. To study the effect of exercise on the extent of branching of regenerating motor axons into BOTH of these branches, we conducted axon reflex experiments. In all animals studied, we found evidence of this branching; a significant EMG response to tibial nerve stimulation was found in the reinnervated TA muscle (Fig. 4A). We measured the amplitudes of these potentials at different times after sciatic nerve transection in Untrained and Trained rats. Stimulation of the tibial nerve in intact rats resulted in a very small potential (<0.005 mV in amplitude) that could be recorded from EMG electrodes in the TA muscle and identified only when using signal averaging (Fig. 4A Intact, open arrow). We assume that this potential represents unavoidable EMG cross-talk (English and Weeks, 1989) and that this very small potential represents the quite laudable extent of fidelity of our recording system. Because it is so small, this data point is not included in Figure 4B. In both groups of animals, slightly larger potentials were recorded in TA over the first 3-5 weeks after nerve injury (Fig 4A: Day 20). Over the next 6-7 weeks, the amplitude of these potentials increased in a linear manner (slope =0.16, $R^2$=0.713, $p<0.008$) in the Untrained rats (Fig. 4B, white symbols),
and by the end of the study period, substantial potentials could be recorded from the TA muscle in response to stimulation of the tibial nerve (Fig. 4A: Untrained, Day 67). In Trained rats a clear difference was found. The amplitude of the potentials did not increase over the last 6-7 weeks of the study period (slope=-0.0023, R^2=0.024, p=0.908) (Fig. 4B, black symbols) and at the end of the study period, significantly smaller potentials were recorded in Trained vs. Untrained rats (unpaired t-test, p<0.007) (Fig. 4A: Trained, Day 67). To the extent that the linear increase in amplitude found in Untrained rats might reflect the addition or retention/maturation of regenerated TA motor units that are shared with targets of the tibial nerve, no such effect was found in Trained rats. Thus, exercise has a positive effect on this form of axon misdirection.

To evaluate a second form of regenerating motor axon misdirection, the routing of motor axons that had coursed in one sciatic nerve branch prior to injury and then were found in the other branch after regeneration, we stimulated fascicles of the sciatic nerve that formed the tibial and common fibular nerves well proximal to the lesion site, using the method described by (Evans et al., 1991), and monitored activity evoked in SOL and TA in Trained and Untrained rats. Data from these experiments were compared to similar data from the contralateral intact TA and SOL muscles, where little or no such misdirection of axons would be expected. Results are shown in Figure 5.

If the sciatic nerve is intact, the evoked response produced by tibial nerve stimulation in SOL and by common fibular stimulation in TA was dominant. This is the response that would be anticipated. Small potentials, constituting 3-4% of the total M response amplitude, were recorded in TA from tibial nerve stimulation and in SOL from common fibular stimulation (Fig. 5B, Intact). These were considered to be the result of either incomplete isolation of the nerve fascicle during dissection or unavoidable spread of stimulus current from the stimulated fascicle in these acute experiments. In contrast, significant potentials could be recorded in BOTH the
reinnervated TA and SOL muscles from stimulating EITHER the tibial or the common fibular nerve (Fig. 5B, TT). Slightly more than half of the total M response found in the reinnervated SOL muscle was produced by axons originating in the tibial branch and slightly more than half of the M response in the reinnervated TA muscle was produced by axons originating in the common fibular nerve (Fig. 5C). Slightly less than half of muscle reinnervation is by axons originating in functionally inappropriate nerves. In both Trained and Untrained rats, these proportions are not significantly different from each other but are significantly different from those found after stimulating the same branches of intact nerves. Thus, as we have asserted previously for mice (English et al., 2009), treadmill training does not affect the extent of misdirection and aberrant reinnervation of muscle targets.

Effects of exercise on locomotor EMG activity - The timing of activation of the TA and SOL muscles during treadmill locomotion on three different slopes was compared among three different treatment groups: Intact, Untrained, and Trained. Representative raw locomotor EMG traces obtained are shown in Figure 6. In both of the panels with recordings from reinnervated muscles, the amplitude of SOL activity is smaller, relative to those found in TA, than found in Intact rats. Note also that the strict reciprocal activation of TA and SOL found in Intact rats is not present in these panels.

We used factor analysis with principal components (PCA) to evaluate the significance of any changes in activation pattern of these two muscles following muscle reinnervation in Trained and Untrained rats. Significant differences between groups were found for all three slopes studied at both survival times. For downslope walking significant differences were found for factor 1 \( (F_{9,36}=3.549, p<0.003063) \) and factor 2 \( (F_{9,36}=8.106, p<0.000002) \). For upslope walking, significant differences were found for factor 2 \( (F_{9,36}=16.717, p<2 \times 10^{-10}) \) and factor 3 \( (F_{9,36}=3.666, p<0.002) \). For level walking significant differences were found for factor 2
Mean factor loadings for factor 2, where significant differences were found for all three slopes, are shown for the four and 10 week survival time groups in Figure 7.

In Intact rats, significant differences in mean factor loadings were found between SOL and TA for all three slopes studied (LSD, average p<0.003) (Fig. 7A, B: Intact). We have argued previously that this very large difference is a reflection of the strict reciprocal activation of TA and SOL found during locomotion in Intact rats (Sabatier et al., 2011b; Thota et al., 2005). In Untrained rats, all of the factor loadings are significantly different from those of both TA and SOL in Intact rats, at both times studied and at all slopes (LSD, average p<0.01) (Fig. 7A, B: UT), consistent with the use of unique patterns of activation during locomotion; one that is different from that of either SOL or TA in Intact rats. In addition, no significant differences in factor loadings between TA and SOL among the Untrained rats were found at any slope (LSD, average p=0.809). We interpret this finding as a reflection of the co-activation of these functional antagonists observed during locomotion in these animals, both here and by others (Gramsbergen et al., 2000). No significant differences were found between the 4 and 10 week times examined.

Factor loadings for TA in the Trained group also were significantly different from those for both TA and SOL of Intact animals at both times studied (LSD, average p<0.004) (Fig. 7 A, B: TT), as were the factor loadings for SOL at the four week survival time (average p<0.03). In contrast, by ten weeks after sciatic nerve transection, all factor loadings (for all PCs) for SOL in the Trained group were not significantly different from those for SOL in the Intact rats at all slopes studied (LSD, average p=0.486). We interpret this finding to mean that moderate exercise applied immediately following sciatic nerve injury has an effect on the timing of activation of the reinnervated SOL during locomotion, at all three slopes studied, at ten weeks
but not four weeks after injury.

*Effects of exercise on hindlimb movements during treadmill locomotion* – We used a global measure of hindlimb movements (Chang et al., 2009) to study the effects of exercise on hindlimb movements following sciatic nerve transection and repair. Marked points on the skin over the greater trochanter and the fifth metatarsophalangeal joint were used to define a limb length position vector. The magnitude of this vector was calculated as the distance between these two skin markers and is termed limb length. The direction of this vector reflects the orientation of the limb to the treadmill belt and is termed limb angle (Fig. 8B: inset).

Changes in these two components of the global limb vector during the step cycle in Intact, Untrained, and Trained rats are shown in Figure 8. In each panel, data points from level, upslope, and downslope walking are indicated by different shading. Some of the data from Intact and Untrained rats have been presented in a slightly different form in previous papers from our lab (Sabatier et al., 2011a; Sabatier et al., 2011b). They form controls against which the data from Trained rats can be compared. In each panel, the magnitude of the global limb vector is displayed as adjusted limb length, the ratio of limb length to femur length, so that animals of different sizes could be compared at different survival times. For each of the two components of the global limb vector, three measures were chosen for parametric analysis. Average, minimum, and maximum values of limb angle and adjusted limb length over the entire step cycle were determined and are indicated by horizontal and vertical lines in Figure 8A. All of these measurements were subjected to a one-way ANOVA. For each parameter, if a significant result was obtained from the omnibus test, paired, post-hoc tests (LSD) were conducted. These data are summarized in Table I. Mean values that are significantly different (p<0.05) from Intact rats are indicated by asterisks.

The most striking outcome of this analysis is that global limb length is conserved by the
recovering animals, as (Chang et al., 2009) have suggested for cats and we have proposed for rats (Sabatier et al., 2011b). Among the 54 paired comparisons of adjusted limb length (three survival times X three parametric measures X three slopes X two treatment groups) made in the recovering rats, only nine significant (p<0.05) differences (17%) were encountered. Most of these differences were for maximum adjusted limb length in Untreated rats. In contrast, significant differences in mean limb angle were found in 38 of the 54 comparisons made (70%). In all of these instances, mean limb angle measures were significantly greater than those found in Intact rats. The implication of this observation is that in both Trained and Untrained rats, a strategy for conserving limb length during locomotion by increasing limb angle is adopted.

A second outcome of this analysis is related to the ability of the rats to conserve limb length when walking on different slopes. When Intact rats walk with the treadmill belt inclined downward, the swing phase is prolonged and the maximum adjusted limb length is significantly greater than found during level walking, as the limb is extended to contact the treadmill belt (Fig. 8A: Maximum Length). This increased limb length persists throughout the step cycle. During upslope walking, maximum length occurs at the end of the stance phase when the angle of the limb is much reduced, as contact with the belt is prolonged (Fig. 8A: Minimum Angle). These adaptive changes to slope walking in Intact rats are not apparent in Untreated rats. There is barely any detectable difference in limb angle or limb length at different slopes (Fig. 8B), despite the very different biomechanical requirements for slope walking. The pattern of limb movement during walking on all three slopes resembles that described above for downslope walking in the Intact rats. As we have argued previously (Sabatier et al., 2011b), the adaptive strategy of conserving limb length by increasing limb angle is very effective during downslope walking. Of the 18 paired comparisons made for downslope walking, only two (maximum angle and average angle at four weeks in the Untrained group) were significant (Table I).
In marked contrast, slope-related adaptive changes in limb movements were observed in
Trained rats (Fig. 7C). The same success in conserving both limb angle and adjusted limb
length as found in Untreated rats was found during downslope walking. In Trained rats,
maximal limb length was not significantly different from Intact rats during both level and upslope
walking, and, at least by the ten week post-transection survival time, this conservation was
achieved by smaller increases in average and minimum limb angle than found in Untrained rats
(Table I). Thus, two weeks of exercise applied immediately following sciatic nerve injury is
sufficient to enable these animals to adapt the movements of their hind limbs to the differing
mechanical demands of walking on different slopes.
Discussion

Moderate exercise in the form of treadmill training has been shown to enhance axon regeneration following peripheral nerve injury in mice (Sabatier et al., 2008, English et al., 2009) and rats (Asensio-Pinilla et al., 2009; Ilha et al., 2008; Seo et al., 2006; Udina et al., 2011), but its full effects on functional recovery are not known. We hypothesized that moderate exercise in the form of daily treadmill training during the first two weeks following transection and repair of the rat sciatic nerve would lead to improved functional recovery. The main finding of this study was that two weeks of daily treadmill training following sciatic nerve injury in rats results in improvements in at least four aspects of functional recovery when compared to Untrained rats receiving the same injury.

Exercise results in enhanced functional muscle reinnervation - The reappearance of an M response and an H reflex in SOL to tibial nerve stimulation in awake behaving animals was used to measure the timing of functional muscle reinnervation. In Trained rats, both evoked potentials re-appeared significantly earlier than in Untrained rats. The simplest explanation for this effect is that exercise promoted regenerating axon elongation, as has been shown anatomically in mice (English et al., 2009; Sabatier et al., 2008). The amplitude of both of these potentials increased linearly in all rats during the 10-15 week study period. This finding also is consistent with our previous anatomical observations in exercised mice (English, 2005a) and following brief electrical stimulation in rats (Al-Majed et al., 2000). Elongation of regenerating axons (Al-Majed et al., 2000), and muscle fiber reinnervation (English et al., 2007b), both are temporally staggered over several weeks following nerve transection and repair. The linear increase in amplitudes of these responses to tibial nerve stimulation is likely the result of some combination of the reinnervation of the SOL muscle by progressively more regenerating motor axons and the enlargement of reinnervated muscle fibers. Because nearly
half of reinnervated SOL muscle fibers stop expressing slow myosin heavy chain isoforms and begin to express fast isoforms following sciatic nerve injury (Mendler et al., 2008), we also cannot rule out a contribution of this fiber phenotype transformation to the increase in evoked EMG amplitudes.

However, the slopes of the lines fitted to the data from Trained rats were significantly greater than the corresponding slopes for Untrained rats, suggesting that, at any given time during recovery, the regeneration process was more mature in the Trained animals. These findings are comparable to the results of nerve conduction studies reported by Navarro and colleagues (Asensio-Pinilla et al., 2009; Udina et al., 2011) who found that four weeks of application of an exercise protocol that was even less intense than the one used here resulted in larger M responses at 60 days after injury. Coupled with the results presented above, that a similar enhancement of evoked EMG amplitudes is found as long as 100 days after injury, we suggest that the effects of moderate exercise after peripheral nerve injury influence the regeneration process for a considerable time after the exercise has ended.

By 11 weeks after sciatic nerve transection and repair, the amplitude of both the SOL M response and the H reflex in Trained rats is much larger than that found in Untrained rats and also nearly twice that found in the same muscle prior to sciatic nerve transection. Others have reported more modest relative activity (Asensio-Pinilla et al., 2009; Udina et al., 2011). We can only speculate as to why such a large increase in amplitudes was found in the Trained rats. Despite finding in our post-mortem examinations that the EMG wires were in the same locations in the muscles as where we had implanted them prior to nerve transection, it seems likely that the geometric relationship between our implanted EMG electrodes and the surrounding muscle fibers may change as the fibers first atrophy with denervation and then recover somewhat after reinnervation. In both Untreated and Treated rats, such a change might mean that the number
of muscle fibers in close proximity to the moderately high spatial resolution EMG electrodes
used in the present study might be greater than encountered in muscles of Intact rats. The
compound muscle action potentials recorded in fully reinnervated muscles, either directly (M
response) or via the H reflex, then might be expected to be larger than those recorded from
activating the same number of motor units in the muscles in Intact rats. We believe that the
larger-than-pre-transection potentials recorded only in Trained rats reflects the faster and more
extensive reinnervation of SOL muscle fibers found with moderate exercise. The restoration of
evoked potential amplitudes to pre-transection levels found in Untrained rats thus might be
interpreted as evidence of incomplete reinnervation.

Exercise restores H reflex efficacy in reinnervated muscles - The ratio of the amplitude
of the largest H reflex recorded to that of the largest M response in the same experiment is
considered a reflection of the efficacy of the reflex, a measure of the portion of the total
available SOL motoneuron pool that is recruited into activity by the electrical stimulation of
peripheral afferent axons (Navarro et al., 2007). Because it is a comparison of the amplitudes
of two potentials, it is assumed to be independent of the amount of muscle reinnervation. This
H$_{\text{Max}}$/M$_{\text{Max}}$ ratio differed markedly in Trained and Untrained rats. In both Trained and Untrained
rats this ratio is initially larger than found in Intact rats, as has been observed by others (Navarro
et al., 2007); (Asensio-Pinilla et al., 2009; Udina et al., 2011). In Untrained rats this very large
response persists for 3-4 weeks and then decreases, at first rapidly and then more gradually
over a four week period, until it reaches a stable ratio significantly below that of Intact rats
(English et al., 2007a). Thus, by the end of the study period, we would conclude that relatively
fewer SOL motor units are recruited into the H reflex in Untrained rats than found in Intact rats.
In Trained rats, the initial H$_{\text{Max}}$/M$_{\text{Max}}$ ratio decreases gradually until it stabilizes at a value similar
to that noted in Intact rats, indicating that a similar proportion of the available motoneuron pool
is recruited into activity by peripheral afferent stimulation in Trained and Intact rats.
Peripheral nerve transection results in a withdrawal of the synaptic inputs onto the somata and proximal dendrites of motoneurons, a phenomenon known as synaptic stripping (Blinzinger and Kreutzberg, 1968). Although many of these withdrawn inputs are later restored, most of those originating from primary sensory neurons, the afferent axons contributing to the H reflex, are not (Alvarez et al., 2010; Alvarez et al., 2011) and this permanent loss of inputs may explain the loss of the stretch reflex in self-reinnervated muscles (Cope et al., 1994). We have found a similar withdrawal of synaptic terminals in untrained mice following sciatic nerve transections, but no evidence for stripped inputs on axotomized motoneurons in mice after moderate daily exercise (English et al., 2011). It is tempting to speculate that this effect of exercise on the structure of circuitry in the CNS might underlie the restoration of the full \( \frac{H_{\text{Max}}}{M_{\text{Max}}} \) ratio in our Trained rats, but more data would be needed to support such a conclusion.

Exercise has a modest effect on locomotor activation patterns of reinnervated muscles -

In Untrained rats, the pattern of activation of the SOL and TA muscles during treadmill locomotion shifts from strict reciprocity (Sabatier et al., 2011b; Thota et al., 2005) to co-activation (Sabatier et al., 2011b) following recovery from sciatic nerve injury. A similar outcome was noted when Trained rats were studied at early reinnervation times but by 10 weeks after nerve repair the pattern of activity of the reinnervated SOL was not significantly different from that of the SOL muscle of Intact rats during locomotion on all three slopes. Because the timing of SOL activity has been shown to differ between level and upslope walking in intact rats (Sabatier et al., 2011b), this alteration in the timing of activity of the reinnervated SOL at all three slopes must reflect at least a modest restoration of the ability of the rat to adapt the pattern of reinnervated muscle activity to the different mechanical demands of slope walking.
Such a change in the timing of activity could be related to the specificity with which regenerating axons reinnervated functionally appropriate vs. inappropriate muscle targets. We have shown here, and elsewhere (English et al., 2009) that exercise has little effect on one form of misdirection of regenerating motor axons, the precision with which those axons reinnervate peripheral targets. However, we also found that a second form of axon misdirection, the branching of regenerating axons to reinnervate BOTH the TA muscle and muscle targets of the tibial nerve was greatly reduced in Trained relative to Untrained rats. The extent of such dual innervation, as noted in the results of tibial nerve axon reflex experiments (Fig. 4), was similar in the two experimental groups until about six weeks after sciatic nerve transection and repair. After that time, the extent of branched innervation increased in the Untrained rats but not in Trained rats. Whether the lesser amount of branched muscle reinnervation found in Trained rats, and by implication a greater ability to activate the TA and SOL muscles independently, is the source of the restored pattern of SOL activation during locomotion is not clear at this time. The contribution of branched muscle reinnervation could be evaluated by study of the activation patterns of individual reinnervated motor units.

A similar effect of training was not found in the reinnervated TA muscle; the locomotor activity pattern of the reinnervated TA muscle at both times studied and on all slopes was not significantly different in Trained and Untrained rats. Thus, SOL and TA are co-activated during locomotion in the Trained rats, but the pattern of co-activation is different from the pattern of co-activation found in Untrained rats at 10 weeks or even that in Trained rats at earlier survival times. The effect of the exercise is a reduction in the amount of SOL activity during the swing phase but co-contraction with TA during the stance phase (see Fig. 6, bottom panel). The fact that nearly half of the reinnervation of both the SOL and TA muscles is from functionally inappropriate motoneurons (see Figure 5) could account for the co-activation of these muscles during walking on all slopes in the Untrained rats, provided the outputs of the spinal circuits that
regulate the timing of activation of the motoneurons reinnervating these muscles during locomotion was not changed. Because the timing of SOL locomotor activity was changed in the Trained rats at 10 weeks, but not 4 weeks after injury, and the misdirection of regenerating axons was the same as found in Untrained rats, we suggest that the outputs of the spinal circuitry that regulate the timing of activation of the motoneurons reinnervating the SOL muscle during locomotion must have been changed by the exercise. In particular, we would speculate that the activation of motoneurons that had previously innervated TA but had regenerated to reinnervate SOL must have been changed in the Trained rats and that the change occurred sometime between four and ten weeks after nerve transection. Testing of this speculative hypothesis awaits further study.

We have postulated (Sabatier et al., 2011b) that the co-activation of the functional antagonistic TA and SOL muscles found in Untrained rats might be a part of an adaptive strategy to stiffen the ankle joint during both the stance and swing phases of the step cycle. In this context, we consider that the improved SOL reinnervation found following only two weeks of moderate daily exercise might not be sufficient to counteract such a strategy following a nerve injury that affects both TA and SOL. In mice, we found that a similar training paradigm to the one employed in this study resulted in more profound effects on axon regeneration in the tibial branch of the sciatic nerve than the common fibular branch, but if the amplitude of the exercise was increased by training on an upslope inclined treadmill, then the effect on axon regeneration in the two nerve branches was more balanced (Sabatier et al., 2010). A future study should investigate the effect of this upslope training on locomotor EMG activity.

Exercise improves hindlimb movement patterns during locomotion on different slopes - Locomotion on different slopes requires different limb movement strategies to cope with the different biomechanical demands (Carlson-Kuhta et al., 1998; Maas et al., 2009). Previous
observations in Untrained rats (Sabatier et al., 2011b) and in cats (Chang et al., 2009) have been used to argue that hindlimb length is a conserved variable in these strategies. Variability in limb length measurements, both between animals and between different steps in the same animals, is very much smaller than any other measure of limb or joint kinematics (Sabatier et al., 2011b). The range of limb lengths encountered during locomotion at all three slope conditions is conserved by changes in the angle of orientation of the limb, especially during level and upslope walking. One goal of this conservation of limb length is proposed to help maintain the coordination of movements of the different limbs (Chen et al., 2011).

Sciatic nerve transection causes paralysis of the muscles that act about the ankle and foot. Walking is accomplished by activation of innervated proximal musculature and little use of the ankle and foot (Bain et al., 1989). The foot lies flat on the treadmill belt during stance but changes in hip and knee movements result in an increase in limb angle and an attempt to maintain the overall length of the hindlimb to match the pattern found in Intact rats (and, presumably, the contralateral hindlimb). This new walking pattern is retained following muscle reinnervation, where it is reinforced by co-activation of functional antagonists (SOL and TA) about the ankle. This compensatory strategy was not changed by our exercise protocol. All measures of limb angle were increased significantly in both Untrained and Trained rats at all times studied. What was changed by exercise was the effectiveness of this strategy to cope with the different mechanical demands of locomotion on slopes. Limb length was conserved successfully by all rats during downslope walking, but conservation of limb length by changing the orientation of the limb was successful during level and upslope locomotion only in the Trained rats.

The exercise protocol used does enhance functional recovery from peripheral nerve injury by enhancing axon regeneration, by facilitating a faster than normal recovery time of
reflexes, and by restoring the efficacy of the H reflex. Although it does not result in a return to full functional recovery, at least as measured during locomotion, the enhancements of muscle and reflex function undoubtedly contribute to a restored ability of the animals to adapt to the different biomechanical demands of slope walking. We conclude that this restored ability represents a modest, but significant improvement over the Untrained group. We feel that exercise as a therapy for peripheral nerve injury has significant potential for translation to clinical use, but more study is needed. It is possible that the brief period of very moderate exercise used in the present study, while sufficient to promote nerve regeneration and modest functional recovery, is not optimal to promote full functional recovery. Different paradigms could have a more pronounced effect on functional recovery. However, because different exercise protocols, including passive limb movements, have resulted in improvements in axon regeneration after peripheral nerve injury (reviewed in (Udina et al., 2011), one also cannot rule out that the effects we have observed are the result of the increased environmental enrichment associated with exercise, as has been suggested by others (Gomez-Pinilla et al., 2011). Identification of the critical features of exercise that promote axon regeneration and improved functional recovery will have to be a priority if our findings are to be translated to clinical use.
Acknowledgements

The authors thank Dr. Michael Kutner for his advice and help with all of the statistical methods used. Dr. Manning Sabatier read and commented on earlier versions of this paper and his help is greatly appreciated.
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Disclosures

No conflicts of interest.
Author contributions:
TB, MI, KM, JN, KP, SJR, & EW: Primary contributors to all aspects of the study
AWE: Assisted and supervised completion of the study
Table I

Limb Kinematic Parameters During Treadmill Locomotion

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English AW. Enhancing axon regeneration in peripheral nerves also increases functionally inappropriate reinnervation of targets. *J Comp Neurol* 490:427-441. 2005b.


Figure Legends

Figure 1. Effects of exercise on restoration of evoked EMG responses. A. Two evoked EMG potentials were found in the SOL muscle in response to stimulation of the tibial nerve: a direct muscle (M) response and, at a slightly longer latency, an H reflex. The different traces show these potentials recorded prior to (Intact) and at different times after transection and repair of the sciatic nerve. All were recorded from a single, Untrained rat and each trace is an average of the responses to 100 stimulus presentations. Grey bars below each trace indicate the time windows chosen for the M responses and H reflexes at the different recovery times. Note that only a stimulus artifact, and no evoked responses were found at the earliest post-injury time point (2 wks.). At the time of earliest recording of restored activity (3.5 wks.), multiple small potentials were recorded. Dividing them into M responses and H reflexes was somewhat arbitrary so that these potentials are referred to as putative M responses and putative H reflexes. B. Mean (+ SD) values for the first day after nerve transection and repair that M responses (left) and H reflexes (right) could be evoked are shown for Untrained rats and rats that had been treadmill trained for two weeks. Small symbols in each bar in these graphs represents the values obtained from individual rats. Different symbols are shown for Sprague-Dawley (HSD) and Lewis rats.

Figure 2. Time course of recovery of the M response in Trained and Untrained rats. A. The mean amplitude of the maximal M response (average rectified activity in the M time window) is shown at different times after sciatic nerve transection and repair for Trained rats (black symbols) and Untrained rats (white symbols). All values are expressed as a proportion of the maximal M response amplitude recorded from that rat prior to nerve transection. The horizontal dashed line is at 1.0, indicating the amplitude of this pre-transection response. Data from
Trained and Untrained groups were fitted with linear least squares regression lines. Equations for the lines and correlation coefficients are shown next to each line. The shaded area at the left of the graph indicates the time course of treadmill training in the Trained animals. B. The mean latency from stimulus onset to the start of the M response is shown at different times after sciatic nerve transection and repair for Trained rats and Untrained rats. The horizontal dashed line represents the M response latency found in intact rats (0.5 msec). Error bars represent the SEM for each time point. No error bars are visible at times longer than five weeks because the SEM is smaller than the symbols used. Data from Untrained animals were offset slightly to the right of data points from Trained animals to avoid overlap.

Figure 3 Time course of recovery of the H reflex in treadmill Trained and Untrained rats. A. The mean amplitude of the maximal H reflex (average rectified activity in the H time window) is shown at different times after sciatic nerve transection and repair for Trained rats (black symbols) and Untrained controls (white symbols). All values are expressed as a proportion of the maximal H reflex amplitude recorded from that rat prior to nerve transection. The horizontal dashed line is at 1.0, indicating the amplitude of this pre-transection response. Data from Trained and Untrained controls were fitted with linear least squares regression lines. Equations for the lines and correlation coefficients are shown next to each line. The shaded area at the left of the graph indicates the time course of treadmill training in the Trained animals. B. The same data are re-expressed as a proportion of the maximal M response amplitude at the different times studied. All values of this \( \frac{H_{\text{MAX}}}{M_{\text{MAX}}} \) ratio are, in turn, expressed as a proportion of the same ratio determined from that rat prior to nerve transection \( \frac{H_{\text{MAX}}}{M_{\text{MAX}}/\text{Intact}} \). The horizontal dashed line is at 1.0, indicating the amplitude of this pre-transection ratio. The sizes of the symbols used are proportional to the SD for each time point. The lines for each group are not fitted to any function, but are included only to emphasize differences between Untrained and
Trained groups. The shaded area at the left of the graph indicates the time course of treadmill training in the Trained animals.

Figure 4  The time course of the amplitude of tibialis anterior muscle responses to electrical stimulation of the tibial nerve distal to transection of the sciatic nerve is shown.  A. Examples of evoked EMG traces (axon reflexes) recorded from TA in an Intact rat and in an Untrained (left) and a Trained (right) rat at an early innervation time, 20 days after nerve transection, and a later time, 67 days after nerve transection.  Each trace represents the average of 100 stimulus presentations.  The open arrow in the top trace points to a very small potential that probably represents unavoidable EMG cross-talk in the Intact rats.  Vertical scale bar in each trace is 0.5 mV.  B. The mean amplitude of the maximal M response (average rectified activity in the M time window) is shown at different times after sciatic nerve transection and repair for Trained (black symbols) and Untrained rats (white symbols).  Different dashed lines do not represent regression lines, but are included only to emphasize differences between Untrained and Trained groups.  The dark arrows point to the time at which the traces in panel A were obtained. The shaded area at the left of the graph indicates the time course of treadmill training in the Trained animals.

Figure 5. Specificity of muscle reinnervation in treadmill Trained and Untrained rats.  A. As part of a terminal experiment, at least 10 weeks following sciatic nerve transection and repair, the tibial and common fibular fascicles of the sciatic nerve were dissected apart above the surgical repair site.  A cuff electrode was placed about each of the dissected nerves (shown about common fibular branch in this diagram) and used to stimulate axons of motoneurons coursing in that branch.  B. Representative potentials recorded from patch electrodes on the soleus (SOL) and tibialis anterior (TA) muscles were made in response to stimulation of the tibial (Tib) and
common fibular (CF) fascicles of the sciatic nerve proximal to the nerve transection. Traces are shown for the Intact side and the reinnervated side (TT) of a rat 15 weeks after transection and repair of the sciatic nerve and two weeks of post-transection exercise. C. Proportions of $M_{\text{Max}}$ in the SOL (top row) and TA (bottom row) muscles produced by stimulating the Tib and CF nerves are shown. In each pie chart, the proportion of the response produced by stimulation of the anatomically appropriate nerve is shown by dark shading. That produced by stimulation of the anatomically inappropriate nerve is shown by lighter shading.

Figure 6  Locomotor EMG activity in soleus (SOL) and tibialis anterior (TA) muscles during treadmill locomotion. Representative samples of activity recorded while walking on a level treadmill at 11 M/min are shown for an Intact rat (top) and for rats 10 weeks after transection and repair of the sciatic nerve and Untrained (middle) or Trained rat (bottom). Activity was synchronized with video recordings. Vertical dashed lines and upward pointing arrows are placed at the onset of the swing phase of the step cycle, when the paw is removed from the treadmill belt.

Figure 7. Results of factor analysis using principal components (PCA) applied to locomotor EMG activity in the soleus (SOL) and tibialis anterior (TA) muscles. Activity during multiple individual step cycles was averaged for both muscles during level, upslope and downslope walking at 11 M/min. Mean values (+ SEM) of factor loadings for factor 2, which accounts for 20% of the variance in the data set, are shown from activity recorded at four weeks (A) and 10 weeks (B) after sciatic nerve transection and repair. Bars representing data from downslope, level, and upslope treadmill locomotion are shown in different shadings. Data from the SOL muscle are shown in the left of each panel. Similar findings from the TA muscle are shown to
the right in each panel. Data from Intact, Untreated (UT) and Treadmill Trained (TT) rats are shown as groups. The data points from Intact rats are the same in both panels A and B.

Figure 8. Whole hindlimb kinematics during treadmill locomotion on different slopes. From two dimensional analysis of video records, a global limb vector of the hindlimb was calculated. The magnitude of this vector is limb length, the distance between skin markers placed over the greater trochanter and the fifth metatarsophalangeal joint, and its direction is limb angle, the orientation of the limb to the treadmill belt (see inset in panel B). For analysis, limb length was scaled to femur length in each rat and is reported as Adjusted Limb Length. In each panel, changes in limb angle during the step cycle are plotted against adjusted limb length. Data from Intact rats are shown in panel A. In panels B and C, data are from Untreated rats and from Treadmill Trained rats, respectively, 10 weeks after transection and surgical repair of the sciatic nerve. Data from downslope, level, and upslope walking at 11 M/min are shown by symbols of different shading. Each of the resulting graphs in each panel forms an irregular shape. Data points in each shape represent the average of several rats (see Table I). Paw contact, the end of the swing phase, is found at the extreme right of each shape (down arrows), and corresponds to the time of maximal Limb Angle. Paw off, the end of the stance phase, is found to the extreme left of each shape (up arrows), and corresponds to the time of minimum Limb Angle. Data points in the swing phase are found along the bottoms of each shape and data points from the stance phase are along the top of each shape. In panel A, the timing of maximum and minimu Adjusted Limb Lengths are indicated by larger filled circles. Average values of limb angle and adjusted limb lengths are indicated by arrows in each panel.
A. $H_{\text{MAX}}$ Amplitude

- Trained: $y = 0.19x - 0.14$, $R^2 = 0.99$
- Untrained: $y = 0.07x - 0.23$, $R^2 = 0.87$

B. $H_{\text{MAX}}/M_{\text{MAX}}/\text{Intact}$

- Trained: $y = 0.19x - 0.14$, $R^2 = 0.99$
- Untrained: $y = 0.07x - 0.23$, $R^2 = 0.87$
A. Sciatic n. 

- Repair site 
- Common fibular n. 
- Tibialis anterior 
- Soleus 

B. Intact 

- Sol 
- TA 

- TT 

- Tib Stimulation 
- CF Stimulation 

C. Soleus 

Intact 

- Tib 94% 
- CF 59% 

Untrained 

- Tib 56% 
- CF 53% 

Trained 

- Tib 56% 
- CF 53% 

Tibialis anterior 

- CF 97%
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| * p<0.05 vs. Intact  
+p<0.05 vs. Untrained