Effects of treadmill training on functional recovery following peripheral nerve injury in rats

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Boeltz T, Ireland M, Mathis K, Nicolini J, Poplavski K, Rose SJ, Wilson E, English AW. Effects of treadmill training on functional recovery following peripheral nerve injury in rats. J Neurophysiol 109: 2645–2657, 2013. First published March 6, 2013; doi:10.1152/jn.00946.2012.—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 1 h/day on a level treadmill, beginning 3 days after unilateral transection and surgical repair of the sciatic nerve, and conducted 5 days/wk for 2 wk. In Trained rats, both direct muscle responses to tibial nerve stimulation and H reflexes in soleus reappeared 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 coactivated 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 downslope 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.

TRAIAMIC PEOPHAL 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 laboratory 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 colleagues (Asensio-Pinilla et al. 2009) studied the effects of treatment of rats with 4 wk of twice daily exercise after sciatic nerve transection and repair. For both the tibialis anterior (TA) 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 2 mo 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). Our laboratory has 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 (body weight ~250 g). 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. Pretransection 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 postinjury period. At the conclusion of the study period, all experimental animals were euthanized using an intraperitoneal injection of 100 mg/kg of pentobarbital. Some of the data from some (n = 4) of the Sprague-Dawley rats in the Untrained group have 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 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 deinsulated tips of the wires outside of the muscle. Wire locations were visualized postmortem 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. 1997), 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 postmortem, 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 no. MS363), ~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 1 h, 5 days/wk. 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 presurgical screenings, at a speed of up to 20 m/min without the need for reinforcement. To avoid any complications of preinjury exercise, rats were not exposed to the treadmill for at least 2 wk before 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 2, 4, and 10 wk after nerve transection and repair, when the rats walked on the treadmill for a total of 10 min over a period of at least 30 min.

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 ~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 before the delivery of each
stimulus. Ongoing EMG activity in the SOL was monitored, and when
such activity was maintained for 10 s within a user-defined voltage
window, a very short (0.1 ms) 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
3 s, 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 (M_{\text{max}}) and the maximum
H reflexes (H_{\text{Hmax}}) 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 ac-
tivity, as our laboratory has described previously (English et al.
2007a). Recordings were made at multiple times over a period of
10–15 wk 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 follow-
ing nerve transection and repair, when treadmill training began in the
Trained group. After the 2-wk treadmill training program ended,
recordings of evoked EMG activity were made 5 days/wk until the
first H reflex could be recorded. Recordings of evoked EMG activity
were made at weekly intervals thereafter. The amplitudes of M_{\text{max}}
responses and H_{\text{Hmax}} reflexes were expressed as a percentage of the
corresponding peak amplitudes obtained in pretranssection recordings.
Average intensities were computed for rats in the Untrained and
Trained groups during weekly intervals after nerve transection and
repair, and the data (posttranssection 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).

Locomotor EMG activity. In Intact rats, SOL and TA are recipro-
cally activated during locomotion (Sabatier et al. 2011b; Thota et al.
2005). However, following transaction and repair of the sciatic nerve
in Untrained rats, coactivation 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 4 and 10 wk after nerve transection and repair in
Trained rats and was 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 laboratory’s previous publications (Sabatier et al. 2011b;
Sabatier et al. 2012). Muscle activity and video records were synchron-
ized 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 classifica-
tion 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 exper-
iment and subjected to Principal Components Analysis (PCA) (Statistica,
StatSoft). 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
during 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 ANOVA, with post hoc
paired [Fisher least significant difference (LSD)] testing, where appro-
priate. 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 mark-
ers 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 hindlimb. 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 loco-
motion using X-ray video recordings was compared with 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 differ-
ences 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-xylazine 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 with that recorded from
contralateral, intact muscles using an unpaired t-test (r = 0.05).

RESULTS

Effects of exercise on restoration of evoked SOL EMG activity. Tibial nerve stimulation evoked an M response and an
H reflex in the SOL muscle of Intact rats (Fig. 1A, Intact). Both
evoked potentials disappear in denervated muscles (Fig. 1A,
2 wk), but later reappear 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 wk). 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 wk and 7 wk). 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 SOL 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 SOL H reflex is first seen significantly earlier (unpaired t-test, \( P < 0.009 \)) in Trained rats compared with Untrained rats (on average, 20.00 days vs. 29.25 days) (Fig. 1B).

The amplitude of the largest restored M_{\text{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 offset slightly to the right of data points from Trained animals to avoid overlap.

Fig. 2. Time course of recovery of the M response in Trained and Untrained rats. A: the mean amplitude of the maximal M response (M_{\text{Max}}; 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 M_{\text{Max}} amplitude recorded from that rat prior to nerve transection. The horizontal dotted line is at 1.0, indicating the amplitude of this pretranssection 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 represent the M-response latency found in Intact rats (0.5 ms). Error bars represent the SE of the mean for each time point. No error bars are visible at times longer than 5 wk, because the SE of the mean 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.

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.
declined more gradually and stabilized at approximately 9 wk after nerve repair surgery. The $H_{\text{Max}} / M_{\text{Max}}$ ratio for Untrained rats (0.52, SD 0.20) remains significantly different from 1.0. Over the same time period, the ratio for Trained rats (0.71, 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 prelesion 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 Fig. 4B.

In both groups of animals, slightly larger potentials were recorded in TA over the first 3–5 wk after nerve injury (Fig. 4A, Day 20). Over the next 6–7 wk, 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 wk 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/maturational 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 with 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 Fig. 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 one-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 one-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 one-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 our laboratory has asserted previously for mice (English et al. 2009), treadmill training augments the specificity of muscle reinnervation in TT and Untrained rats.

Fig. 4. The time course of the amplitude of tibialis anterior (TA) muscle responses to electrical stimulation of the Tib 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 $M_{\text{Max}}$ (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 A were obtained. The shaded area at the left of the graph indicates the time course of treadmill training in the Trained animals.

Fig. 5. Specificity of muscle reinnervation in TT and Untrained rats. A: as part of a terminal experiment, at least 10 wk following sciatic nerve transection and repair, the Tib and common fibular (CF) fascicles of the sciatic nerve were dissected apart above the surgical repair site. B: representative potentials recorded from patch electrodes on the SOL and TA muscles were made in response to stimulation of the Tib and 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 wk after transection and repair of the sciatic nerve and 2 wk of posttransection 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.
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 Fig. 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 ($F_{9,36} = 9.520, P < 0.0000004$). Mean factor loadings for factor 2, where significant differences were found for all three slopes, are shown for the 4- and 10-wk survival time groups in Fig. 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. 7, A and B, Intact). Our laboratory has 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. 7, A and 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 coactivation of these functional antagonists observed during locomotion in these animals, both here and by

![Fig. 6. Locomotor EMG activity in SOL and 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 wk after transection and repair of the sciatic nerve and Untrained (middle) or Trained rat (bottom). Activity was synchronized with video recordings. Vertical dotted 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.](http://jn.physiology.org/)

![Fig. 7. Results of factor analysis using principal components (PCA) applied to locomotor EMG activity in the SOL and 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 (+SE) of factor loadings for factor 2, which accounts for 20% of the variance in the data set, are shown from activity recorded at 4 wk (A) and 10 wk (B) after sciatic nerve transaction and repair. Bars representing data from downslope, level, and upslope treadmill locomotion are shown in different shadings. Data from the SOL muscle are shown to 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 TT rats are shown as groups. The data points from Intact rats are the same in both A and B. *$P < 0.05$ vs. Intact SOL & Intact TA and NS vs. each other. **$P < 0.05$ vs. Intact TA and NS vs. Intact SOL.](http://jn.physiology.org/)
others (Gramsbergen et al. 2000). No significant differences were found between the 4- and 10-wk 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 and B, TT), as were the factor loadings for SOL at the 4-wk survival time (average \( P < 0.03 \)). In contrast, by 10 wk 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 10 wk, but not 4 wk, 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 MP 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 Fig. 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 laboratory (Sabatier et al. 2011a, 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 Fig. 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 1. 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 our laboratory has proposed for rats (Sabatier et al. 2011b). Among the 54 paired comparisons of adjusted limb length (three survival times \( \times \) three parametric measures \( \times \) three slopes \( \times \) 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 Untrained 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

![Fig. 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 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 A. In B and C, data are from UT rats and from TT rats, respectively, 10 wk 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 1). 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 A, the timing of maximum and minimum 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.](http://jn.physiology.org/doi/10.1152/jn.00946.2012/fig/8)
and minimum limb angle than found in Untrained rats (Ta-
ing, and, at least by the 10-wk posttransection survival time,
different from Intact rats during both level and upslope walk-
as found in Untreated rats was found during downslope walk-
success in conserving both limb angle and adjusted limb length
movements were observed in Trained rats (Fig. 8
. The same
in the Untrained group) were significant (Table 1).
walking, only two (maximum angle and average angle at 4 wk
by increasing limb angle is very effective during downslope
adapt the movements of their hindlimbs to the differing me-
sciatic nerve injury are sufficient to enable these animals to
ce the biomechanical demands of walking on different

B

to slope walking in Intact rats are not apparent in Untreated

A

Moderate exercise in the form of treadmill training has been
shown to enhance axon regeneration following peripheral
nerve injury in mice (English et al. 2009; Sabatier et al. 2008)
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 exer-
cise in the form of daily treadmill training during the first 2 wk
following transection and repair of the rat sciatic nerve would
lead to improved functional recovery. The main finding of this
study was that 2 wk of daily treadmill training following sciatic
nerve injury in rats results in improvements in at least four
aspects of functional recovery compared with 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 reappeared 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- to 15-wk study
period. This finding also is consistent with our laboratory’s
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

Table 1. Limb kinematic parameters during treadmill locomotion

<table>
<thead>
<tr>
<th></th>
<th>Time, wk</th>
<th>n</th>
<th>Average Angle, °</th>
<th>Minimum Angle, °</th>
<th>Maximum Angle, °</th>
<th>Average Length</th>
<th>Minimum Adjusted Length</th>
<th>Maximum Adjusted Length</th>
</tr>
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<tbody>
<tr>
<td>Intact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Downslope</td>
<td>2</td>
<td>7</td>
<td>100.87 ± 9.35</td>
<td>68.27 ± 14.12</td>
<td>133.49 ± 4.96</td>
<td>1.75 ± 0.35</td>
<td>1.20 ± 0.30</td>
<td>2.31 ± 0.43</td>
</tr>
<tr>
<td>Level</td>
<td>2</td>
<td>10</td>
<td>99.94 ± 12.34</td>
<td>66.01 ± 17.79</td>
<td>133.86 ± 6.89</td>
<td>1.79 ± 0.21</td>
<td>1.18 ± 0.07</td>
<td>2.40 ± 0.37</td>
</tr>
<tr>
<td>Upslope</td>
<td>2</td>
<td>10</td>
<td>107.98 ± 11.22*</td>
<td>69.32 ± 13.52*</td>
<td>135.48 ± 7.88*</td>
<td>1.70 ± 0.19</td>
<td>1.52 ± 0.34</td>
<td>2.18 ± 0.15*</td>
</tr>
<tr>
<td>Untrained</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Downslope</td>
<td>2</td>
<td>6</td>
<td>106.05 ± 7.35*</td>
<td>67.07 ± 6.73*</td>
<td>140.14 ± 7.32*</td>
<td>1.73 ± 0.45</td>
<td>1.53 ± 0.39</td>
<td>2.17 ± 0.46</td>
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<tr>
<td>Level</td>
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<td>10</td>
<td>105.90 ± 8.91*</td>
<td>73.93 ± 6.75*</td>
<td>138.95 ± 10.68*</td>
<td>1.90 ± 0.17*</td>
<td>1.55 ± 0.25</td>
<td>2.46 ± 0.32*</td>
</tr>
<tr>
<td>Upslope</td>
<td>2</td>
<td>6</td>
<td>110.46 ± 14.92*</td>
<td>69.19 ± 17.38*</td>
<td>151.72 ± 14.88*</td>
<td>1.65 ± 0.33</td>
<td>1.17 ± 0.29</td>
<td>2.06 ± 0.26*</td>
</tr>
<tr>
<td>Trained</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Downslope</td>
<td>2</td>
<td>5</td>
<td>94.05 ± 6.76</td>
<td>66.62 ± 7.37</td>
<td>121.47 ± 8.20†</td>
<td>1.66 ± 0.12</td>
<td>1.25 ± 0.15</td>
<td>2.08 ± 0.14</td>
</tr>
<tr>
<td>Level</td>
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<td>10</td>
<td>91.85 ± 5.29</td>
<td>60.34 ± 14.41</td>
<td>123.51 ± 9.26†</td>
<td>1.85 ± 0.44</td>
<td>1.35 ± 0.33</td>
<td>2.34 ± 0.56</td>
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<tr>
<td>Upslope</td>
<td>2</td>
<td>5</td>
<td>101.80 ± 9.19*</td>
<td>68.71 ± 12.88*</td>
<td>134.89 ± 8.80</td>
<td>1.62 ± 0.17*</td>
<td>1.27 ± 0.14</td>
<td>1.96 ± 0.21</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. Length measurements are adjusted limb lengths. See text for details. *P < 0.05 vs. Intact. †P < 0.05 vs. Untrained.
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 one-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 Untreated 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 4 wk 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 wk 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 Untreated 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 postmortem 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 Trained 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 Untreated 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 Untreated rats. We believe that the larger-than-pretransection 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 pretransection levels found in Untreated 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 $H_{\text{Max}}/M_{\text{Max}}$ differed markedly in Trained and Untreated rats. In both Trained and Untreated rats, this ratio is initially larger than found in Untreated rats, as has been observed by others (Asensio-Pinilla et al. 2009; Navarro et al. 2007; Udina et al. 2011). In Untreated rats, this very large response persists for 3–4 wk and then decreases, at first rapidly and then more gradually over a 4-wk 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 Untreated rats than found in Intact rats. In Trained rats, the initial $H_{\text{Max}}/M_{\text{Max}}$ 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 Untreated 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, 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 Untreated 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 central nervous system might underlie the restoration of the full $H_{\text{Max}}/M_{\text{Max}}$ 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 Untreated 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 coactivation (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 wk after nerve repair, the pattern of activity of the reinnervated SOL was not significantly different from that of the SOL muscle of Untreated 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. Our laboratory has 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 6 wk 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 coactivated during locomotion in the Trained rats, but the pattern of coactivation is different from the pattern of coactivation found in Untrained rats at 10 wk, 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 cocontraction with TA during the stance phase (see Fig. 6, bottom). The fact that nearly one-half of the reinnervation of both the SOL and TA muscles is from functionally inappropriate motoneurons (see Fig. 5) could account for the coactivation 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 wk, but not 4 wk 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 regulates 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 4 and 10 wk after nerve transection. Testing of this speculative hypothesis awaits further study.

Our laboratory has postulated (Sabatier et al. 2011b) that the coactivation 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 2 wk 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 coactivation 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.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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