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Recovery of Electromyographic Activity After Transection and Surgical Repair of the Rat Sciatic Nerve

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English AW, Chen Y, Carp JS, Wolpaw JR, Chen XY. Recovery of electromyographic activity after transection and surgical repair of the rat sciatic nerve. *J Neurophysiol* 97: 1127–1134, 2007. First published November 22, 2006; doi:10.1152/jn.01035.2006. The recovery of soleus (SOL), gastrocnemius (GAS), and tibialis anterior (TA) electromyographic activity (EMG) after transection and surgical repair of the sciatic nerve was studied in Sprague–Dawley rats using chronically implanted stimulation and recording electrodes. Spontaneous EMG activity in SOL and GAS and direct muscle (M) responses to posterior tibial nerve stimulation persisted for ≤2 days after sciatic nerve transection, but SOL and GAS H-reflexes disappeared immediately. Spontaneous EMG activity began to return 2–3 wk after transection, rose nearly to pretransection levels by 60 days, and persisted for the duration of the study period (120 days). Recovery of stimulus-evoked EMG responses began about 30 days after sciatic nerve transection as multiple small responses with a wide range of latencies. Over time, the latencies of these fractionated responses shortened, their amplitudes increased, and they merged into a distinct short-latency component (the putative M response) and a distinct long-latency component (the putative H-reflex). The extent of recovery of stimulation-evoked EMG was modest: even 100 days after sciatic nerve transection, the responses were still much smaller than those before transection. Similar gradual development of responses to posterior tibial nerve stimulation was also seen in TA, suggesting that some regenerating fibers sent branches into both tibial and common peroneal nerves.

INTRODUCTION

In adult laboratory animals, both sensory and motor axons are capable of regenerating and reinnervating their targets after injury to a peripheral nerve. Despite this capacity for axonal regeneration, poor functional recovery after injuries to peripheral nerves remains an important clinical problem. Only a small proportion of human subjects experiencing peripheral nerve injuries recover full function (Brushart 1998). The poor functional outcomes noted clinically are often attributed not only to the relatively slow growth of regenerating axons, but also to a decline in the specificity of reinnervated neuromuscular connections (Fawcett and Keynes 1990). In the rat (Brushart et al. 1983) and mouse (English 2005), transection and repair of the sciatic nerve results in the reinnervation of muscles by substantial numbers of motoneurons that, because of their spinal locations, are judged to have innervated different muscles or muscle groups before nerve injury. Efforts aimed at enhancing the growth of regenerating axons have all resulted in an increase in the amount of this topographically inappropriate motor reinnervation (English 2005).

This perturbation of connectivity in the peripheral nervous system can affect the functioning of spinal circuits that regulate movement and can thereby impair functional recovery. Perhaps the best known of these effects is that long-term self-reinnervated muscles of both rats and cats lack stretch reflexes (Cope et al. 1994; Haftel et al. 2005). After transection and surgical repair of muscle nerves, stretch-evoked postsynaptic potentials are absent from reinnervating motoneurons well after the return of effective motor reinnervation and of the ability of the primary afferent axons to transmit muscle length information to the CNS (Haftel et al. 2005). These findings imply that the peripheral lesion results in a marked central inhibition of at least one reflex pathway in the spinal cord, despite the regeneration of axons in the peripheral nervous system (Haftel et al. 2005).

The time course of the loss of this simple behavior after nerve transection and repair is not well known. It is possible that the central inhibition of the reflex pathway observed well after reinnervation actually begins at the time of the injury and, despite any restoration of peripheral connections that occurs later, the function of these reflex pathways is never restored. It was previously shown that, after peripheral nerve transection, synapses on the somata and dendrites of the axotomized motoneurons are displaced by intervening cellular processes of astrocytes (Alvarez et al. 1997; Brannstrom and Kellerth 1998). When axons in the cut nerve are allowed to reinnervate muscles, these synapses are reformed, but their relative densities at different somatodendritic locations suggest that their functions have not been totally restored (Brannstrom and Kellerth 1999). Alternatively, once the peripheral components of the stretch reflex pathway are restored by muscle fiber and muscle spindle reinnervation, a reasonable facsimile of the stretch reflex might return, only to be lost later. The H-reflex, the electrical analog of the monosynaptic portion of the stretch reflex, is evident 90 days after sciatic nerve crush in rats (Valero-Cabre and Navarro 2001), but the time course of this H-reflex recovery is not known.

Some researchers studied plasticity in the H-reflex in monkeys, rats, and mice using operant conditioning methods and showed that it can effectively be gradually increased or decreased (Chen et al. 2001, 2003a). After peripheral nerve injury, one strategy to compensate for the decline in neuro-

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muscular specificity, and even the loss of the stretch reflex, might be application of reflex pathway-altering operant conditioning methods, using electrically evoked responses as endpoints. However, the suitability of such an approach cannot be assessed until the time course of the return of those responses is better known. In this study, we examined spontaneous EMG activity and electrically evoked muscle responses over long periods after transection and surgical repair of the sciatic nerve in rats. We report here that responses to nerve stimulation are found relatively soon after such an injury, that they recover gradually to resemble the timing of responses in intact rats, but that the amplitude of the restored reflex is much reduced over that of intact animals. A preliminary report was recently published (Chen et al. 2006).

METHODS

Subjects were six male Sprague–Dawley rats weighing 379 (±56 SD) g at the beginning of the study. All procedures conformed to the *Guide for the Care and Use of Laboratory Animals* of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Academy Press, Washington, DC, 1996). They were reviewed and approved by the Institutional Animal Care and Use Committees of Emory University and the Wadsworth Center. The methods for construction of the chronic nerve stimulating cuff, chronic electrode implantation, EMG recording in freely moving rats, M-response and H-reflex elicitation, and sciatic nerve transection and repair were previously described in detail (Chen and Wolpaw 1995, 1997, 2002, 2005; Chen XY et al. 2003b; Chen Y et al. 2005; English 2005; Wolpaw and Chen 2006; Wolpaw et al. 1993) and are summarized here.

Each rat was implanted under general anesthesia [sodium pentobarbital, 60 mg/kg, administered intraperitoneally (ip), supplemented as needed]. All surgical procedures were performed under aseptic conditions. To record EMG activity, pairs of multistranded (10 × 50 gauge) stainless steel fine-wire electrodes stripped of their final 0.5 cm of Teflon insulation were implanted in the right soleus (SOL) and right medial and lateral gastrocnemius (GAS) (SOL–GAS, four rats) or the right SOL and right tibialis anterior (TA) (SOL–TA, two rats) muscles. To elicit the direct muscle response (M response) and H-reflex from SOL and GAS to nerve stimulation, a silicone rubber nerve cuff (with inside diameter about twice the diameter of the nerve) containing a pair of stainless steel multistranded fine-wire electrodes was placed on the right posterior tibial nerve and was closed by a suture that encircled the cuff. The Teflon-coated wires from the muscles and the nerve cuff passed subcutaneously to a connector secured to the skull with stainless steel screws and dental cement. In four of the six rats (2 SOL–GAS and the 2 SOL–TA rats), the sciatic nerve was transected and surgically repaired at the time of electrode implantation. In the other two SOL–GAS rats, electrodes were implanted 45 days before the nerve transection surgery. In all rats, the sciatic nerve was exposed proximal to the branching of the sural nerve and cut completely with sharp scissors. The distal stump was immediately aligned with the proximal stump using surface landmarks such as blood vessels and connective tissues as a rough guide; the two stumps were then secured using fibrin glue (English 2005).

Immediately after surgery, the rat was placed under a heating lamp and given an analgesic (Demerol, 0.2 mg, administered intramuscularly). Once awake, it received a second dose of analgesic and was returned to its cage and given free access to food and water.

Each rat lived in a standard rat cage with a 40-cm flexible cable attached to the skull-mounted connector. The cable, which allowed the animal to move freely in its cage, conveyed the wires from the electrodes to a commutator above the cage that connected to EMG amplifiers and a nerve-cuff stimulation unit. Each rat had free access to water and food throughout the experiment. Animal well-being was carefully checked several times each day and body weight was measured weekly. Laboratory lights were dimmed between 2100 and 0600 h daily.

In the two rats in which electrodes were implanted before sciatic nerve transection and repair, spontaneous EMG activity and evoked responses were recorded for >10 days before transection surgery and immediately after the animal recovered from anesthesia. In the other four rats, data collection began 1–2 days after the electrode implantation–nerve transection surgery. In five animals, EMG recording continued for ≥120 days after nerve transection. In one SOL–GAS rat, recording was stopped 67 days after nerve transection as the result of skull connector failure.

A computer-based data-acquisition system continuously monitored SOL and GAS or TA spontaneous (i.e., ongoing) EMG activity and controlled the nerve-cuff stimulus, 24 h/day, 7 days/wk, for the entire experiment. It sampled ongoing SOL and GAS or TA EMG activity at 5 kHz and calculated its absolute value (equivalent to full-wave rectification). Nerve stimulation was delivered and evoked responses were recorded when the absolute value of the ongoing SOL EMG activity remained within a defined range. This range was set daily based on each rat's spontaneous EMG activity levels so that each animal received about 9,000 stimuli a day. Thus the background EMG activity (i.e., the EMG activity level at the time of nerve stimulation) necessarily differed pre- and postnerve transection and during the course of subsequent recovery. When the absolute value of the background SOL EMG activity remained within range for a randomly varying 2.3- to 2.7-s period, the computer saved the most recent 100 ms (i.e., the background EMG activity interval) of background EMG activity from both implanted muscles and delivered a stimulus pulse (usually 0.5 ms, but 0.1 ms if the 0.5-ms pulse produced a large stimulus artifact) to the tibial nerve cuff. The computer then collected and saved EMG activity from both implanted muscles for another 200 ms. The prestimulus 100 ms and poststimulus 200 ms of recorded EMG activity constituted a single trial. The prestimulus background EMG activity time requirement of ≥2.3 s combined with the poststimulus interval and processing time ensured that the shortest possible interstimulus interval was 2.75 s.

Before nerve transection in the two rats previously implanted with stimulating and recording electrodes, pulse amplitude was adjusted by the computer after each trial to maintain the M-response amplitude associated with a maximum H-reflex. After nerve transection and when the evoked response had disappeared, all rats were tested for about 15 min each day using tibial nerve stimulation at an intensity three- to fivefold that sufficient to elicit an EMG response in a normal animal. (There was no evidence of discomfort with this stimulus in the animals with nerve transection.) Once an EMG response could be elicited (i.e., 2–3 wk after sciatic nerve transection), our standard computer-controlled nerve-cuff stimulation protocol was enabled (Chen and Wolpaw 1995, 2002, 2005; Wolpaw and Chen 2006). That is, the computer adjusted the stimulus after each trial to maintain the average EMG amplitude at the time of the M response at 30–50 μV above the background EMG level.

At the end of study, each rat received an overdose of sodium pentobarbital (ip) and was perfused through the heart with saline followed by 3% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3), so that the spinal cords could be harvested for use in another study. The integrity of the EMG electrodes, nerve cuff, and tibial nerve were examined and the SOL muscles from both sides were removed and weighed.

RESULTS

Animals remained healthy and active throughout data collection. Body weight increased from 338 to 476 g at the time of implantation surgery to 537–665 g at the time of perfusion.

In each rat, the nerve cuff remained at the location in which it had been implanted and was covered by connective tissue. The tibial nerve was well preserved inside the cuff. However, the weight of the SOL muscle from the transected side [mean = 0.19 (±0.05 SD) g] was significantly less ($P < 0.01$ by paired *t*-test) than that from the intact side [mean = 0.25 (±0.04) g].

Spontaneous EMG activity

Spontaneous EMG amplitude decreased rapidly after sciatic nerve transection in all three muscles. The time course of this reduction and its subsequent recovery were most easily observed in the two SOL–GAS rats implanted with EMG wires before nerve transection surgery. The average daily SOL EMG amplitude (as a percentage of its pretransection 10-day average amplitude) is shown in Fig. 1*A* for a rat during the10 days before and 120 days after nerve transection. Activity in SOL dropped dramatically after the nerve transection, began to return about 3 wk after transection (Fig. 1*A*, arrow), and rose to near pretransection levels in about 60 days. Activity remained slightly below the pretransection level (roughly 80% of the pretransection level in this example; Fig. 1*A*, dashed line) for 3–4 mo after the nerve transection.

Similar results were observed in the other animals. Average (±SE) daily spontaneous SOL EMG amplitude (as a percentage of each animal's final 10-day average EMG amplitude) is shown for all six rats during 120 days after the nerve transection in Fig. 1*B*. Activity in SOL increased until about 90 days after nerve transection (Fig. 1*B*, arrow). The rate of recovery was slow initially: the increase was only about 20% during the first 30 days. Recovery subsequently accelerated: the increase was about 50% in the second 30 days. Spontaneous EMG activity continued to increase more gradually, gaining about 20% in the third 30 days before reaching an apparent plateau.

Average (± SE) spontaneous EMG activity for GAS (filled circles, four rats) and TA (open squares, two rats) as a percentage of the final 10-day average amplitude is shown for each 5-day period during the 120 days after nerve transection in Fig. 1*C*. Activity in GAS and TA, like that of SOL, increased for about 90 days after the nerve transection.

Evoked EMG responses

Stimulation of the posterior tibial nerve in an awake rat with an intact sciatic nerve typically elicits an M response in SOL and GAS at a latency of about 2.0 to 4.5 ms and an H-reflex at a latency of about 6 to 10 ms (Chen and Wolpaw 1995). Similar results were observed in the two rats in which chronic electrodes were implanted before sciatic nerve transection (see example in Fig. 2).

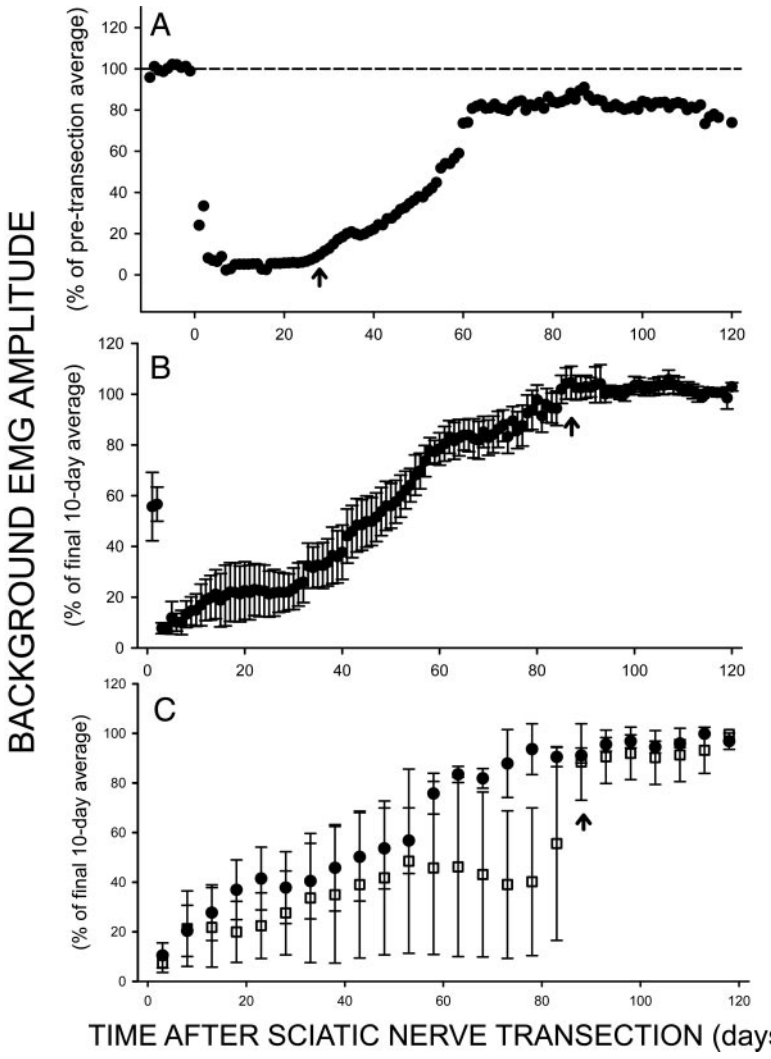

FIG. 1. Recovery of spontaneous electromyographic (EMG) activity after sciatic nerve transection and repair. *A*: average daily soleus (SOL) EMG activity (as a percentage of the pretransection 10-day average) for one rat during 10 days before and 120 days after sciatic nerve transection. Background SOL EMG activity virtually disappears by 2 days after nerve transection, begins to recover 3 wk after transection (arrow), and recovers to near pretransection level by about 60 days. *B*: average (±SE) daily SOL EMG activity (as a percentage of the final 10-day average) for all 6 rats during 120 days after nerve transection. Arrow points to the time of maximal restitution of spontaneous EMG activity. *C*: average (±SE) gastrocnemius (GAS, filled circles, 4 rats) and tibialis anterior (TA, clear squares, 2 rats) EMG activity (as a percentage of the final 10-day average) for each 5-day period for 120 days after nerve transection. Arrow points to the time of maximal restitution of spontaneous EMG activity.

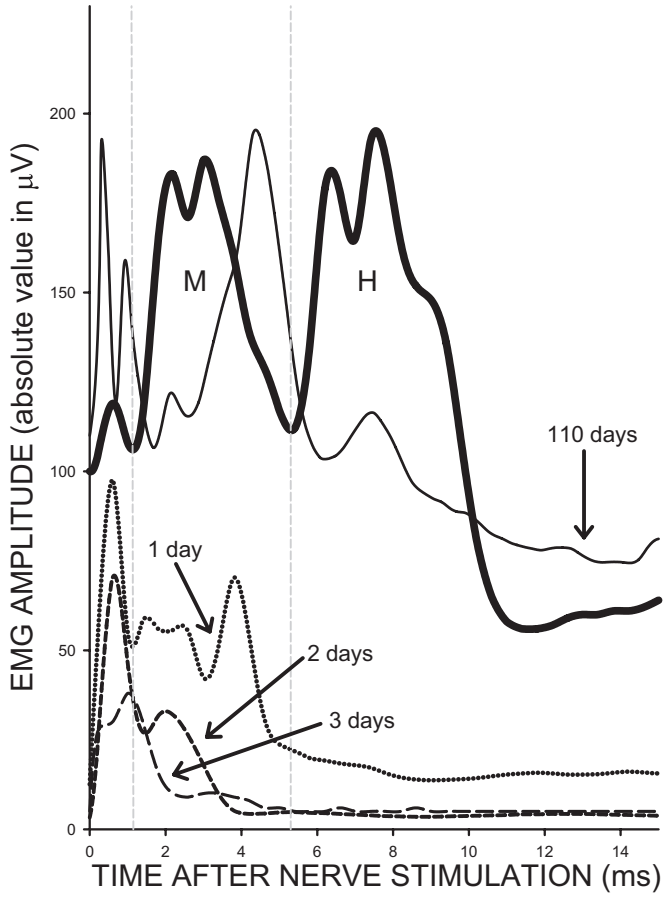

FIG. 2. Average daily poststimulus SOL EMG activity before and after sciatic nerve transection and repair in one rat. Pretransection evoked response (*thick black trace*) consists of a short-latency M response (labeled M) and a longer-latency H-reflex (labeled H). These M- and H-response intervals are delineated by the vertical dashed lines. Portion of each trace to the *left* of the vertical dashed lines is the stimulus artifact. Resting EMG activity in SOL decreases and the SOL H-reflex disappears immediately after transection. An M response persists for up to 2 days (*dashed traces*). By 110 days after transection (*thin solid trace*), the level of resting EMG activity recovers to close to that before transection (compare starting times of *thick* and *thin solid traces*) and a putative direct muscle (M) response can be evoked that is similar in amplitude to the M response recorded preoperatively. A longer-latency response (the putative H-reflex) can be recorded but its amplitude is much smaller than the H-reflex amplitude before transection.

Posterior tibial nerve stimulation elicited M responses that could be recorded as soon as the animals recovered from nerve transection and repair surgery. The amplitude of these M responses diminished and eventually disappeared during the next 3 days in all animals. The average daily evoked responses from the SOL muscle in one rat before and after nerve transection are shown in Fig. 2. Activity within the M-response interval (between the two vertical dotted lines in Fig. 2) decreases and nearly disappears over the next 3 days, even though the stimulus intensity during these days increased about 30%.

Also shown in Fig. 2 is that sciatic nerve transection causes an immediate loss of the SOL H-reflex. This did not arise from inadequate stimulation because the M response was evident for 2 days after the nerve transection before disappearing.

Responses to stimulation of the posterior tibial nerve started to reappear 2–3 wk after sciatic nerve transection and repair. Two temporally distinct groups of responses emerged during the recovery period. The first group appeared 23 days (±4 SD; range 19–28) after sciatic nerve transection in SOL muscle and 26 days (±5; range 20–29) after sciatic nerve transection in GAS muscle and appeared in all rats studied. During the first week of their appearance, variable latencies to peak amplitude were found in these evoked responses, ranging from about 3 to 10 ms. During the subsequent 2 wk, the latency-to-peak amplitude of these responses decreased to a range of 2–7 ms. These responses presumably were the direct response of efferent fibers to the nerve stimulation (i.e., a putative M response) and indicated muscle reinnervation.

The second group of evoked responses was observed in four of the six rats studied. They started to appear 32–70 days after the nerve transection. These responses had longer and more variable latencies, with latencies to peak amplitude in a range from about 8 to 15 ms. These late components are likely to reflect (at least in part) a spinally mediated response component (i.e., a putative H-reflex). Although the time course and the magnitude of recovery of the responses varied across animals, the recovery always started as small responses at latencies much longer than those found in intact rats (Fig. 3, arrows). Over time, the latencies of these responses shortened until a single response at a fairly constant latency was observed (Fig. 3: 43 days) that was distinct from the shorter-latency responses (putative M responses).

Responses recorded from SOL (*left*) and GAS (*right*) in response to posterior tibial nerve stimulation are shown in Fig. 4 for one rat 50 days after sciatic nerve transection and repair. Recovery of the long-latency part of the response (the putative H-reflex) is modest. In the two rats in which pretransection data were collected, the amplitude of the second response 110 days after nerve transection was ≤10% of that of the pretransection H-reflex (see example in Fig. 2).

Recovery of TA responses

An unexpected finding was that in both of the SOL–TA rats, stimulation of the tibial nerve elicited responses not only in the SOL, but also in the TA. Responses of SOL and TA to the tibial nerve stimulation for select 10-day periods during 20–100 days after transection and surgical repair of the sciatic

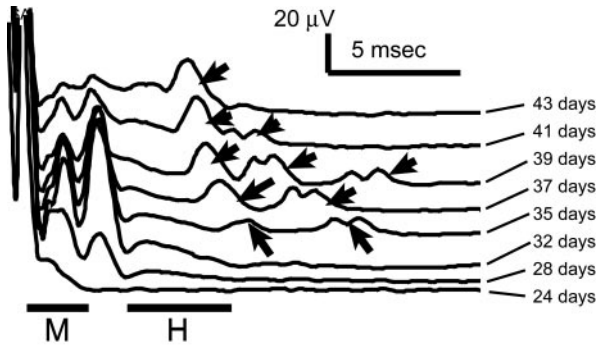

FIG. 3. Time course of recovery of SOL responses to tibial nerve stimulation. Individual traces in this figure have been offset slightly for purposes of clarity. Recovery of reflex activity is first observed as multiple small responses of varying latencies (arrows). Over time, only 2 distinct responses remain and their latencies shorten and their amplitudes increase. Short-latency component is a putative M response and the long-latency one is a putative H-reflex. Horizontal bars indicate the timing M response (M) and H-reflex (H) intervals of normal rats (Chen and Wolpaw 1995).

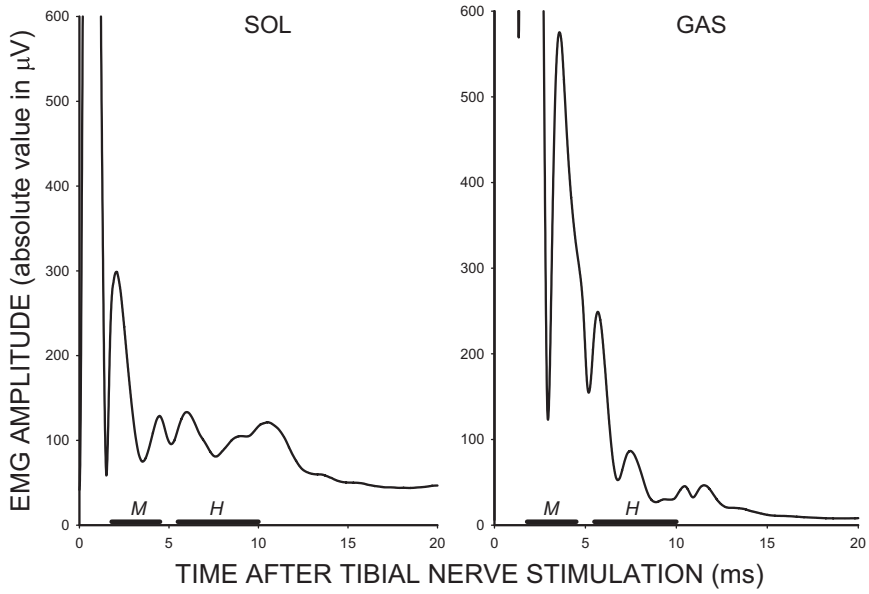

FIG. 4. Average daily poststimulus EMG activity showing SOL (*left*) and GAS (*right*) responses to tibial nerve stimulation in a rat 50 days after sciatic nerve transection and repair. Horizontal bars indicate the M response (M) and H-reflex (H) intervals of normal rats (Chen and Wolpaw 1995).

nerve are shown in Fig. 5. A clear short-latency response appeared in SOL 21–30 days after the transection and repair, but no longer-latency response appeared in SOL until 31–40 days posttransection. In TA, a small short-latency response appeared 31–40 days posttransection, and the long-latency evoked reflex response appeared after 80 days. During the period from 40 to 80 days posttransection, the amplitudes of both responses increased in SOL, but not in TA. Dramatic increases in the TA responses occurred after 80 days. Although the stimulus pulse amplitude and the responses in SOL remained largely unchanged, the responses in TA increased almost 10-fold from 81 to 90 days to 91 to 100 days posttransection. This implies that the EMG activity recorded from TA was not cross talk from SOL (and/or GAS), but was rather actual TA activity.

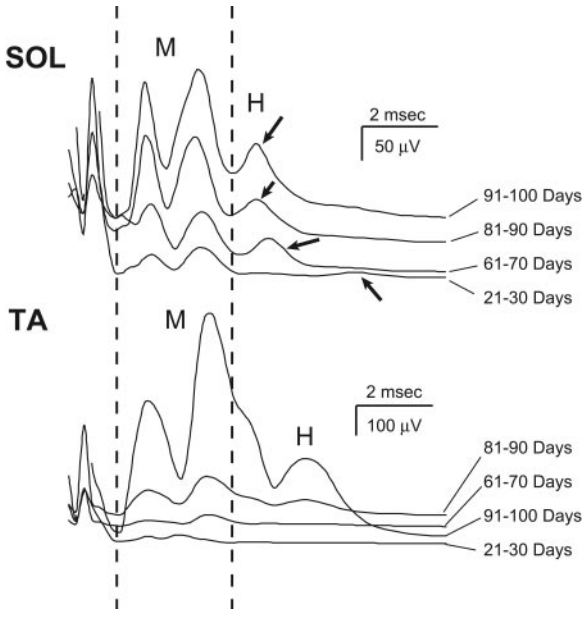

FIG. 5. Average SOL and TA EMG activity of a rat at select 10-day periods between 20 and 100 days after sciatic nerve transection and repair. *Top*: SOL short-latency (putative M) response begins to recover between 21 and 30 days posttransection and continues to increase in amplitude throughout the 100-day study period. Latency to a putative H-reflex (arrows) decreases dramatically and increases in amplitude over the same time period. *Bottom*: a mild direct response to posterior tibial nerve stimulation is found in TA beginning at 61 days and increases during the remainder of the experiment, especially after the direct response in SOL has already stabilized. An H-reflex was found in this muscle, beginning at 80 days postoperative, well after the H response in SOL had matured. Thus the increases in the amplitudes of the TA responses are not concurrent with the increase in the SOL responses. This implies that the TA response is mediated by aberrant reinnervation and is not merely cross talk from SOL or GAS.

DISCUSSION

In this study two electrically evoked short-latency EMG responses in muscles of unanesthetized rats were found beginning as early as 1 mo after transection and surgical repair of the sciatic nerve. The earlier responses (found in all six rats) and the later responses (found in four of six rats) appear to represent M responses and H-reflexes, respectively, although their latencies are slightly longer than those of M responses and H-reflexes in normal animals (Chen and Wolpaw 1995). Similar longer latencies were reported for restored H-reflexes by others in the self-reinnervated muscles of anesthetized rats (Valero-Cabre and Navarro 2001).

The most novel aspect of this study is the view it provides of the redevelopment of the reflex. By continuous monitoring of EMG activity throughout the 4-mo posttransection period, we were able to detect the initial return of the H-reflex and follow its subsequent development. The first detection of a putative reflex response to stimulation of the posterior tibial nerve occurred at least 1 wk after return of a putative M response. The response was first observed as a series of small evoked potentials at varying latencies after stimulation, all of which were longer than that of the H-reflex in intact rats. Over time, the latencies of these responses shortened and the number of separate responses declined until a single reflex response was evident. We interpret these early EMG responses cautiously as the earliest evidence of the restoration of a reflex, but other possible explanations need to be considered. The most likely alternative explanation is that antidromic activation of motoneurons by our test stimulus resulted in a slightly later second discharge of those motoneurons that we recorded as a long-

latency EMG response. In the human EMG literature, such responses are known as F waves (e.g., Lomen-Hoerth and Aminoff 1999). In human muscles, F waves are generated only at relatively high stimulus intensities and even then their nature is variable and they occur infrequently. The stimulus intensity that we used to evoke EMG responses during reinnervation was purposefully kept small in an attempt to evoke a maximal H response. The EMG responses reported above each represent the averages of several thousand stimulus presentations. Thus generation of repetitive firing of motoneurons by our test stimulation would have to occur both at low-stimulus strengths and very consistently to be observed in our data. We did not stimulate at high intensity to look for the presence of F waves. Thus although we cannot rule out the possibility that single stimulus applications to regenerating axons of very excitable motoneurons might result in F waves, we think that this explanation for our putative H-reflex is highly unlikely.

Although the amplitude of the M response returned to near-normal values, the amplitude of the presumed H-reflex remained much smaller than its pretransection level. Thus restoration of the putative H-reflex after peripheral nerve injury seems to occur gradually and the form of the reflex changes considerably during this recovery. This progressive recovery presumably reflects both axon regeneration in the periphery and restoration of functioning afferent synapses in the spinal cord.

It is well established that the regeneration of axons in peripheral nerves is not temporally synchronous, but rather is dispersed over a period of several weeks (Al-Majed et al. 2000; Brushart et al. 2002; English 2005). If the sensory axons forming the afferent limb of the H-reflex are among those axons regenerating later, they might not reach the stimulation site on the posterior tibial nerve until after earlier regenerating motor axons had reinnervated muscle fibers. It is not known whether afferent axons regenerate more slowly than motor axons or even whether the low threshold afferent axons associated with the H-reflex regenerate more slowly than axons not contributing to the short -latency reflex response. In addition, the conduction velocities of regenerating axons are slower than those of intact axons. Over time these velocities increase and return to near-normal values (Foehring et al. 1986; Haftel et al. 2005). If the restoration of conduction velocities were also dispersed in time, this could contribute to both the longer latencies of the restored H-reflexes and to the multiple small responses seen early in the restoration period. Further study will be needed to determine whether the magnitude of any temporal heterogeneity in restoration of axon conduction velocities is sufficient to explain the temporal features of H-reflex restoration.

After transection of peripheral nerves, synapses on the somata and proximal dendrites of the axotomized motoneurons are altered. Processes of glial cells were previously observed interposed between pre- and postsynaptic elements (Alvarez et al. 1997; Brannstrom and Kellerth 1998). When axons in the cut nerve have regenerated and reinnervated muscles, these disrupted synapses are reformed (Brannstrom and Kellerth 1999). However, the density and locations of the restored synapses are not identical to those found in controls, suggesting that the communication between afferent neurons and motoneurons is altered. The precise time course of restoration of synapses onto motoneurons after peripheral nerve injury is not well established, but it has been suggested that the restoration of central connectivity occurs only after muscle reinnervation by regenerating motoneurons (Sumner and Sutherland 1973). Thus one might expect that the reappearance of an M response would precede the reappearance of an H-reflex. Temporal dispersion in the reformation of synaptic connections between sensory axons and motoneurons in the spinal cord that occurs in parallel with the temporally dispersed regeneration of peripheral processes of sensory axons (see above) could contribute to both the longer latencies and the temporal segmentation of the restored reflex found early in the regeneration process.

An issue of some importance is whether the stimulation of the tibial nerve that was used to test for EMG responses might have contributed to the finding of a small putative H-reflex. Electrical stimulation of the proximal stump of cut peripheral nerves has been used to promote enhanced axon regeneration (Al-Majed et al. 2000; English 2005). However the pattern of stimulation (20 Hz continuously) used in these experiments was much more intense than our test stimulation, which was regulated to be no more rapid than 0.3 Hz, and in fact occurred at an average rate of <0.1 Hz over any 24-h period. In addition, electrical stimulation has been observed to shorten the period over which axons regenerate, thereby compressing the period of temporally staggered growth (Al-Majed et al. 2000; English 2005). We did not observe such an effect, either on the time course of restoration of resting EMG activity or the evoked EMG responses. In fact the prolonged time course of restoration of activity that we observed is compatible with the temporally prolonged regeneration observed by others. Thus although we cannot entirely rule out an effect of the test stimulation on axon regeneration, we believe that it was highly unlikely.

Even though we were able to demonstrate putative H-reflex restoration in most animals studied, and even though a putative M response of near-normal amplitude could be recorded in all rats, the amplitude of this putative H response at all stages of recovery studied was significantly smaller than the amplitude of the H-reflex recorded either before nerve transection in the two rats of this study or in intact rats in previous studies (Chen and Wolpaw 1995, 1997, 2002, 2005; Chen XY et al. 2003b; Chen Y et al. 2005; Wolpaw and Chen 2006; Wolpaw et al. 1993). The ability of posterior tibial nerve stimulation to evoke a reflex response clearly decreases. This decline in efficacy could be the result of rewiring of the peripheral and/or central circuitry underlying the reflex. After transection and surgical repair of the mouse sciatic nerve, $\geq$20% of the reinnervation of muscles is by motoneurons that had formerly innervated functionally antagonistic muscles (English 2005). The extent to which sensory axon regeneration in the sciatic nerve might also be topographically inappropriate is unknown, but Brushart and colleagues (2005) showed that as many as 60% of muscle afferent axons in the cut femoral nerve are routed to cutaneous targets on regeneration. This amount of loss of sensory and motor neuromuscular specificity might be expected to substantially reduce H-reflex amplitude.

In the CNS, changes in the extent and organization of synapses onto motoneurons after nerve injury (Brannstrom and Kellerth 1999) will affect motoneuron function. The best studied such effect is on the stretch reflex. In long-term self-reinnervated muscles after transection and surgical repair

of peripheral nerves, Cope et al. (1994) first showed that reflex responses to stretch were diminished or lost. The functional consequences of this loss of ongoing proprioceptive feedback are profound (Abelew et al. 2000). Although regeneration of axons in the cut nerves is sufficient to restore motor unit and muscle contractile properties (Foehring et al. 1986), muscle spindle reinnervation (Haftel et al. 2005; Wolf and English 2000), effective coding of length information by stretch-sensitive afferent neurons (Haftel et al. 2005; Johnson et al. 1995), and electrically evoked postsynaptic potentials in the previously axotomized motoneurons (Collins et al. 1986; Haftel et al. 2005), stretch-evoked excitatory postsynaptic potentials could not be recorded from these motoneurons. This led Cope and colleagues (Haftel et al. 2005) to postulate that the loss of the stretch reflex in long-term self-reinnervated muscles is the result of a central inhibition of the reflex pathway. The small amplitude of the restored H-reflex in our study might be attributable to such central inhibition, but at present this interpretation is speculative. It is possible that increased central inhibition might arise as an adaptive response to the change (i.e., loss) in specificity of the regenerating axons.

We interpret our finding that large M responses could be evoked in both SOL and TA in two rats by stimulating regenerating axons in the posterior tibial nerve to mean that some motor axons had reinnervated both of these functionally antagonistic muscles. Because a robust evoked EMG response was found in SOL but not TA at postoperative times shorter than 80 days, we believe that an implication of our findings is that these branches of regenerating motoneurons had established significant numbers of synaptic contacts with TA muscle fibers only after they had already formed functional connections with SOL muscle fibers. We think that an alternate explanation of our finding, that it results from activation of newly regenerating TA (only) motoneurons by passive spread of stimulus current to the common fibular nerve, is unlikely. Our test stimulus intensity was intentionally kept very small, both in an attempt to evoke a maximal reflex response and to avoid passive current spread, and the stimulating cuff on the tibial nerve was observed in postmortem analysis of these animals to be firmly attached to the tibial nerve, so that its position was unlikely to have changed after 80 postoperative days. Using electrophysiological methods similar to ours, others (Evans et al. 1991; Valero-Cabre et al. 2004) provided evidence for the branching of the same regenerating axons from the injured sciatic nerve into both tibial and common fibular branches. Using retrograde fluorescent labeling, a small proportion of motoneurons were labeled from both nerves at $\leq$90 days after sciatic nerve injury (Valero-Cabre et al. 2004). However, whether this amount of axon branching could account for the observations we have made in two rats is not clear. We cannot rule out the possibility that such passive current spread contributed to the responses we observed. A firm resolution of this issue will have to await the results of study of more animals that also includes retrograde labeling experiments.

These observations on the restoration of reflex responses after different forms of nerve injury contrast with those of Valero-Cabre and Navarro (2001). Although these authors also found that restored EMG responses to nerve stimulation had longer than expected latencies, they reported a single H-reflex potential at the earliest times after nerve injury that they were able to evoke an M response. The earliest time at which they first reported a restored M response (and H-reflex) (30 days) is similar to the time course we observed for a restored reflex (32–70 days), but well after the earliest time that we first observed a restored M response (19 days). This difference in results presumably reflects the difference in recording protocol. By continuously monitoring resting EMG, we were able to observe the process of evoked response recovery in more detail than in studies using intermittent EMG recordings. This allowed us to detect response restoration at earlier times and to distinguish the time course of motor and reflex recovery. Another difference between our study and that of Valero-Cabre and Navarro (2001) was that in their study the restored H-reflexes were significantly larger than those recorded preoperatively. This difference could be a manifestation of the different conditions under which the evoked responses were recorded, in that our observations were made in awake, behaving animals, whereas theirs were made under general anesthesia.

H-reflex amplitude can be substantially increased or decreased using operant conditioning methods (Chen and Wolpaw 1995). Such conditioning could be used to treat the functional consequences of the disordered peripheral specificity associated with axonal regeneration and reinnervation. That is, operant conditioning might be used to increase H-reflexes in agonist muscles (i.e., the muscles normally innervated by the nerve being stimulated) and/or to decrease them in antagonist muscles. However, this approach would be possible only if H-reflexes are present after nerve regeneration. We report here that a peripherally evoked reflex is often restored after transection and surgical repair of the rat sciatic nerve; that its reappearance is not noted until well after the restoration of muscle fiber reinnervation; and that even though it returns initially as a series of small responses, it then coalesces into a recognizable single response. These encouraging findings provide an impetus for developing operant conditioning-based interventions that focus on restoring more normal spinal reflex specificity after peripheral nerve injury and subsequent regeneration.

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