Development/Plasticity/Repair

Central Suppression of Regenerated Proprioceptive Afferents

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Long after a cut peripheral nerve reinnervates muscle and restores force production in adult cats, the muscle does not respond reflexively to stretch. Motivated by the likelihood that stretch areflexia is related to problems with sensing and controlling limb position after peripheral neuropathies, we sought to determine the underlying mechanism. Electrophysiological and morphological measurements were made in anesthetized rats having one of the nerves to the triceps surae muscles either untreated or cut and immediately rejoined surgically many months earlier. First, it was established that reinnervated muscles failed to generate stretch reflexes, extending observations of areflexia to a second species. Next, multiple elements in the sensorimotor circuit of the stretch reflex were examined in both the PNS and CNS. Encoding of muscle stretch by regenerated proprioceptive afferents was remarkably similar to normal, although we observed some expected abnormalities, e.g., increased length threshold. However, the robust stretch-evoked sensory response that arrived concurrently at the CNS in multiple proprioceptive afferents produced synaptic responses that were either smaller than normal or undetectable. Muscle stretch failed to evoke detectable synaptic responses in 13 of 22 motoneurons, although electrical stimulation generated monosynaptic excitatory postsynaptic potentials that were indistinguishable from normal. The ineffectiveness of muscle stretch was not attributable therefore to dysfunction at synapses made between regenerated Ia afferents and motoneurons. Among multiple candidate mechanisms, we suggest that centrally controlled neural circuits may actively suppress the sensory information encoded by regenerated proprioceptive afferents to prevent recovery of the stretch reflex.

Key words: **regeneration; plasticity; synapses; spinal cord; motoneurons; primary afferents; muscle spindles; reinnervation**

Introduction

Skeletal muscle reinnervated by a cut muscle nerve does not regain the ability to initiate a stretch reflex many months after recovery of force generation (Cope and Clark, 1993; Cope et al., 1994; Huyghues-Despointes et al., 2003). Loss of the stretch reflex, even when restricted to only a few muscles, is associated with deficits in limb movement, including reduction in weight support and loss of interjoint coordination in locomoting cats (Abelew et al., 2000). Loss of sensory feedback from muscle stretch is also likely to result in altered proprioception and in problems with controlling limb position observed in human subjects with large fiber neuropathies (Rothwell et al., 1982; Sainburg et al., 1995).

Mechanisms underlying stretch areflexia are not yet established, although previous studies limit the possibilities. There is ample evidence that cut motor axons can reinnervate muscle and extensively restore force production (Gordon and Stein, 1982; Gordon, 1987). Motoneurons themselves recover the normal range of excitability (Foehring et al., 1986) and fire reflexively in response to activation of uninjured sensory afferents (Cope and Clark, 1993). In contrast with the nearly complete motor recovery, there is only partial recovery in sensory encoding of muscle stretch. Abnormalities reported for spindle afferents in response to stretch of a reinnervated muscle include firing rates that are slower at the peak of ramp stretch and/or slower or unsustained during static stretch (Brown and Butler, 1976; Gregory et al., 1982; Banks and Barker, 1989; Lewin and McMahon, 1991). These and other aberrations (Decherchi et al., 2001) focus attention on incomplete recovery of proprioceptive feedback as the basis for stretch areflexia.

Nonetheless, many afferents supplying reinnervated muscle do respond to stretch, and some of them generate approximately normal responses (citations given above). It is surprising, therefore, that stretch not only fails to initiate reflex contraction but that it is also ineffective in modulating contraction initiated by other afferent or central pathways (Cope and Clark, 1993; Cope et al., 1994; Huyghues-Despointes et al., 2003). Thus, even when other sources of synaptic excitation bring motoneurons closer to or above firing threshold, muscle stretch is ineffective in both recruiting motoneurons to fire and altering their firing. These findings suggested to us that the CNS may contribute to areflexia by suppressing incoming sensory information.

It is currently not possible to assign relative importance to peripheral versus central mechanisms in causing stretch areflexia. One basis for uncertainty is that, for cut and regenerated afferents, there is little quantitative data on those response properties

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that are most relevant to generating the stretch reflex (Banks and Barker, 1989). Additionally, we are unable to find any reports on synaptic potentials produced by stretch of reinnervated muscle. To provide this information, we examined multiple sensorimotor elements of the stretch reflex for rat muscles long after reinnervation by their own severed nerve. The results suggest that mechanisms in the CNS limit recovery of the stretch reflex that should otherwise result from substantial recovery of proprioceptive feedback.

Materials and Methods

Data were collected from adult female Wistar rats as approved by the Emory University Institutional Animal Care and Use Committee. Two groups of rats were used in the terminal experiments described below. Rats in one group were anesthetized with pentobarbital (Nembutal; 35 mg/kg, i.p.) and subjected to a single survival surgery in which the nerve supplying the left medial gastrocnemius (MG) or the left lateral gastrocnemius-soleus (LGS) muscles was exposed through a skin incision, completely severed close to the muscle by scissors, and immediately rejoined by one suture of 10-0 ethilon. All muscle and skin incisions were closed by sutures, and the rats were returned to their cages until the terminal experiments performed 9–14 months later. Rats in the other group were untreated before the terminal experiments.

Terminal experiments. Physiological data were collected from each rat in one of three different kinds of experiments described below. Body temperature was monitored and maintained at 37°C by radiant heat, mean blood pressure monitored at the carotid artery exceeded 60 mmHg, and/or end-tidal CO_2 ranged from 20 to 30% throughout data collection. All rats were killed at the end of these experiments by barbiturate overdose (Nembutal; 150 mg/kg, i.p.).

Muscle stretch reflexes. The purpose of these experiments was to quantify deficits in the stretch reflex of reinnervated muscles. Reflex contraction was compared within each rat between the left, reinnervated MG muscle and the right unoperated MG muscle. The three rats used in these experiments had the nerve to the left MG muscle cut and surgically rejoined 9, 12, and 13 months earlier. Surgical preparation in the terminal experiment was performed with rats anesthetized by isofluorane (1.5–2% in room air). Each rat was fixed in a recording frame, and each femur and tibia was fixed rigidly to the frame. Both left and right MG muscle tendons of insertion were exposed through skin incisions, detached from the calcanei, and tied in series to a servomotor through a strain gauge. Suture ties in the MG tendon and on connective tissue near the ankle were positioned to line up at the MG muscle resting length (Lr) attained with knee and ankle joints at angles of ~90°. One pair of wire electrodes was inserted into each MG muscle to measure electromyographic (EMG) activity. The caudal cutaneous sural nerves in each limb were exposed through a skin incision, freed from surrounding tissue, and placed on bipolar hook electrodes. Next, a portion of the calvarium was removed, and all brain tissue was aspirated rostral to an intercollicular transection of the brainstem. Decerebration permitted removal of the gaseous anesthesia, thereby making it possible to obtain reflex contraction in response to muscle stretch.

Muscle force and EMG (each sampled digitally at 5 kHz and stored on computer for later analysis) were measured from MG muscles in response to ramp–hold–release stretches. Even for the unoperated MG muscle, stretch alone was often insufficient to yield any reflex contraction that was distinguishable from the passive response to stretch of the muscle, verified by the absence of any EMG response. In contrast, both MG muscles consistently contracted reflexively in response to electrical stimulation of the sural nerve at 100 Hz. These observations led us to the strategy of evoking MG muscle contraction by sural nerve stimulation just before and throughout four successive trials (4–5 s intervals) of muscle stretch (Fig. 1*A*, *C*). Under these conditions, the unoperated MG muscle plainly responded with additional reflex contraction during stretch, again as verified by EMG. Stretches 1 mm in amplitude and 20 mm/s in velocity were selected because these parameters yielded readily discernable reflex responses in the unoperated MG muscle.

Multiple trials of ramp–hold–release stretch presented alone or superimposed on sural reflexes were obtained from the unoperated and reinnervated MG muscles in each rat. Responses from both muscles were compared over similar ranges in background force, which was measured just before the transient stretch trials and which was attributable to a combination of the passive force of the muscle when held at Lr and the active force produced by the sural reflex. The magnitude of the stretch reflex was obtained by subtracting the passive response to ramp–hold–release stretch from the responses to stretches applied during the sural reflex (Fig. 1*B*). The passive stretch response used in these subtractions was taken from the average of several trials immediately preceding and after the stretch plus sural trials. It was determined, however, that individual passive responses were not constant in amplitude, and this factor

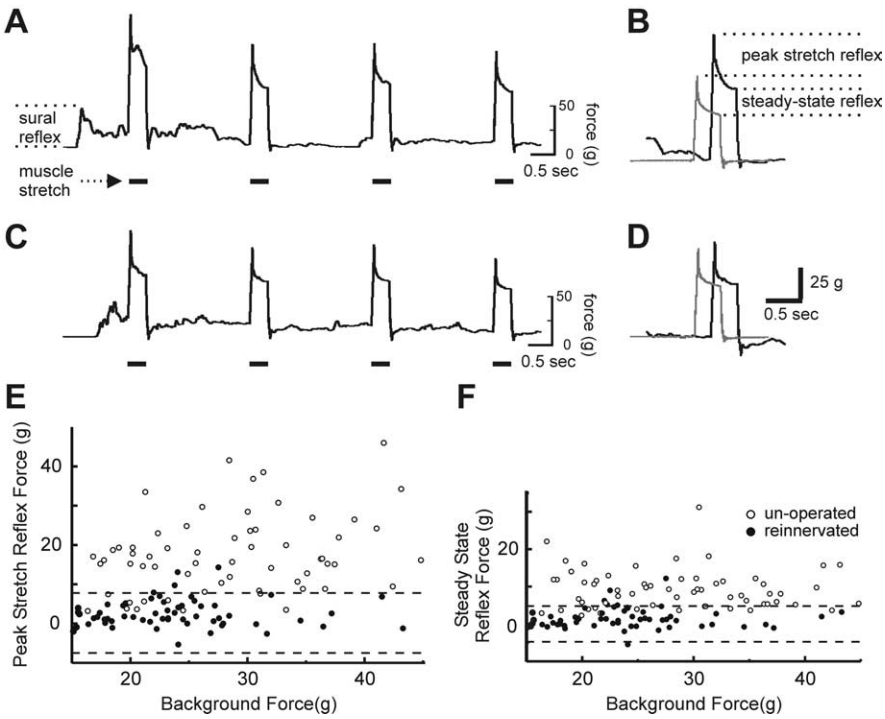

Figure 1. Regenerated proprioceptive afferents are ineffective in generating muscle stretch reflexes. Records of isometric force for the right, unoperated MG muscle (***A***, ***B***) and for the left, reinnervated MG muscle (***C***, ***D***) from one rat 13 months after nerve section and surgical reunion are shown. Traces in ***A*** and ***C*** show continuous force records obtained during four successive trials of ramp–hold–release stretch of the MG muscles. Black bars marking occurrence of stretch are aligned vertically at 0 g absolute force. Stretches applied during ongoing reflex contraction evoked by electrical stimulation of sural nerve (sural reflex) in both ***A*** and ***C***. Darker traces in ***B*** and ***D*** taken from the second stretch trial in ***A*** and ***C***, respectively; lighter traces are "passive" responses measured from separate trials in which stretch elicited no reflex. Subtraction of passive stretch responses from ones measured during sural reflex yielded net peak and steady-state reflex forces that were evident for unoperated MG muscles (***B***) but were not expressed by reinnervated MG muscle (***D***). Plots ***E*** and ***F*** show data taken from multiple (~20) stretch trials in unoperated and reinnervated MG muscles for each of three rats. Reflex forces at peak (***E***) and steady state (***F***) for each stretch trial are plotted against force measured just before the stretch trial (background force). Horizontal dashed lines delimit region on either side of 0 g force in which variation [greater for peak (***E***) than for steady-state (***F***) values] in passive stretch responses (lighter traces in ***B***, ***D***) introduces uncertainty in the occurrence of net reflex. Positive peak and steady-state reflex forces (values outside the limits of uncertainty) were typical for unoperated muscles (open circles) but rare for reinnervated muscles (filled circles).

introduced error in our measurements of stretch-reflex amplitude. This error was estimated from the maximum difference between the individual components making up the averaged passive response to stretch for both peak and steady-state values (Fig. 1*E*,*F*, horizontal dashed lines).

Neural innervation of muscle spindles. At the end of the experiments, the MG muscles were removed and weighed. Muscle wet weights for reinnervated and unoperated muscles, respectively, were 0.70 and 0.71 g, 0.83 and 1.03 g, and 0.90 and 1.17 g, indicating substantial reinnervation. Muscles were then placed in 4% paraformaldehyde solution for fixation, washed in sucrose solution, and cut on a cryostat into sections 100 μm thick. Sections were processed for immunohistochemistry for PGP (protein gene product) 9.5 to study the neural innervation of muscle spindles (Matsuo et al., 2000; Santiwong et al., 2002). Axons were labeled with a rabbit polyclonal antibody against PGP 9.5 (1:500; Biogenesis, Poole, UK). Labeling was visualized using fluorescein-conjugated secondary antibodies (1:100; Jackson ImmunoResearch, West Grove, PA). All sections were carefully scanned for fluorescent labeling of muscle spindles through an upright microscope equipped with a motorized stage. *z*-axis stacks of images at sequential focal planes (1 μm separation) were obtained from all detected muscle spindles. Images were captured using a spinning-disk confocal attachment to the microscope. Illustrated images show single plane views from three-dimensional reconstructions of the acquired stacks.

Afferent firing responses to muscle stretch. Another purpose of these experiments was to quantify the extent of recovery in afferent firing responses to muscle stretch. Data were collected in the present study from regenerated afferents supplying the left, reinnervated triceps surae muscles, MG, or LGS in 12 rats 12–16 months after nerve section and reunion. Comparisons were made against data from the left, unoperated MG or LGS muscles in 17 rats obtained in a previous study (Haftel et al., 2004) using identical methods. Briefly, rats were anesthetized throughout surgical preparation and data collection by pentobarbital (induced with 35 mg/kg, i.p., Nembutal and supplemented as needed to maintain deep anesthesia). Rats were fixed in the recording frame, and reinnervated MG or LGS muscles were attached to a servomotor as described above. With the exception of the nerve supply to the muscle under study, all other nerves in the hindlimb were severed. Laminectomy exposed the dorsal roots (L5 and L6) containing the axons of regenerated triceps surae afferents. Individual axons were penetrated with glass micropipettes (2 M K-acetate; 40–80 MΩ) in the dorsal roots to study their firing responses to various stimuli. First, we stimulated the peripheral nerve to initiate muscle twitch contractions during which we determined whether the firing rate of an afferent decelerated, as typically observed for spindle afferents, or accelerated, as normally expected for tendon-organ afferents (Matthews, 1972). Next, afferent firing was measured in response to muscle stretch. All stretches were superimposed on a resting muscle length held at Lr. In addition to the ramp–hold–release stretches applied as described in the section above, repeated trials of symmetric triangular stretch and release were applied as described by Haftel et al. (2004). Triangular stretches extended to 3 mm in length at constant stretch and release velocity of 4 or 16 mm/s. Records of afferent action potentials, muscle length, and muscle force were collected, digitized (22 kHz), and stored on computer for later analysis.

Synaptic potentials evoked in motoneurons by muscle stretch. These experiments were designed to test the synaptic responses of motoneurons under the same conditions in which we observed that stretch activation of regenerated muscle afferents virtually failed to elicit reflex contraction (Fig. 1). Data were compared from two groups of rats: six rats had the left MG nerve cut and surgically rejoined (see above) 9–12 months before the terminal experiment; seven rats were untreated before terminal experiments. Rats were made decerebrate, and the left MG muscle was attached to a servomotor following procedures described above. The lumbosacral enlargement of the spinal cord was exposed by laminectomy of vertebrae L4–L7 to provide recording access to the MG motoneuron cell bodies.

Intracellular records were measured from motoneurons penetrated by glass micropipettes (5–15 MΩ; 2 mM K-acetate) as described by Seburn and Cope (1998). MG motoneurons were distinguished by action potentials generated both antidromically after electrical stimulation of the MG nerve (2.5× threshold; 40 μs duration) and orthodromically in averaged traces recorded extracellularly in the MG nerve and evoked by current injection in MG motoneurons. Several measurements were taken from MG motoneurons for as long as recording quality permitted, i.e., when antidromic action potentials checked intermittently throughout data collection were larger than 65 mV. First, stretch-evoked synaptic potentials (SSPs) were measured in response to repeated trials (20 ms intervals) of ramp–hold–release muscle stretches with parameters identical to those used to study stretch reflexes (initial length at Lr, 1 mm stretches with 20 mm/s ramp and release, 1 s hold phase). Next, we measured rheobase current (current threshold for action potential, 50 ms square current pulses) and half-decay time of afterhyperpolarization (action potentials activated by 0.5 ms current pulses). Finally, EPSPs were produced by electrical stimulation of the MG nerve [1 pulse per second (pps)] at stimulus strengths just below motoneuron firing threshold and not exceeding 2.5× threshold for volleys recorded from dorsal roots. Deterioration of recording quality prevented acquisition of a complete set of parameters for some motoneurons. Records of motoneuron membrane potential, electrode current, and muscle length were collected, digitized (22 kHz), and stored on computer for later analysis. Synaptic responses were averaged to improve resolution.

Statistical comparisons were made throughout using one-way ANOVA applied to data sets pooled from rats with reinnervated versus unoperated muscles. Values are reported as means ± SD.

Results

Stretch reflex is virtually absent in reinnervated rat muscle

Reflex behavior of the reinnervated and the contralateral unoperated MG muscles was examined in each of three rats. Figure 1, *A* and *C*, shows that both reinnervated and unoperated MG muscles contracted reflexively in response to electrical stimulation of the sural nerve. Although not systematically studied, the force of sural reflex contraction for the reinnervated MG muscles was 63, 81, and 101% of the contralateral unoperated MG muscles. Therefore, the reinnervated MG muscles were capable of generating force, and the regenerated MG motoneurons were responsive to synaptic excitation from uninjured cutaneous afferents. Figure 1 shows that these conditions, although necessary, were not sufficient to restore the stretch reflex to reinnervated muscle.

Stretch was profoundly ineffective in either eliciting reflex contraction of a reinnervated muscle at rest or modulating ongoing contraction during a sural nerve reflex (Fig. 1*B*,*D*). Both peak and steady-state stretch reflex force measured over the same range of background force (passive and sural reflex forces combined) tended toward lower values for reinnervated than for unoperated muscles across multiple stretch trials in each of three rats (Fig. 1*E*,*F*). Group means were significantly smaller ($p < 0.0001$) for reinnervated than for unoperated MG muscles, respectively, both for peak reflex force (2.7 ± 3.6 vs 18.6 ± 12.1 g) and for steady-state reflex force (1.3 ± 3.2 vs 10.1 ± 7.9 g). For reinnervated muscles, reflex forces in the majority of stretch trials (56 of 60 for both peak and steady-state reflex force) fall between the horizontal dashed lines that delimit the range of measurement error over which we are not confident that any reflex was expressed (Fig. 1*E*,*F*). Thus, only ~5% of all stretch trials yielded any substantial reflex force in the reinnervated muscles.

The lower values of stretch reflex magnitude for reinnervated muscles were not attributable to saturation in force production. The total force generated by stretch reflexes superimposed on submaximal sural reflexes never exceeded the maximum reflex force recorded in separate tests of sural stimulation alone, and neither was the absence of stretch reflexes explained by a change in length dependence of the stretch reflex for reinnervated muscles: stretch reflex magnitude was not increased by altering the background muscle length (up to 3 mm longer or shorter than Lr). These results demonstrate that the stretch reflex is virtually

absent long after a cut nerve reinnervates its original muscle. In addition, the findings are comparable with those from cat (Abelew et al., 2000; Huyghues-Despointes et al., 2003), suggesting that the inability to recover stretch reflexes after nerve section can be generalized across mammalian species.

The studies described below were performed in an attempt to identify the mechanism(s) underlying stretch areflexia of reinnervated muscles. Properties of spindle afferents and stretch-evoked synaptic potentials in motoneurons are considered in turn.

Table 1. Spindle innervation in the MG muscle

	Unoperated MG muscle	Reinnervated MG muscle		
	Innervated spindles	Innervated spindles	ASEs present	ASEs absent
Rat 1	21	18	14	4
Rat 2	20	18	14	4
Rat 3	21	16	11	5

Data were taken from the three rats used in reflex studies (see Fig. 1). Nerves to the MG muscle were cut and immediately rejoined by suture in the left hindlimb and unoperated in the right 9–13 months before data collection. Values are the number of spindles innervated by neural processes stained positive for PGP 9.5. All spindles in unoperated muscles had annulospiral endings (ASEs) in the equatorial region of the spindle receptor (see Fig. 2*A*). Innervated spindles from reinnervated muscle had either annulospiral endings (see Fig. 2*B*) or no annulospiral endings (see Fig. 2*C*).

Recovery of proprioceptive afferents/receptors

Reinnervation of muscle spindle receptors

Muscle spindle morphology was examined in reinnervated and in unoperated MG muscles in the three rats used in the reflex study described above. Attention focused on annulospiral endings in the equatorial region of the spindles, and innervation by γ-motoneurons was not studied. For unoperated MG muscles, the number of spindles with annulospiral endings stained positive for PGP 9.5 matched the number reported previously for MG muscles in control rats (Table 1) (Werner, 1973; Sekiya et al., 1986). These annulospiral endings were formed by transverse terminal bands that wrapped around the equatorial region of intrafusal muscle fibers in the spindle receptor (Fig. 2*A*), and, in normal animals, these endings are generated by group Ia afferents (Barker, 1974). In the contralateral MG muscles that were self-reinnervated after nerve cut, we found that most muscle spindles (>75%) (Table 1) received a nerve supply (Banks et al., 1985; Ip et al., 1988). More than half of these spindles exhibited regular transverse bands of annulospiral endings in the equatorial region of intrafusal fibers similar to normal spindles (Table 1; Fig. 2*B*). A fraction of the spindles were abnormal in appearance, exhibiting only thin neural projections resembling free nerve endings (Table 1; Fig. 2*C*). These features of muscle spindles reinnervated after nerve section are documented in several previous reports (Banks et al., 1985; Ip et al., 1988; Banks and Barker, 1989; Dieler and Schroder, 1990; Dieler et al., 1992; Barker et al., 1993). Banks and Barker (1989) give evidence that regenerated annulospiral endings in the equatorial region of intrafusal fibers are likely produced by regenerated group Ia afferents, although reinnervation by other proprioceptive afferents, i.e., groups Ib and II, cannot be ruled out. We cannot be certain, therefore, about the origin of the spindle innervation, but we can conclude that stretch areflexia was not the result of a complete failure of the regenerating nerve to innervate muscle spindles.

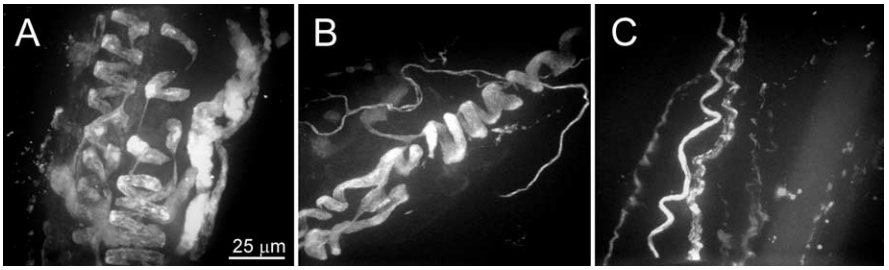

Figure 2. Reinnervation of spindle receptors. Immunolabeling (PGP 9.5) of neural innervation in the equatorial regions of spindle receptors sampled from MG muscles in one rat that were unoperated (***A***) or reinnervated (***B***) by the MG muscle nerve that was cut 13 months earlier (***B***, ***C***) is shown. Some spindles in the reinnervated muscle exhibited annuolspiral nerve endings with transverse bands similar to normal (***B***), whereas others had thin axons that failed to form normal spiral endings and that were not structurally associated with the intrafusal muscles fibers (***C***). Spindles were examined in unoperated and reinnervated MG muscles in all three rats used to study stretch reflexes (see Fig. 1) and were counted and categorized according to the three categories of spindles in ***A–C***. Note that the number of spindles receiving some form of innervation in the reinnervated muscle was 75% or more than that in the contralateral control muscle (Table 1).

Response properties of regenerated muscle spindle afferents

In a different set of 12 rats (see Materials and Methods), the firing of 40 single afferents was recorded from dorsal roots in response to stretch of reinnervated MG or LGS muscles. Figure 3 illustrates responses of nine different spindle afferents sampled from one reinnervated MG muscle. All afferents fired in response to muscle stretch. Most of the afferents (seven of nine) exhibited initial burst firing (high firing rates at stretch onset) that is typical of group Ia spindle afferents (Proske et al., 1993), and they fired throughout stretch, achieving peak rates ranging from 96 to 204 pps. Two afferents (conduction velocities of 72 and 43 m/s) had abnormally high length thresholds described previously for reinnervated spindles (see below). These observations give direct evidence that this sample of ~30% of the original number of group Ia and II spindle afferents (normally $n = 30$) (Zelena, 1994) generated distinct firing responses to stretch of the reinnervated muscle.

The total activity combined across multiple afferents formed a substantial population response to stretch, as shown in Figure 3*C*, in which the spikes produced by all nine afferents in one rat are summed into 50 ms bins. The number of spikes in all bins during stretch rose well above the background firing observed before stretch was applied (horizontal dashed line). It is important to emphasize that the afferent firing responses were measured in the dorsal roots, meaning that the stretch-evoked discharge from multiple afferents arrived concurrently up to the point of entering the CNS. Data presented next suggest that the population response was indistinguishable from normal.

Additional examination of the sensory encoding of muscle stretch focused on dynamic response properties, i.e., firing during the constant velocity rise in triangular stretches. Pooled data sets from rats with unoperated versus reinnervated muscles are compared in Figure 4. There was no significant group difference in the number of spikes and their average firing frequency during ramp stretch or in the instantaneous frequency at the onset (initial burst firing) or peak of stretch, whether for stretches that were 1 mm ($p > 0.5$ in all comparisons) or 3 mm in amplitude (data not shown). The proportion of afferents expressing initial burst firing was nominally smaller for rats with reinnervated (24 of 40, 60%) compared with unoperated (37 of 48, 77%) muscles, but

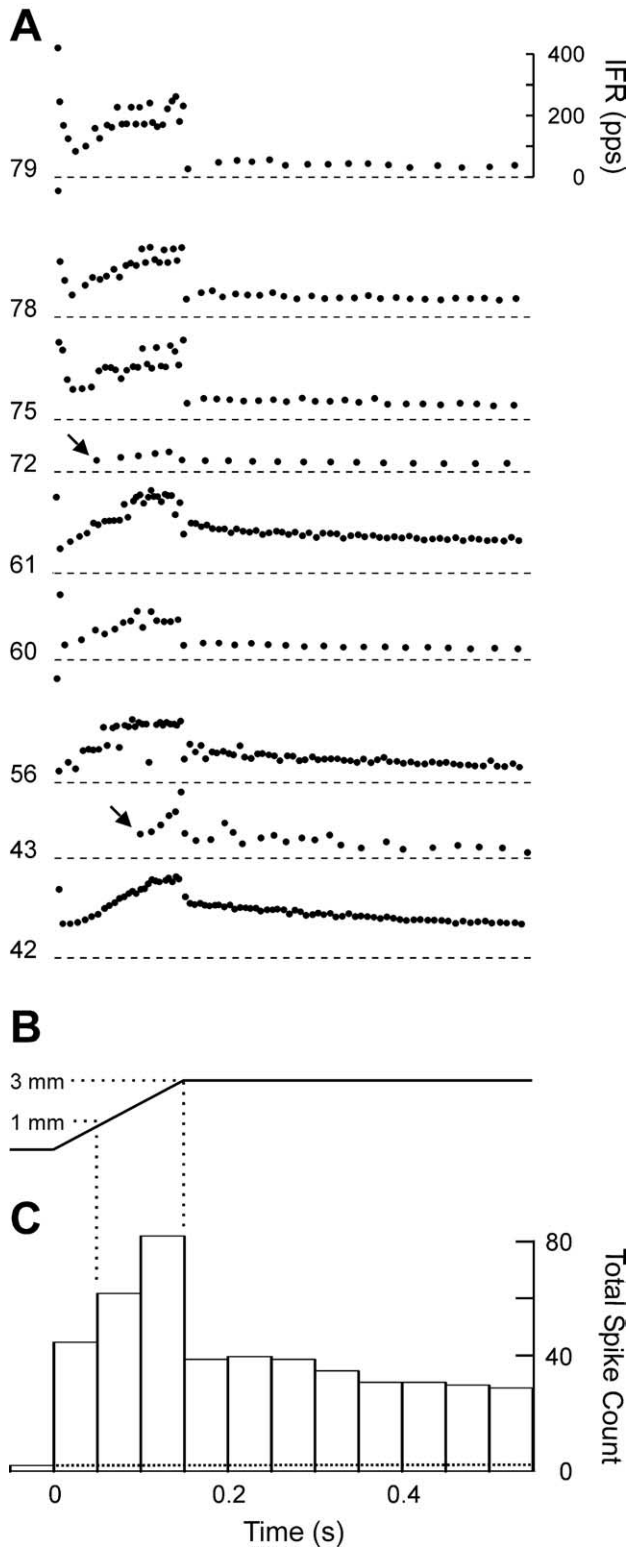

Figure 3. Multiple regenerated proprioceptive afferents generate vigorous firing responses to muscle stretch. The instantaneous firing rate (IFR) plotted versus time for each of nine afferents (***A***) in response to ramp–hold–release stretch (***B***) of one MG muscle studied 14 months after nerve section and surgical reunion is shown. Horizontal dashed lines identify boundary between afferents and numbers to left give axonal conduction velocity in meters per second. Arrows for two afferents indicate that firing begins at stretch lengths that are longer than normal. The histogram in ***C*** contains the total number of spikes in 50 ms bins from all nine afferents. One bin before stretch onset (time = 0) indicates the low level of firing before stretch (dashed horizontal line) found for only two afferents (data not shown in ***A***). Note that the total number of spikes for this sample far exceeds the background at both 1 and 3 mm stretch amplitudes.

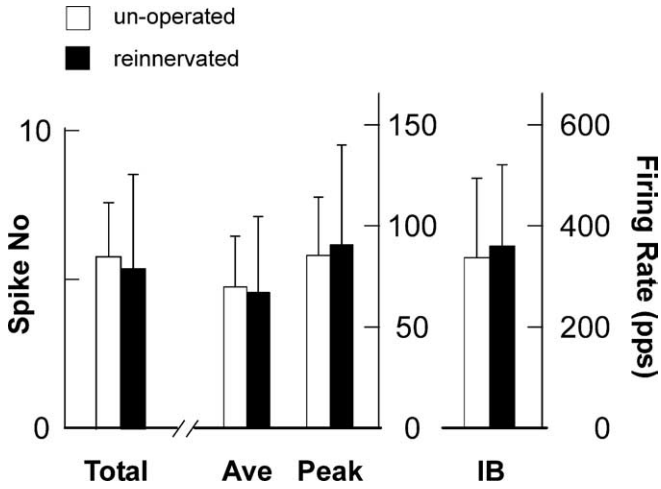

Figure 4. Dynamic responses recovered by regenerated spindle afferents. Means ± SDs for various firing properties measured during ramp stretch (dynamic responses) for spindle afferents ($n = 48$ and $n = 40$, respectively) pooled from rats with unoperated versus long-term reinnervated muscles are shown. Properties from left to right are total number of spikes (Total), average (Ave) and peak (Peak) firing rates, and maximal initial burst (IB) firing rates, all measured during rising phase of triangular muscle stretch (3 mm, 16 mm/s). No significant group differences ($p > 0.5$) were found.

these proportions were not significantly different (Fisher's exact test, two-tailed; $p = 0.106$).

Two of the primary parameters normally encoded by spindle afferents are muscle length and stretch velocity (Matthews, 1972), and Figure 5 illustrates that this encoding was comparable for unoperated and reinnervated muscles. Increasing muscle length from 1 to 3 mm increased afferent firing in both groups along a similar trajectory (Fig. 5*A*). Figure 5*B* shows similarity in the relationship between the rate of afferent firing and the rate of muscle stretch for both unoperated and reinnervated groups over a fourfold range of stretch velocity.

There were some differences in the response properties of spindle afferents from unoperated and reinnervated muscles. Figure 5*A* shows that four afferents from reinnervated muscles did not achieve firing threshold at 1 mm stretch (Fig. 5*A*, filled circles aligned vertically at 0 pps). This observation brought us to examine the length thresholds that were measurable for afferents that were not firing before transient muscle stretch (78 vs 42% of the samples for rats with reinnervated vs unoperated muscles, respectively). Similar to previous demonstrations (Gregory et al., 1982; Banks et al., 1985; Banks and Barker, 1989; Lewin and McMahon, 1991), length thresholds were significantly greater ($p = 0.0015$) for afferents from reinnervated (0.39 ± 0.65 mm; $n = 31$) than for ones from unoperated (0.05 ± 0.02 mm; $n = 20$) muscle. This increase in length threshold had little effect on the firing responses of afferents in rats with reinnervated muscles (Fig. 4). At stretch velocity of 16 mm/s, the time taken to stretch from 0.05 to 0.39 mm (the group difference in length threshold) is only 21 ms, which is time for the occurrence of only approximately one less action potential for afferents from reinnervated muscles firing at the average rate of 67 pps (Fig. 4, see average firing rate). This probably explains the slightly smaller total number of spikes and lower average firing rate during ramp stretch for afferents supplying reinnervated compared with unoperated muscle (Fig. 4). Reducing this group difference are the slightly higher peak and initial burst firing rates in rats with reinnervated muscles (Fig. 4). Figure 5*A* also shows that many afferents from

reinnervated muscle achieved rates similar to those from unoperated muscle by the time stretch reached 1 mm in amplitude.

Group differences in both length threshold and background firing might be explained by the incomplete recovery of γ-motoneuron influences on spindle afferents described in previous studies of reinnervated muscle (Brown and Butler, 1976; Gregory et al., 1982). To test this possibility, we eliminated all γ-motoneuron input to muscle spindles by sectioning the ventral roots acutely at the time of data collection in three rats with unoperated MG muscle nerves. Mean length threshold for five afferents from this group (0.17 ± 0.10 mm; $n = 5$) were significantly greater ($p = 0.0015$) than for unoperated rats with ventral roots intact but not significantly different ($p = 0.47$) from the length thresholds of afferents from reinnervated muscles (values given in previous paragraph). These observations are consistent with the idea that the increased length threshold of spindle afferents results from incomplete recovery of γ-motoneuron drive to reinnervated muscle spindles.

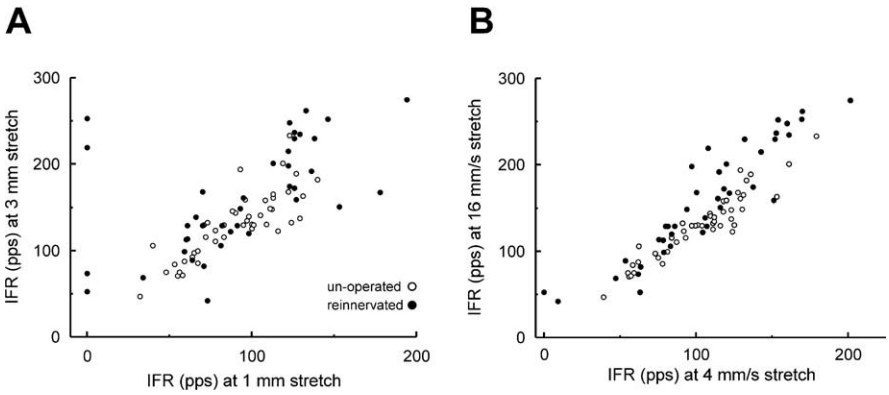

Figure 5. Sensory encoding of muscle length and stretch velocity recovered by regenerated spindle afferents. The instantaneous firing rate (IFR) for individual spindle afferents measured for stretch amplitudes of 3 versus 1 mm (***A***) and for stretch velocities of 16 versus 4 mm/s (***B***) is shown. Increases with length and velocity are comparable for spindle afferents in rats with unoperated and long-term reinnervated muscles.

Response properties of tendon-organ afferents

Tendon-organ afferents are relevant to the present study because they respond to muscle stretch and, through their synaptic influences on motoneurons, have the potential to influence the stretch reflex (Jami, 1992). Our sample from rats with reinnervated muscles included 17 afferents that accelerated firing during isometric muscle twitch and would be classified on this basis as tendon organ afferents. However, 7 of 17 of these afferents exhibited initial burst firing (Fig. 6*B*), which was never observed in our sample of tendon-organ afferents ($n = 24$) from normal muscles. We considered the possibility that these seven afferents with initial bursts were actually innervating muscle fibers, possibly spindle receptors, and were not tendon-organ afferents, based on strong evidence that the initial burst in afferent firing is imparted by properties of intrafusal muscle fibers (Proske et al., 1993). Consistent with this possibility, firing properties of these seven afferents (Fig. 6, gray bars) were not significantly different from the responses measured for spindle afferents in unoperated rats (Fig. 6, cross-hatched bars).

Figure 6*B* illustrates the similarity in stretch responses, including the initial burst, between a spindle afferent from an unoperated rat and an afferent provisionally classified as a tendon organ based on accelerated firing evoked by contraction of a reinnervated muscle. The response properties (average and peak rates) of the 10 afferents that accelerated firing during twitch contraction and that did not express initial burst firing were not significantly different from those from tendon-organ afferents in rats with unoperated muscles (Fig. 6*A*, white vs black bars, *B*, individual examples). These observations indicate that at least some tendon-organ afferents achieve good recovery of their normal responses to muscle stretch, although recovery appears incomplete for their responses to active contraction (Scott et al., 1996).

Stretch-evoked synaptic transmission is suppressed in the spinal cord

To examine the central synaptic effects of stretch-activated afferents, membrane potential was recorded intracellularly from MG motoneurons while stretching the MG muscle. The SSPs were recorded in response to ramp–hold–release stretches (Fig. 7*A4*) identical to those used in studying stretch reflexes (see above). In rats with unoperated MG muscles, SSPs had a distinctive profile (Fig. 7*A1*): after rapid depolarization at stretch onset, depolarization rose to a peak value at the end of ramp stretch and then fell to a lower steady-state level maintained throughout the hold phase of stretch (Westbury, 1972). Motoneurons that responded with larger peak SSP also tended to express larger steady-state values (Fig. 7*C*). Additionally, the peak SSP values for each motoneuron tended to increase as the amplitude to ramp–hold–release stretch increased from 1 to 3 mm (Fig. 7*D*).

The SSP from rats with reinnervated muscles differed sharply from those just described for unoperated muscles. Figure 7, *A2* and *A3*, illustrates the SSPs recorded from two different MG motoneurons in one rat with a reinnervated MG muscle. In one case, the SSP was small and not sustained throughout stretch (Fig. 7*A2*), and, in the other, no SSP was detected (Fig. 7*A3*). More than half of the motoneurons (13 of 22) yielded no SSP in response to 1 mm muscle stretch (Fig. 7*B*). In contrast, the failure to respond to muscle stretch was a rare occurrence in rats with unoperated muscles (1 of 16 motoneurons) (Fig. 7*B*). Among the nine motoneurons that generated an SSP at the peak of stretch, only four sustained steady-state depolarization (Fig. 7*C*). The peak SSP from rats with reinnervated muscles were on average ~12% the amplitude of those from rats with unoperated muscles in response to 1 mm stretch–hold–release (0.34 ± 0.55 vs 2.82 ± 1.87 mV, respectively; $p < 0.001$). Increasing ramp–hold–stretch amplitude from 1 to 3 mm (Fig. 7*D*) for 20 motoneurons in rats with reinnervated muscles increased SSP amplitude for nine motoneurons but failed to have any effect on nine motoneurons and actually produced inhibitory potentials (Fig. 7*A5*) in two motoneurons; the average SSP in response to 3 mm stretches for rats with reinnervated versus unoperated muscles were 0.48 ± 0.91 mV, $n = 18$ versus 4.19 mV ± 2.47, $n = 10$, respectively ($p < 0.001$). None of the SSP differences between rats with reinnervated versus unoperated muscles could be explained by group differences in the properties of the motoneuron samples; there were no significant group differences in the action potential amplitude ($p = 0.141$), conduction delay ($p = 0.433$), rheobase current ($p = 0.606$), or afterhyperpolarization half-duration ($p = 0.189$).

Recording stability permitted measurement of electrically evoked EPSPs from 7 and 15 MG motoneurons from rats with unoperated and reinnervated MG muscles, respectively. Electri-

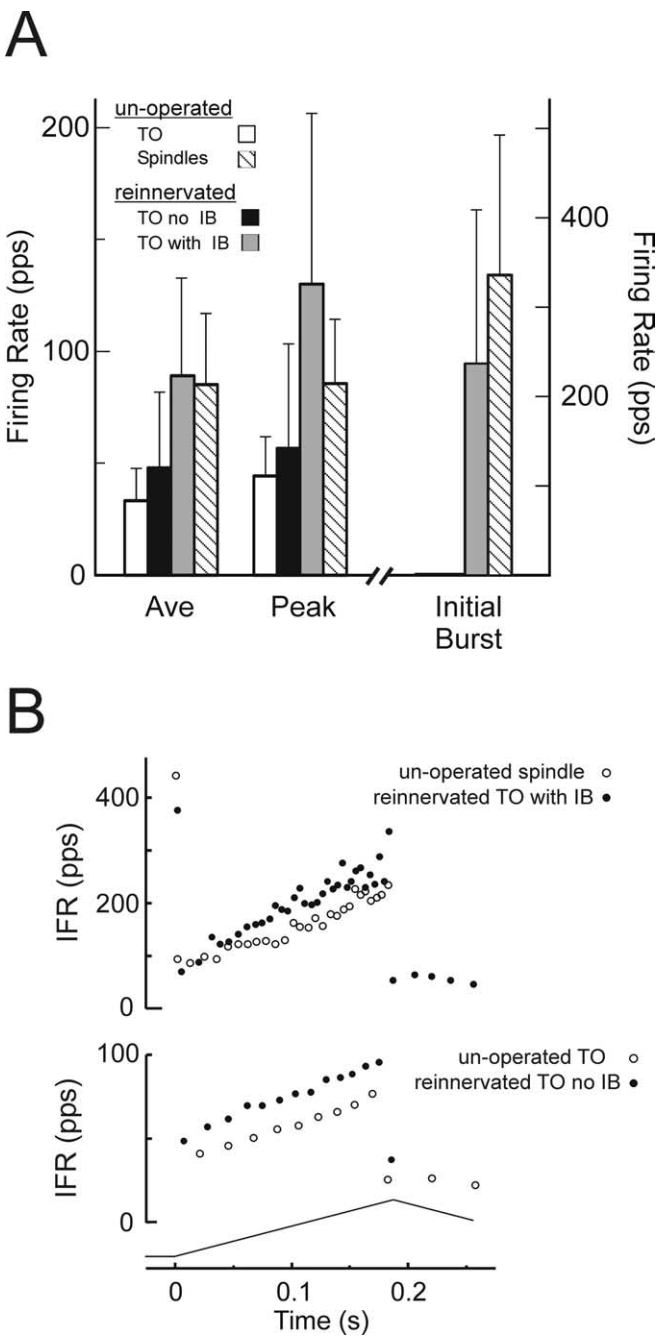

Figure 6. Stretch responses of tendon-organ afferents. ***A***, Means ± SDs for average (Ave), peak (at peak stretch), and initial burst firing rates measured during ramp stretch. There were no significant differences ($p > 0.5$) between unoperated and reinnervated rats in the response properties of those afferents that were classified as tendon organs (TO; $n = 24$ and $n = 10$, respectively) based on accelerated firing during twitch contraction and no initial burst (IB) firing at the onset of muscle stretch. Firing responses for afferents from reinnervated rats that exhibited accelerated firing during twitch and initial burst firing at stretch onset (gray bars; $n = 7$) were similar to firing responses for spindle afferents from unoperated rats (cross-hatched bars; $n = 48$). In the latter comparisons, only peak firing rate was significantly different between groups ($p = 0.006$). Data suggest recovery of normal dynamic responses for properly classified tendon-organ afferents. ***B***, Instantaneous firing rates (IFR) plotted versus time for four different MG afferents over a portion of their response to triangular stretch (3 mm; 16 mm/s) applied to MG muscles (bottom trace). Top plot illustrates similarity in firing, including IB, for one spindle afferent from an unoperated rat and one afferent classified as tendon organ based on accelerated discharge during muscle contraction (data not shown). The middle plot shows similarity in firing (no initial burst) for afferents classified as tendon organ and sampled from one unoperated and one reinnervated muscle.

cal stimulation of the regenerated MG nerve produced an EPSP in all cases tested, even for MG motoneurons in which stretch of the reinnervated MG muscle failed to yield any detectable SSP. The inset in Figure 7*A3* illustrates data from one of eight motoneurons that generated an electrically evoked EPSP but no stretch-evoked potential. No statistically significant group differences were found between EPSP amplitude (3.71 ± 1.54 vs 3.36 ± 1.90 mV, unoperated vs reinnervated, respectively; $p = 0.674$) or EPSP time-to-peak (1.24 ± 0.45 vs 1.16 ± 0.40 ms; $p = 0.691$).

Discussion

The results provide new observations on the central synaptic actions of cut and regenerated proprioceptive afferents. We found that stretch of a reinnervated muscle failed to produce either reflex contraction or synaptic excitation in a large fraction of homonymous motoneurons. This collapse seems surprising given that many large-diameter, regenerated afferents are responsive to muscle stretch and that the motoneurons fire in response to previously uninjured synaptic inputs and are capable of initiating muscle contraction. In the discussion that follows, we examine candidate mechanisms that alone or in combination could account for the ineffectiveness of stretch in producing synaptic potentials and reflex contraction.

Response of regenerated spindle afferents to muscle stretch

There is some uncertainty about the extent to which the population of regenerated spindle afferents recovers normal function. On the one hand, it is clear that recovery is not complete (Brown and Butler, 1976; Gregory et al., 1982; Banks and Barker, 1989; Lewin and McMahon, 1991). The number of innervated spindle receptors is reduced to as few as 75% of normal (Table 1) (Banks et al., 1984; Barker et al., 1985, 1988; Scott, 1987; Dieler and Schroder, 1990), and there is indirect evidence that those receptors that are reinnervated may be supplied by the wrong class of afferents (Collins et al., 1986; Banks and Barker, 1989). These deficiencies in peripheral regeneration probably underlie the failure that we observed in 10% of stretch-sensitive afferents to respond with more than a few spikes. On the other hand, many of the regenerated group I and II afferents sampled in the present study (all of the 30% sampled in one rat) (Fig. 3) exhibited a suite of stretch responses typical of normal spindle afferents (Fig. 4). Although nonspecific reinnervation of spindle receptors makes it possible that a fraction of the stretch signal was conducted centrally through pathways that do not normally produce excitatory SSPs (Rymer et al., 1979), one study of long-term reinnervated muscle in cat (Collins et al., 1986) demonstrates that spindle-like responses were recovered by substantial fractions (~50%) of the regenerated group Ia afferents that terminate in the homonymous motor pool. These findings support the assertion that a normal stretch signal was carried centrally by a substantial fraction (at least ~30%) of spindle afferents.

If this stretch signal arrives at excitatory monosynaptic connections with motoneurons, we estimate that it should produce detectable, albeit smaller, SSPs. Even if the number of monosynaptic connections carrying a normal stretch signal was reduced to 30% of normal, one might expect from rough approximation that the SSP should be ~30% of the normal SSP amplitude (2.8 mV), or 0.8 mV, which is well above the smallest SSP measured here (0.2 mV). This estimation is at odds with the complete absence of SSPs that we observed in the majority of motoneurons in every treated rat. Based on this reasoning, we argue that the magnitude of the estimated reduction in the stretch signal occurring

after reinnervation cannot solely account for the complete absence of the monosynaptic component of SSPs. However, polysynaptic components of SSPs might be eliminated by a weaker-than-normal stretch signal, which may fail to activate interneurons interposed in the synaptic pathways to motoneurons. Unfortunately, the relative roles of monosynaptic versus polysynaptic pathways in determining the motoneuron synaptic response to ramp–hold muscle stretch are not well established.

Functional competence of monosynaptic transmission and motoneuron firing

In contrast with the poor recovery from nerve injury for SSPs, there was very good recovery for the monosynaptic EPSPs evoked by low-frequency (0.5 pps) electrical stimulation. This is consistent with previous demonstrations that electrical stimulation of regenerated muscle nerves produces EPSPs in cat motoneurons (Eccles et al., 1960, 1962; Thulin, 1961; Goldring et al., 1980; Mendell et al., 1995) and H-reflexes in rats (Valero-Cabre and Navarro, 2001, 2002). Our observations establish that monosynaptic transmission was operational in the rat. However, we did not study and cannot be certain that transmission was normal at the higher-stimulation frequencies typical of those occurring during muscle stretch. At these higher frequencies, it is possible that axonal conduction or synaptic transmission may fail. In the cat, however, Ia monosynaptic EPSPs do not exhibit abnormally high-frequency depression after regeneration (Mendell et al., 1995). Although we lack direct evidence to rule out this possibility for the rat, it seems likely that the same is true for rat monosynaptic EPSPs, particularly because high-frequency modulation of monosynaptic EPSPs is comparable in normal adult rats and cats (Collins et al., 1984; Peshori et al., 1998; Seburn and Cope, 1998). These observations lead us to suggest that regenerated spindle afferents in the rat are competent to transmit monosynaptic excitation onto motoneurons during muscle stretch.

As for the excitability of the regenerated MG motoneurons, their capacity to fire repetitively in response to synaptic current is established by the reflex contraction elicited in the reinnervated MG muscle by cutaneous stimulation. However, we cannot rule out the possibility that some abnormality in the regenerated motoneurons contributes to the ineffectiveness of muscle stretch in eliciting reflex contraction. Recent studies demonstrate that modulation of motoneuron firing rate relies on the enhancement of synaptic current by voltage-gated conductance intrinsic to motoneurons (Binder, 2002). A deficit in this mechanism may be necessary to account for the observation that the SSPs observed in some motoneurons (9 of 22), although smaller than normal, produced essentially no modulation of ongoing contraction and, by inference, no modulation of motoneuron firing (Prather et al., 2001).

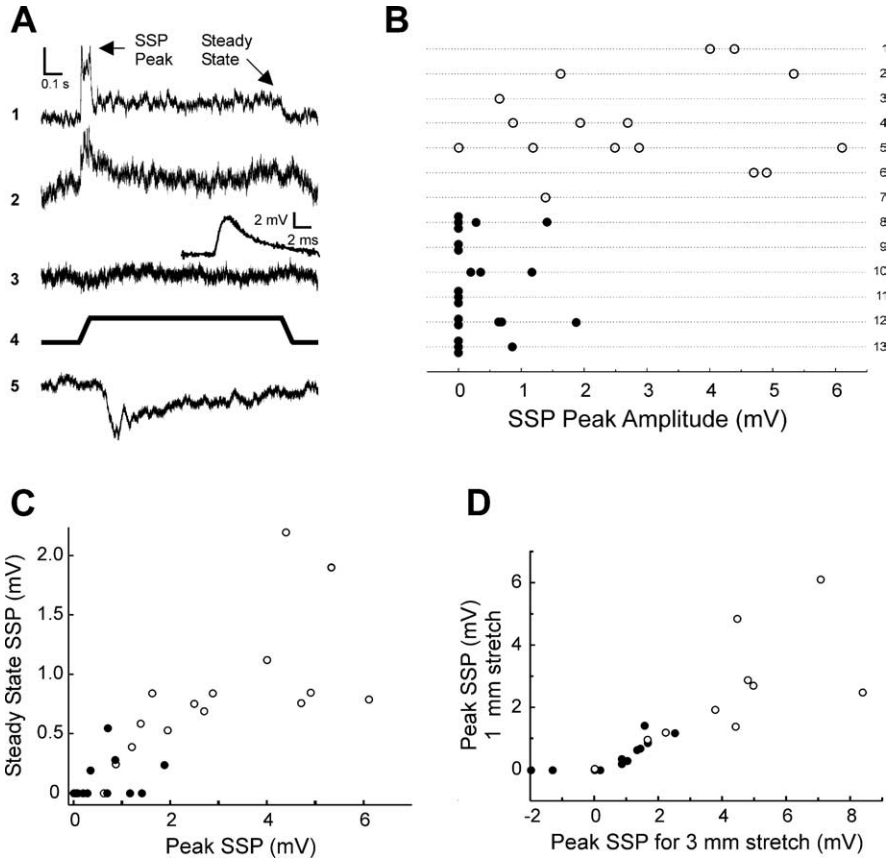

Figure 7. Central suppression of synaptic input from regenerated proprioceptive afferents. ***A***, Records of motoneuron membrane potential averaged over 13–16 trials of ramp– hold–release stretch (trace 4 is 1 mm stretch amplitude, 20 mm/s ramp and release velocity) applied to MG muscle. Trace 1 shows SSP from one unoperated rat; arrows point to measurements of SSP peak (peak depolarization at end of rising phase of ramp stretch) and steady state (depolarization at end of hold phase of stretch). Traces 2 and 3 show SSPs produced in two motoneurons by stretch of reinnervated MG muscle in one rat. Stretch responses of the same regenerated proprioceptive afferents that generated the SSP in one motoneuron (trace 2) produced no response in another motoneuron (trace 3), although a normal-sized EPSP was elicited by electrical stimulation of the MG nerve (inset in trace 3). The failure of motoneurons to respond to proprioceptive input through transmission-competent synapses is strong evidence for suppression by mechanisms in the spinal cord. Trace 5 shows that the 3 mm stretch of a reinnervated muscle evoked hyperpolarization that was never observed with stretch of unoperated muscle. SSP amplitude calibration bar shown in trace 1 is 2 mV for trace 1 and 1 mV for traces 2, 3, and 5. ***B***, Values observed for SSP peak amplitude for all motoneurons recorded from each of 13 different rats (open circles, unoperated; filled circles, reinnervated). Note the tendency toward smaller SSPs in rats with reinnervated muscle and absence of any response to stretch for 13 of 22 motoneurons. ***C***, Plot of steady-state versus peak SSP values for 1 mm stretches. ***D***, Plot of peak SSP values measured for 1 versus 3 mm stretches.

Active suppression of Ia motoneuron transmission by extrinsic neural circuits

Despite the arrival of discreet stretch-evoked discharge of large-diameter afferents and despite the capacity for synaptic transmission between regenerated proprioceptors and motoneurons, no SSP could be resolved in 60% of the motoneurons sampled. Together with evidence that essentially all MG motoneurons normally receive monosynaptic group I excitatory input in the rat (Miyata and Yasuda, 1988; Manabe et al., 1989; Seburn and Cope, 1998), these finding suggest that additional mechanisms may operate to suppress SSPs after regeneration. In principle, suppression of Ia motoneuron transmission might derive from presynaptic and/or postsynaptic inhibition. In the case of presynaptic inhibition, there is evidence that, several weeks after nerve crush in the cat, primary afferent depolarization (PAD) of the damaged group Ia afferents is sustained by certain segmental sources (Enriquez-Denton et al., 2004) and enhanced by descending pathways, i.e., the reticulospinal tract (Enriquez et al., 1996). If

presynaptic inhibition is the mechanism that suppresses SSPs, then it remains to be explained why electrically evoked monosynaptic EPSPs are not also blocked but are instead normal in size. Because SSPs and EPSPs both seem to rely at least in part on transmission from Ia afferents (Nichols, 1999), it seems that SSP suppression is not solely attributable to tonic presynaptic inhibition of regenerated Ia afferents. A mechanism involving presynaptic inhibition may instead require stretch of the reinnervated muscle to drive the interneurons that mediate depolarization of the regenerated Ia afferents. Support for this possibility will require evidence that PAD is produced in regenerated Ia afferents by stretch activation of homonymous afferents.

As for postsynaptic inhibitory mechanisms, a remote, shunting inhibition might suppress the SSP without producing any detectable change in motoneuron membrane potential in response to muscle stretch, just as we observed in most cases (Coombs et al., 1955). In other cases, we found that increasing muscle stretch produced a hyperpolarizing inhibition. Either mechanism is a viable candidate for explaining SSP and stretch-reflex suppression. One likely source of postsynaptic inhibition is from stretch activation of group Ib afferents (Jami, 1992) and possibly even from group Ia afferents, which in cat are shown to generate disynaptic inhibitory potentials in homonymous motoneurons (Fetz et al., 1979).

For either presynaptic or postsynaptic inhibitory mechanisms to explain our results, they must have the capacity to suppress even the earliest component of the SSP, which is presumably generated by the group Ia monosynaptic pathway in normal animals and which should emerge ~2–4 msec before being suppressed by polysynaptic inhibitory pathways (Eccles et al., 1961; Watt et al., 1976; Jankowska et al., 1981). The latter condition may be met because muscle stretch produces a relatively desynchronized barrage of afferent activity, so the earliest monosynaptic potentials may be small and quickly overcome by transmission through presynaptic or postsynaptic inhibitory pathways.

Maladaptive plasticity

Soon and for weeks after peripheral axotomy, some muscle afferents generate spontaneous discharge (Michaelis et al., 2000), which is necessarily unrelated to stretch of the detached muscle. This abnormal afferent activity is conducted centrally, and its suppression would reduce the chance of inappropriate activation of motoneurons (Enriquez et al., 1996), including synergistic motoneurons whose peripheral nerves are uninjured. Continued suppression seems maladaptive, however, when the correct signal is substantially restored by regeneration of the cut nerve into the muscle. The notion of maladaptive suppression of spinal circuits has been considered as the basis for limiting recovery of movement after spinal cord injury. Tillakaratne et al. (2000) show that the loss of purposeful movement in chronic spinal cats is associated with increased staining in motor pools of the spinal ventral horn for glutamic acid decarboxylase-67 (GAD_{67}), a synthetic enzyme for the inhibitory neurotransmitter GABA that mediates presynaptic inhibition. Interestingly, rehabilitation of stepping in these animals through training is associated with decreased staining for GAD_{67} (Tillakaratne et al., 2002). By extension, some degree of proprioception might be restored after peripheral nerve injury or disease, e.g., Guillain-Barre syndrome, if central suppression of input from these afferents could be relieved.

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