

# Recovery of proprioceptive feedback from nerve crush

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**Non-technical summary** Regeneration of muscle nerves damaged by crush reconnects the peripheral limb of neural circuits that pass through the spinal cord, but the mechanisms underlying functional recovery remain uncertain. We examined the actions of natural muscle stretch that initiates muscle contraction, i.e. the stretch reflex, through a spinal circuit that aids in adjusting body movement and posture in response to destabilizing forces in the external environment. Stretch applied to muscles reinnervated by crushed nerves produced reflexive contraction that was more forceful than normal, despite yielding less than normal synaptic excitation to spinal motoneurons. Incomplete recovery of synaptic function by stretch-activated sensory neurons means that the enhanced stretch reflex contraction necessarily involves additional neural adaptations, possibly increased motoneuron excitability. These findings give further support to the importance of the central nervous system in restoring the ability of the regenerated neuromuscular system to respond to external disturbances of movement and posture.

**Abstract** Sensorimotor functions are restored by peripheral nerve regeneration with greater success following injuries that crush rather than sever the nerve. Better recovery following nerve crush is commonly attributed to superior reconnection of regenerating axons with their original peripheral targets. The present study was designed to estimate the fraction of stretch reflex recovery attributable to functional recovery of regenerated spindle afferents. Recovery of the spindle afferent population was estimated from excitatory postsynaptic potentials evoked by muscle stretch (strEPSPs) in motoneurons. These events were measured in cats that were anaesthetized, so that recovery of spindle afferent function, including both muscle stretch encoding and monosynaptic transmission, could be separated from other factors that act centrally to influence muscle stretch-evoked excitation of motoneurons. Recovery of strEPSPs to 70% of normal specified the extent of overall functional recovery by the population spindle afferents that regained responsiveness to muscle stretch. In separate studies, we examined recovery of the stretch reflex in decerebrate cats, and found that it recovered to supranormal levels after nerve crush. The substantial disparity in recovery between strEPSPs and stretch reflex led us to conclude that factors in addition to recovery of spindle afferents make a large contribution in restoring the stretch reflex following nerve crush.

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**Abbreviations**  $L_0$ , resting muscle length; MG, medial gastrocnemius; PIC, persistent inward currents; strEPSP, excitatory postsynaptic potential evoked by muscle stretch.

## Introduction

Persistent deficits in sensory feedback contribute in limiting recovery of normal motor function following regeneration of a severed peripheral nerve (Brink & Mackel, 1987; Lundborg, 2000, 2004; Jaquet *et al.* 2001; Rosen & Lundborg, 2001; Duff, 2005). Despite good recovery of force generation (for review see Gordon *et al.* 2004), reinnervated muscle does not regain its stretch reflex (Cope *et al.* 1994; Huyghues-Despointes *et al.* 2003; Haftel *et al.* 2005; Maas *et al.* 2007). Even under favourable conditions in which the nerve to a single muscle is cut and allowed to regenerate over short distance with minimal delay to reinnervate its original muscle, the affected limb exhibits abnormal joint coordination more than 1 year after nerve transection (Abelew *et al.* 2000; Maas *et al.* 2007; Chang *et al.* 2009). Losses in joint coordination and the stretch reflex reflect losses in the effectiveness of proprioceptive feedback that are not fully understood and that are the subject of the present report.

Recovery of the muscle stretch reflex clearly requires that regenerated spindle afferents regain their responsiveness to muscle stretch. After nerve section, the afferent signal for muscle stretch recovers, but only partially (Gregory *et al.* 1982; Banks & Barker, 1989; Lewin & McMahon, 1991; see for review Scott, 1996 and Zalena 1994), because some proprioceptors either fail to reconnect with receptors or are misrouted to the wrong receptor type (Collins *et al.* 1986; Munson *et al.* 1988; Banks & Barker, 1989). While it is possible for some spindle afferents to express mechanosensitivity without reconnecting to receptors (Johnson & Munson, 1991; Proske *et al.* 1995), the prevalence and long-term stability of this condition is unknown. If the degree of reconnection between sensory afferents and their cognate receptors determines the extent of functional recovery, then the stretch reflex should recover much better from nerve crush. Nerve crush, or axonotmesis, severs axons, but unlike nerve transection may not interrupt the endoneurial tubes that can physically guide regenerating axons back to their original targets (e.g. Sumner, 1990; Fournier & Strittmatter, 2002; Nguyen *et al.* 2002). Superior reconnection with spindle receptors probably explains better recovery of spindle afferent responsiveness to muscle stretch after nerve crush (Brown & Butler, 1976; Hyde & Scott, 1983; Barker *et al.* 1985, 1988) compared to cut (see above), and probably also to better recovery of the stretch reflex.

Recovery of the stretch reflex may also depend on neural changes induced in the central nervous system by peripheral nerve injury (e.g. Valero-Cabre & Navarro, 2002; Haftel *et al.* 2005; Navarro, 2009; for review see Navarro, 2009). Postsynaptic integration of stretch-evoked synaptic input by polysynaptic pathways, e.g. presynaptic inhibition, and by properties intrinsic to motoneurons, e.g. voltage-sensitive conductances, can substantially

amplify or suppress the stretch reflex (Heckman *et al.* 2005; Jankowska & Edgley, 2010). An effect of injury on these mechanisms has the capacity to influence stretch reflex recovery, and there is evidence that peripheral nerve injury alters the strength of segmental spinal circuits (see Navarro, 2009). Central mechanisms are recognized, therefore, for their potential importance to stretch reflex recovery, but their significance relative to peripheral mechanisms remains unknown. The question remains whether the recovery of afferent peripheral responsiveness, although necessary, is sufficient to provide the full explanation for stretch reflex recovery from nerve crush, i.e. is stretch reflex recovery fixed by the fraction of afferents that regain stretch responsiveness; to what extent is recovery boosted by additional mechanisms?

The present study was designed to estimate the portion of stretch reflex recovery attributable to the fraction of spindle afferents that regain stretch responsiveness following nerve crush. The aggregate action of all spindle afferents responding to muscle stretch was measured from the strEPSPs they produced in motoneurons in normal cats *versus* cats studied approximately 1 year after nerve injury. Confounding contributions from other factors, e.g. transmission in polysynaptic pathways and ionic currents intrinsic to motoneurons (see above and Discussion), were reduced by measuring strEPSPs in cats that were anaesthetized. In separate studies, the full set of factors required to produce the stretch reflex were allowed expression by removing anaesthesia in acutely decerebrated cats. Comparison of the findings from these studies showed that afferent function reflected in strEPSPs recovered to only 70% of normal and fell far short of accounting for recovery of the stretch reflex in decerebrate cats that exceeded normal levels. These findings suggest that mechanisms in addition to recovery of spindle afferent function contribute significantly to reflex recovery, and our findings aid in constraining possible candidates which are considered in the Discussion.

## Methods

Studies were performed on nine cats as approved by the Laboratory Animal Care and Use Committees at Emory University and Wright State University. All were mixed-breed, adult females (weighing 2.5–3.5 kg). Six received nerve treatment in survival surgery, three served as untreated controls, and all were studied in terminal experiments after which they were killed by barbiturate overdose (150 mg kg<sup>-1</sup>).

## Survival surgery

In six cats, the nerve supplying the medial gastrocnemius (MG) muscle in the left leg was crushed

and allowed to regenerate in order to examine the capacity for select measures of sensorimotor function to recover. Nerve injury was produced using sterile surgical procedures in a dedicated surgical suite with the cat under deep anaesthesia induced and maintained by isoflurane inhalation (1.5–2.5% in 100% O<sub>2</sub>). Oxygen saturation, pulse rate, respiration rate and body temperature were monitored throughout the procedure, which was typically completed within *ca.* 1.5 h. The MG nerve in the popliteal fossa was exposed by skin incision (*ca.* 5 cm) and gently separated from surrounding tissues within 1 cm of its entry into the MG muscle. The MG nerve was crushed between the prongs of no. 5 microsurgical forceps with firm pressure applied for *ca.* 10 s, leaving the nerve intact but visibly compressed at the injury site. Following MG nerve crush, the incision was closed by suture and analgesics were administered before discontinuing anaesthesia. Cats were continuously monitored, kept warm until regaining sternal posture, and then returned to their housing and monitored for signs of discomfort or infection. All cats quickly recovered normal behaviour and activity in their open room housing. Within a few weeks, movements of the treated and untreated hind limbs were indistinguishable in casual visual inspection. Each cat received scheduled veterinary examination and daily care without any experimental study until their use in terminal experiments performed approximately 1 year post-operatively.

### Terminal studies

All nine cats were studied in one of the two kinds of terminal experiments described below. Several features were common to both experiments. Cats were deeply anaesthetized (absent withdrawal and corneal reflexes) and fitted with monitors of vital signs, including blood pressure obtained through a left carotid cannula (mean 60–120 mmHg), rectal temperature (35–38°C), pulse rate (100–160 beats min<sup>-1</sup>) and blood O<sub>2</sub> saturation (90–100% measured by a light-emitting clip placed on a front paw), and expired CO<sub>2</sub> (3–4%) sampled from a tracheal cannula. Fluids (10 ml h<sup>-1</sup> lactated Ringer solution and/or barbiturate anaesthetic as necessary) were delivered by cannula through the left external jugular vein. Delivery of anaesthetic, Ringer solution, radiant heat and/or artificial respiration used in some cases were adjusted to maintain vital signs within the physiological ranges indicated above. Anaesthesia level and vital signs were maintained from the beginning of terminal experiments through surgery and data collection up to 20 h later when cats were killed.

In all experiments, the study focused on responses (either synaptic or muscle reflex) to controlled stretch applied to the MG muscle. With one or both hind limbs dissected to expose triceps surae muscles and nerves (see

below), cats were secured in a rigid frame and the hind limb was clamped on the distal femur and tibia holding the knee and ankle joints at approximately 135 deg and 90 deg, respectively. The MG was detached from its insertion on the calcaneus and attached by rigid linkage to a force transducer in series with a length-servo motor. During data collection (described below), static muscle length was held near resting muscle length ( $L_0$ ) measured by markers positioned in the limb and on the MG muscle before its detachment. The amount of passive force at  $L_0$  was approximately 100 g for all cats.

Ramp–hold–release stretches of the MG muscle were used to study both synaptic and reflex responses (see below). Muscle length was ramped from  $L_0$  to 2–3 mm at constant velocity (30–40 mm s<sup>-1</sup>) over 100 ms, held for 300 ms, and then released back to  $L_0$  at constant velocity over 100 ms. These stretch parameters are within the normal physiological range of hind limb muscle length changes observed in walking cats (Goslow *et al.* 1973; Walmsley *et al.* 1978; Maas *et al.* 2007), and are similar to those used in earlier studies of physiological responses to natural muscle stretch (Westbury, 1972; Nichols, 1989). Stretches were repeated at 4 s intervals so as to minimize history-dependent effects (Nichols, 1989).

To investigate the limits of recovery from nerve injury, all cats with nerve crush were studied more than 1 year post-operatively, as previous studies report nearly complete restoration of electrically evoked contraction of reinnervated cat muscles at this recovery time (Gordon & Stein, 1982; Foehring *et al.* 1986). Evidence of successful and selective self-reinnervation of the MG muscle was obtained in terminal experiments for all six cats following MG nerve crush. First, the reinnervated MG was observed to contract forcefully and exclusively to electrical stimulation of the MG nerve at a site rostral to surgical nerve injury. Second, the masses of MG muscles reinnervated after nerve crush were within 1% wet weight of their contralateral control MG muscles in all six cats, and an earlier report shows that cat MG muscles which recover weight following nerve crush also recover tetanic force (Rafuse & Gordon, 1996).

### strEPSP experiments

Seven cats (4 with nerve crush, 3 untreated controls) were prepared for intracellular recording from MG motoneurons as follows. Deep anaesthesia was maintained by barbiturate (Nembutal, 40 mg kg<sup>-1</sup> i.v.) in order to reduce excitation of motoneurons through either polysynaptic pathways or intrinsic currents and, thereby, to focus study on monosynaptic excitation from stretch-activated primary afferents onto motoneurons. In one of three control cats, anaesthesia was maintained by inhalation of isoflurane (1.5–2.5%) carried in equal parts of compressed room air and compressed O<sub>2</sub> combined

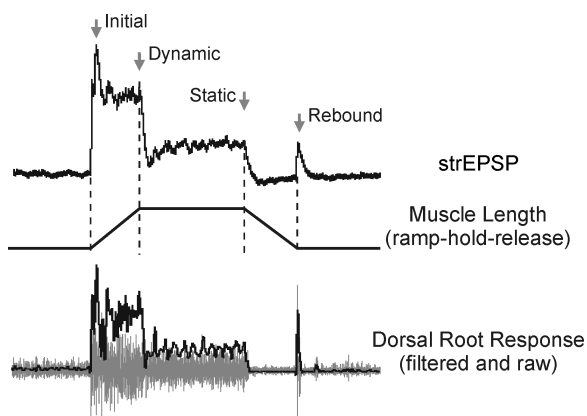
through an air mixer. Data collected from this cat were not significantly different from those collected in the two barbiturate-anaesthetized controls (see Results).

Intracellular recording from MG motoneurons supplying the left hind limb proceeded as detailed in our earlier reports (Prather *et al.* 2001). Briefly, removal of the laminae of vertebrae L4–S1 exposed the dorsal spinal cord, permitting access to MG motoneurons by glass micropipettes (5–10 M $\Omega$ , 2 M potassium acetate) and to MG afferents recorded extracellularly by bipolar electrodes placed on dorsal roots L7 and S1. Motoneurons were positively identified as innervating the MG muscle when antidromic action potentials were generated by bipolar electrical stimulation of the MG nerve and were studied in detail when the intracellular action potentials exceeded 60 mV in amplitude. MG motoneurons were characterized by injecting depolarizing current pulses, which generated action potentials used to determine rheobase current (50 ms pulse duration, at threshold current strengths which produced action potentials in some, but not all, trials) and afterhyperpolarization (AHP) half-decay time (1 ms pulse duration, suprathreshold current strength typically 5 $\times$  rheobase current), measured as the time taken for motoneuron potential to decay from the peak to half-amplitude of the AHP. The remaining recording time was devoted to measuring strEPSPs produced by MG motoneurons in response to repeated trials of MG muscle

stretch applied through the servomotor as described above.

Amplified and filtered records of motoneuron membrane potential (10 $\times$ , DC–10 kHz), microelectrode current, dorsal root activity, muscle length and force were sampled (20 kHz) through separate A/D channels (Cambridge Electronic Design Power 1401) and stored on computer for later analysis using CED Spike 2 software. Events were averaged over replicate stimulus trials to improve the signal-to-noise ratio over multiple trials during which motoneuron resting membrane potential varied by no more than 4 mV. Voltages were measured from strEPSPs at the initial phase of stretch, at the end of the ramp or dynamic phase of stretch, and at the end of the hold or static phase of stretch (Fig. 1). At these points and throughout their entire duration, the strEPSPs closely resembled the waveform for rectified and integrated afferent activity evoked by MG muscle stretch (Fig. 1). The observed afferent activity profile matched that expected for group IA afferents in response to ramp–hold–release stretch (Matthews, 1972), including: (a) marked increase in firing rate near the onset or ‘initial’ phase of stretch, (b) increasing firing rate during the ‘dynamic’ rising phase of the ramp to its peak, (c) a sharp drop in firing rate after ramp peak, (d) sustained firing throughout the ‘static’ or hold phase of stretch, (e) cessation of firing during ramp release and rebound firing soon thereafter. These features suggest that in barbiturate-anaesthetized cats, the strEPSPs recorded in MG motoneurons were produced predominantly by monosynaptic excitation from spindle afferents.

Measures of strEPSPs and motoneuron electrical properties were pooled within treatment groups (control or crush-regenerated), and treatment effects were tested statistically using nested ANOVA (individual experiments nested inside treatment groups). Tukey’s honestly significant difference (HSD) *post hoc* test was used to test for the significance of comparisons of treatment effects (SYSTAT; Systat Software, Point Richmond, CA, USA). Data are reported as mean  $\pm$  SEM unless indicated otherwise.



**Figure 1. Stretching the MG muscle resulted in population-level activation of stretch-sensitive afferents and generation of an aggregate stretch-evoked excitatory post-synaptic potential (strEPSP) in MG motoneurons**  
In an anaesthetized control animal, the intracellularly recorded strEPSP (top trace) averaged of 10 sequential trials of ramp–hold–release (middle trace) comprised distinct components in relation to the stretch stimulus (dashed vertical lines). The magnitude of the strEPSP was measured at specific points within each component (indicated by arrows and labels). The profile of the strEPSP closely resembled the profile of the rectified and filtered voltage recorded by bipolar electrodes positioned on the dorsal root (bottom trace, raw record in light trace superimposed by rectified and filtered record in dark line trace). Data were collected in cats anaesthetized by Nembutal.

### Stretch reflex experiments

In two cats with nerve crush, we directly determined whether reinnervated MG muscles recovered a stretch reflex, i.e. whether these muscles contracted in response to stretch. Methods employed are detailed in our earlier reports (Huyghues-Despointes *et al.* 2003). Briefly, cats were anaesthetized by inhalation of isoflurane (1.5–2.5%) carried in equal parts of compressed room air and compressed O<sub>2</sub> combined through an air mixer. They were fitted with monitors for vital signs and prepared for study of the MG muscles in both hind limbs as described in the previous section, but no laminectomy was performed.



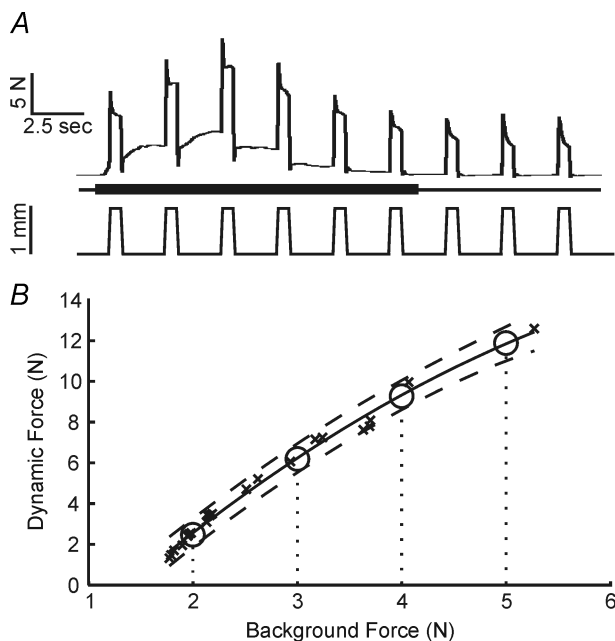
Next, cats were decerebrated by removal of brain tissue rostral to mid-collicular transection in order to render them reflexive yet insensate when the gaseous anaesthesia was discontinued.

The extent of stretch reflex recovery was assessed by bilateral comparisons within each individual cat of the reinnervated MG in the left hind limb and the normally innervated MG in the right hind limb. With right and left legs secured in fixed positions and the MG muscles attached through a force transducer to length-servo motors (described above; linear motors with encoder resolution  $0.1\ \mu\text{m}$  and maximum acceleration  $ca. 50\ \text{m s}^{-2}$ ) to deliver ramp–hold–release stretches (amplitude 2 mm, ramp and release rate  $40\ \text{mm s}^{-1}$ , hold duration 0.3 s) to the MG muscle at 4 s intervals. Stretches were applied before, during and after activation of the MG muscle in a crossed-extension reflex evoked by electrical stimulation (100 Hz) of the contralateral posterior tibial nerve (Fig. 2A).

Records of MG muscle force obtained during muscle stretch were amplified ( $5\times$ ), filtered (DC to 1 kHz),

digitized (sampling rate 1 kHz), and stored on computer for off-line analysis. Forces were measured at the maximum excursion of the ramp (dynamic force) and from the end of the hold phase of stretch (static force) corresponding to those same points measured from strEPSPs (see below). Force responses were then computed by subtracting the background force measured 50 ms prior to the ramp from the dynamic and static forces. Best polynomial fits (within 95% confidence limits) were obtained in plots of dynamic or static forces vs. background force (Fig. 2B; Huyghues-Despointes *et al.* 2003), and stretch forces were then measured from these polynomial functions for right and left MG muscles at selected background forces.

In order to compare the extent of reflex action in the right and left MG muscles, force responses were plotted as a function of background force, and these relationships were compared between the two muscles (*cf.* Fig. 6). The stretch reflex increases with background force as progressively larger motor units are recruited (Matthews, 1986) and as intrinsic stiffness increases, so comparisons are only valid at matched background forces. Since the force responses consist of intrinsic and reflex components (Nichols & Houk, 1976; Huyghues-Despointes *et al.* 2003), it was also important to verify that the intrinsic mechanical properties of the muscles were not altered as a result of the recovery from nerve crush. We compared the intrinsic mechanical properties by measuring the force responses 10 ms after the beginning of ramp stretch, corresponding to initial or short-range stiffness (Fig. 6B). These responses were due solely to the intrinsic properties of the motor units active prior to stretch, since the stretch reflexes of triceps surae muscles have latencies of approximately 20 ms (Nichols & Houk, 1976). Since the muscles in this study were reflexive, it was not possible to measure the dynamic or static intrinsic response. In order to provide an estimate of the intrinsic components corresponding to the dynamic force responses, we used data obtained from MG muscles reinnervated following transection and repair of the muscle nerve (Huyghues-Despointes *et al.* 2003) (Fig. 6A). Although muscles reinnervated by cut nerves generated graded contraction in crossed-extension reflexes, their responses to stretch applied during these reflexes yielded force increments entirely attributable to intrinsic muscular stiffness as judged by comparison with responses obtained from muscles following dorsal rhizotomy (Huyghues-Despointes *et al.* 2003).



**Figure 2.** Stretch reflex measurements in decerebrate cats

A, muscle force (top trace) was measured from MG muscles bilaterally during ramp–hold–release stretches (bottom trace). Background force was modulated using the crossed-extension reflex elicited by stimulating the contralateral posterior tibial nerve (dark bar middle trace). B, the force of the muscle stretch response during the dynamic component of the stretch (defined as in Fig. 1A) varied as a function of the background force. This plot shows the data taken from stretch responses in one trial of crossed-extension reflex in an MG muscle reinnervated after nerve crush (continuous line is best polynomial fit, dashed lines are 95% confidence interval; dotted vertical lines point to intersection with polynomial fit (open circles) where dynamic forces were identified at selected background forces (2, 3, 4 and 5 N).

## Results

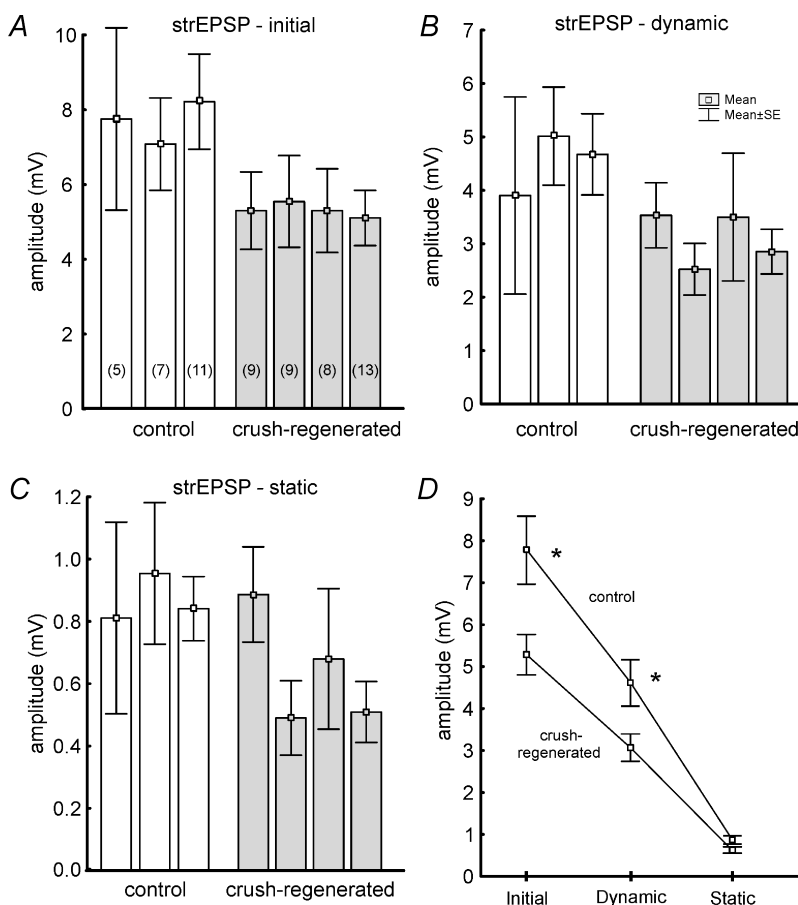
### strEPSP recovery from nerve crush

The goal of experiments reported in this section of the paper was to determine what fraction of regenerated spindle afferents regained both responsiveness

to muscle stretch and monosynaptic transmission onto motoneurons. These functions were measured from strEPSPs (see Fig. 1) recorded in cats that were anaesthetized in order to reduce confounding contributions from interneurons and motoneuron active conductances. Figure 3 shows that recovery of strEPSPs and their component responses was substantial but incomplete. The mean amplitudes of strEPSP components for each of four cats following nerve crush was smaller than for each of three control cats, with the exception of the static component in one cat. This tendency was expressed in comparisons of the pooled group means (Fig. 3D), which achieved statistical significance (nested ANOVA) for initial ( $P = 0.001$ ) and dynamic ( $P = 0.03$ ) components. The initial, dynamic and static components of strEPSPs following nerve crush ( $n = 39$ ) recovered to, respectively, 68%, 68% and 72% of their corresponding values of control strEPSPs ( $n = 23$ ). From these findings we suggest that roughly 70% of the spindle afferent population recovered function following nerve crush regeneration (see Discussion).

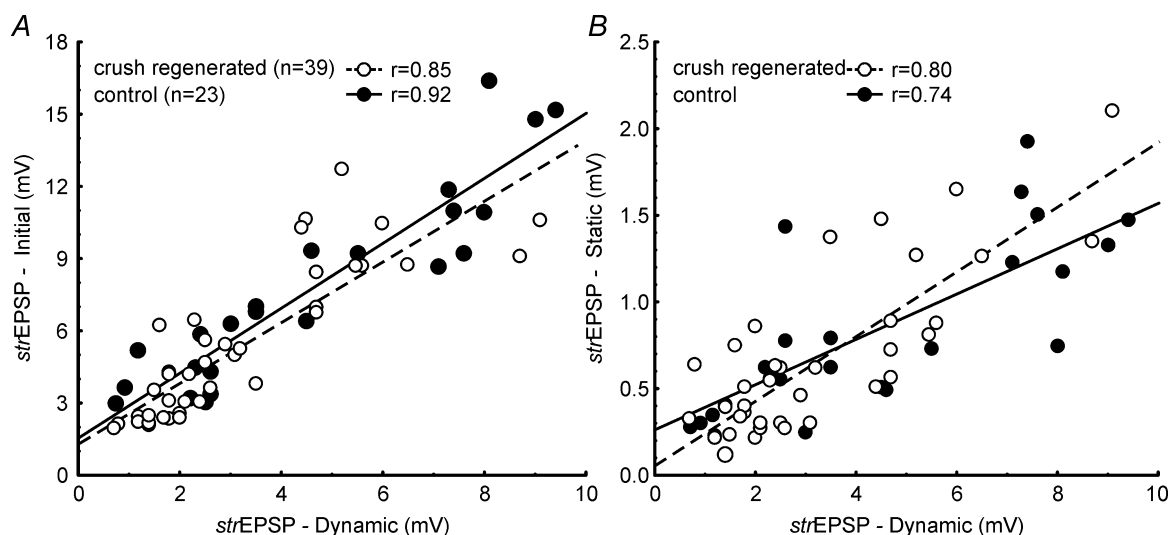
### strEPSP component changes proportionally after nerve crush

Figure 3 shows that on average, regenerated afferents sustained transmission throughout the duration of muscle stretch. That analysis, however, might have obscured incomplete recovery of the transmitted stretch signal resulting from the known failure of some spindle afferents to sustain firing during stretch (Brown & Butler, 1976; Hyde & Scott, 1983; Barker *et al.* 1988) or possible changes in the activity dependence of monosynaptic transmission (Mendell, 1988). These possibilities were tested by examining the relationships among strEPSP components. Figure 4 shows that initial, dynamic and static components of individual strEPSPs co-varied after crush regeneration as they did in untreated controls. There were no significant differences in slopes of the linear regressions (ANCOVA,  $P > 0.005$ ) between initial *vs.* dynamic or static *vs.* dynamic components for the nerve crush group compared with the control group.



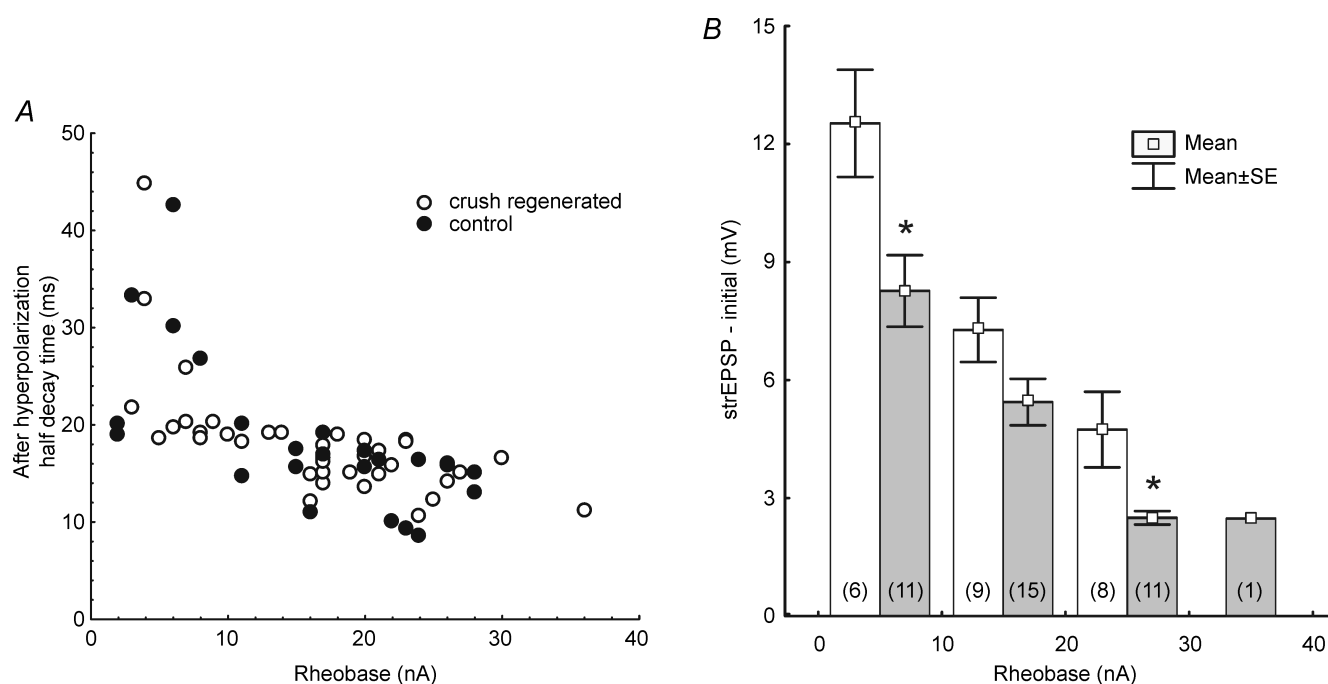
**Figure 3. strEPSPs recovered partially following crush, but failed to return to control values**

The tendency for incomplete strEPSP recovery was evident in the initial (A), dynamic (B) and static (C) components of the strEPSP (each column represents an experimental animal and numbers of strEPSPs sampled in each are shown in parentheses; all strEPSPs recorded in MG motoneurons in response to MG muscle stretch. D, summary of the three conditions shown in panels A–C further illustrates the changes in strEPSP magnitude in each component of the strEPSP (values are means  $\pm$  SE; asterisks designate statistical significance ( $P < 0.03$ ; nested ANOVA with Tukey *post hoc* test). Data were collected in cats anaesthetized by Nembutal.



**Figure 4. Relationships among strEPSP components (initial, dynamic and static) were similar to normal after crush regeneration**

For all linear regressions (continuous and dashed lines) correlation coefficients ( $r$ ) were significant ( $P < 0.05$ ; Pearson correlation); slopes and elevations of regression lines were not significantly different between groups ( $P < 0.005$ , ANCOVA) for initial vs. dynamic components (A) or for static vs. dynamic (B). Data were collected in cats anaesthetized by Nembutal.



**Figure 5. Motoneuron electrical properties returned to normal and were not associated with the decrease in strEPSPs after crush regeneration**

A, rheobase currents ( $I_{rh}$ ) and after-hyperpolarization (AHP) half-decay times co-varied and overlapped between normal and crush-regenerated samples of MG motoneurons. B, initial phase of strEPSPs declines with rheobase, and mean values in each 10 nA bin were smaller in crush-regenerated (filled bars) than in control (open bars). Statistical comparisons between groups were made using one-way ANOVAs ( $*P < 0.05$ ).

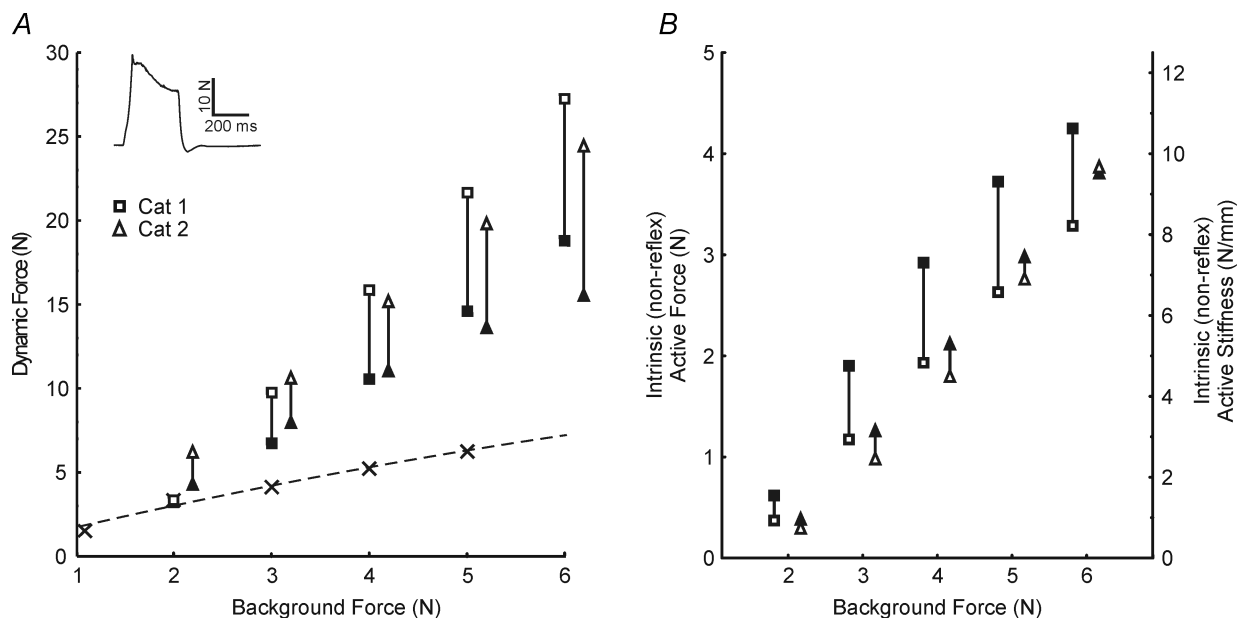
### strEPSPs changed independently of motoneuron electrical properties

Motoneurons sampled from control and nerve crush groups were characterized by properties that normally co-vary with monosynaptic EPSP size (Burke & Rymer, 1976; Zengel *et al.* 1985). Figure 5A shows nearly complete overlap in rheobase current and AHP half-decay time for motoneurons sampled from the two groups. Moreover, the normal tendency for these properties to vary inversely was evident after nerve crush. The motoneurons sampled from control and crush-regenerated groups were not significantly different, respectively, for either rheobase current ( $14.6 \pm 1.7$  and  $16.5 \pm 1.3$  nA; ANOVA,  $P = 0.771$ ) or AHP half-decay time ( $19.1 \pm 1.6$  and  $18.1 \pm 0.9$  ms;  $P = 0.842$ ). These observations suggest that motoneurons were similar in the two samples and that crushed motoneurons regained normal properties. Despite the similarity of motoneuron samples, Fig. 5B shows that strEPSPs in the crush group tended toward smaller values across the range of motoneuron rheobase current. These findings suggest that the decrease in

strEPSP size was not explained by corresponding changes in postsynaptic properties.

### Stretch reflex recovery from nerve crush

In a different experiment, stretch reflex contraction was measured (Fig. 2) from two cats with the left MG nerve regenerated 1 year after nerve crush identical to that described above. The decerebrate condition allows expression of all factors required for generated stretch reflexes, including monosynaptic input to motoneurons from primary afferents. Recovery of the stretch reflex was assessed by comparing the stretch reflex force bilaterally for MG muscles that were untreated in the right leg and reinnervated following nerve crush in the left leg. Figure 6A illustrates the dynamic component of the reflex force produced by ramp–hold–release stretch. Reflex force increased as expected with increasing levels of background muscle force, i.e. with increasing activation of MG motoneurons generated by the crossed extension reflex (see Methods, Fig. 2).



**Figure 6. Stretch reflexes recover to greater-than-normal levels following nerve crush**

In two acutely decerebrate cats, stretch forces generated during ramp–hold–release stretch (inset) were compared bilaterally for MG muscles that were untreated in the right leg (filled symbols) and reinnervated after nerve crush 1 year earlier in the left leg (open symbols). A, plot shows the dynamic component of the muscle force at the peak of ramp stretch vs. background muscle force immediately preceding stretch (*cf.* Fig. 2). The reflex component of dynamic force was that which exceeded the active intrinsic force estimated by the polynomial fit (dashed line) to data collected in an earlier study from a reinnervated MG muscle in cat (Huyghues-Despointes, 2003, see Methods). Dynamic reflex force was greater in reinnervated than in the contralateral untreated MG muscle at each of several background forces (vertical lines join bilateral forces measured from at 2, 3, 4, 5 and 6 N for each cat). B, plots of force responses from MG muscles in cats 1 and 2 obtained 10 ms following initiation of ramp stretch correspond to initial intrinsic (non-reflex) stiffness (see Methods). Data are expressed as force responses (y axis on left of plot) and as stiffness (force response divided by 0.4 mm stretch; y axis on right of plot). Initial stiffness was similar in both muscles in cat 2 and greater in the untreated muscle in cat 1. Therefore, differences in dynamic force between crush-reinnervated vs. normal muscles seen in A were due almost entirely to differences in the reflex component.



At almost all background forces in both cats, stretch reflex force was actually *greater* for MG muscles reinnervated by their crushed nerves than it was for the contralateral normally innervated MG muscles. At maximum background forces (6 N), the dynamic reflex force generated by the reinnervated MG muscles was 145% of the contralateral untreated MG for cat 1 and 157% for cat 2. The large left–right reflex differences for these two cats were probably not attributable to differences in muscle force-generating capacity, since left–right MG muscle masses were within 1% of one another (Rafuse & Gordon, 1996). Neither were there differences in passive intrinsic stiffness of reinnervated and normal muscles, since left–right differences in dynamic force were relatively small at the lowest background forces (2 N, Fig. 6A) in which responses are dominated by intrinsic muscle stiffness (Hoffer & Andreassen, 1981). Changes in the passive intrinsic responses due to crush would have been indicated by a vertical shift in the data points at all force levels. Instead, the data from treated and untreated muscles converged at low force. Active intrinsic stiffness was also unlikely to have been affected significantly by the treatment. Intrinsic responses measured shortly after the initiation of ramp stretch and before any reflex action was possible (corresponding to initial or short-range stiffness (Huyghues-Despointes, 2003) were, if anything, slightly greater for the untreated muscles (Fig. 6B). These data indicate that the reflex components of the responses were substantially larger on the treated side (Fig. 6A). An estimate of dynamic intrinsic stiffness (denoted by the crosses in Fig. 6A, see Methods) from a cat MG muscle is provided to show that the force responses of both treated and untreated muscles were accounted for largely by reflex components at intermediate and higher values of background force. These findings demonstrate the capacity for complete, in fact supranormal, recovery of stretch reflexes after nerve crush in sharp contrast to the complete failure of recovery with nerve-cut regeneration (Cope & Clark, 1993; Cope *et al.* 1994; Huyghues-Despointes *et al.* 2003; Haftel *et al.* 2005; Maas *et al.* 2007).

## Discussion

Sensorimotor function recovers well from nerve crush, certainly better than when regeneration follows nerve cut injuries (e.g. de Medinaceli *et al.* 1982; Zelena, 1994; Scott *et al.* 1996; Bervar, 2000; Lundborg, 2004), and the widely repeated explanation is that regenerating nerves are more successful after crush in reconnecting the periphery with the appropriate specialized sensory receptors (de Medinaceli *et al.* 1982; Zelena, 1994; Scott *et al.* 1996; Bervar, 2000; Lundborg, 2004). Results presented here follow this explanation, with evidence suggesting that the sensory processes leading up to and including

synaptic transmission with motoneurons are restored to the level specified by the fraction of spindle afferents that regain stretch sensitivity in the periphery. Sensory recovery from nerve crush was nonetheless incomplete, amounting to little more than two-thirds of normal and falling far short of the greater-than-normal recovery of stretch reflex contraction measured in the present study. The magnitude of the disparity between strEPSPs and stretch reflex recovery assigns substantial significance to mechanisms beyond recovery of regenerated spindle afferents.

## Recovery of strEPSPs

The present studies focused on the strEPSPs produced in motoneurons by the population of homonymous afferents responding to stretch before and after nerve injury. These synaptic potentials are directly relevant to reflex recovery, because they are a key participant in the initiation and gradation of stretch reflex contraction (see below). In addition, they can be assessed in relation to recovery of their component cellular processes as described next.

The strEPSPs examined in this study were probably produced through the monosynaptic circuit made by muscle spindle afferents and spinal motoneurons. The muscle stretch protocol used here is effective in activating spindle afferents (Matthews, 1972), which individually generate monosynaptic EPSPs in homonymous motoneurons (Mendell & Henneman, 1971) and which should collectively contribute substantially to the aggregate strEPSP. Consistent with this notion, we found that strEPSP waveform closely resembled the afferent firing pattern induced by muscle stretch (Fig. 1). Specifically, the initial, dynamic and static phases of afferent firing, as well as the steep adaptation in firing rate at the end of the ramp and the rebound firing typical of spindle afferent firing were all directly reflected in the strEPSPs. The reflection of spindle afferent firing in strEPSPs seen in normal cats was retained after nerve injury, since strEPSPs components varied together as they did normally (Fig. 4). From these observations, we posit that strEPSPs studied under the experimental conditions used here were generated predominantly by spindle afferents, both group IA and group II (Westbury, 1972).

Assuming the primacy of monosynaptic excitation from spindle afferents in generating strEPSPs, these events are determined by the product of peripheral factors (the number of afferents that connect with spindle receptors and the responsiveness of each to muscle stretch) and central factors (the number of afferents that connect with motoneurons and the transmission efficacy of each). Recovery of these factors has been documented or estimated following nerve crush. Among those individual

spindle afferents that respond to muscle stretch, most fire with temporal patterns and rates similar to normal (Hyde & Scott, 1983; Barker *et al.* 1986), and all transmit excitation to motoneurons with normal connectivity and efficacy (Gallego *et al.* 1980). Thus, the extent of strEPSP recovery would appear to rest on the proportion of spindle afferents that regain stretch sensitivity following nerve crush. For unspecified group I and II muscle afferents, Gallego *et al.* (1980) estimate that 30% lose stretch responsiveness, half of which fail to regenerate across the site of crush injury. The remaining 70% of the proprioceptive afferent population responds to levels of muscle stretch that typically activate spindle afferents. This estimate matches the 70% recovery we observed for strEPSPs, thereby suggesting that following nerve crush, recovery of spindle afferent encoding, axonal conduction and synaptic transmission of the muscle-stretch signal, all depend on the afferents' ability to recover stretch responsiveness.

Recovery of stretch responsiveness does not necessarily require that spindle afferents physically reconnect with spindle receptors, because the disconnected afferents can produce static firing in response to stretch (Johnson & Munson, 1991; Proske *et al.* 1995). However, these reports give no evidence that severed afferents produce the initial burst of high-frequency firing at the onset of stretch that is typical of intact afferents and presumably imparted to afferents through their connection with intrafusal muscle fibres (Proske *et al.* 1993). As strEPSPs exhibited an initial component of depolarization that corresponded to afferent initial burst firing (Fig. 1) and that maintained its normal relations to subsequent dynamic and static components (Fig. 4), we suggest that the strEPSPs were produced by afferents that reconnected with spindle receptors, for which there is ample evidence (reviews by Zelena, 1994; Scott, 1996). By this reasoning, we suggest that recovery of sensory feedback from nerve crush depends entirely on reconnection of regenerating spindle afferents with spindle receptors.

### Recovery of stretch reflex

Partial recovery of strEPSPs, unless compensated by other mechanisms, should result in partial recovery of the stretch reflex. In normal animals, the strength of stretch reflex contraction varies with the strength of monosynaptic excitation of motoneurons by spindle afferents. Motor units that are already firing in response to suprathreshold excitation produced by, for example, crossed-extension reflexes employed in these studies, have their firing rates and contraction forces modulated in direct relation to EPSPs in the size range observed in the present study (Gustafsson & McCrea, 1984). Also, the force of stretch reflex contraction among triceps surae muscles in the

normal cat varies in proportion to the sizes of monosynaptic EPSPs produced by group I electrical stimulation (Nichols *et al.* 1999). Thus, all other factors being equal after nerve regeneration, including recovery of the muscle's normal capacity for force generation (e.g. Rafuse & Gordon, 1996), one would predict that the strength of stretch reflex contraction should decline in parallel with strEPSP reduction following nerve injury.

We were tentative about the conclusions of others that the stretch reflex recovered from nerve crush, because they were based on indirect observations or rudimentary measurements. Complete recovery of the stretch reflex from nerve crush has been inferred by some (Valero-Cabre & Navarro, 2001; Navarro *et al.* 2007) based solely on the recovery of response properties by spindle afferents. Supranormal recovery of the electrically evoked H reflex (Valero-Cabre & Navarro, 2001, 2002; Navarro, 2009) parallels our results, but does not incorporate the contribution of peripheral recovery of stretch-sensitive afferents, which was one of our central aims. The stretch reflex was more directly examined in one study that demonstrated that the knee jerk in acutely decerebrated rabbits produced movement in the hind limb that fully recovered, and that were temporarily supranormal following nerve crush (Barker & Young, 1947). In the present study, we measured the force of reflex contraction elicited by muscle stretch to find that the stretch reflex did in fact recover, and to levels that were substantially greater than normal about 1 year after the nerve injury. We conclude, therefore, that recovery of stretch reflexes far exceeded the subnormal recovery of strEPSPs, which represent the population of afferents that recovered sensory responsiveness and monosynaptic excitation of motoneurons.

With the fractional contribution of strEPSPs accounted for, we turn attention to plausible candidate mechanisms underlying the sizeable remaining portion of stretch reflex recovery. These supplemental mechanisms are ones that were minimized by anaesthesia in our studies of strEPSPs (Hultborn *et al.* 1971; Taylor *et al.* 2000; Heckman *et al.* 2005), but that were released to express themselves in the unanaesthetized decerebrated cats used in our studies of stretch reflexes. One possible mechanism involves fusimotor drive, whereby gamma or beta motoneurons modulate the stretch sensitivity of afferents, in turn affecting the strength of synaptic excitation delivered to motoneurons by those afferents (Matthews, 1972). By this mechanism, the stretch reflex could increase if the central nervous system were to respond to crush injury by increasing fusimotor drive, which recovers well from nerve crush (Brown & Butler, 1976; Hyde & Scott, 1983; reviewed by Zelena, 1994; Scott, 1996). Another mechanism that might augment the stretch reflex is an increase in net excitation of motoneurons mediated through stretch-activated polysynaptic pathways

(Jankowska & Edgley, 2010). Although not established for polysynaptic influences on the stretch reflex, altered transmission has been documented through other segmental motor circuits (e.g. Girlanda *et al.* 2000; Valero-Cabre & Navarro, 2002; for review see Navarro, 2009). In the case of nerve-cut regeneration, we have proposed that alteration of polysynaptic circuits might eliminate stretch reflexes by suppressing strEPSPs (Haftel *et al.* 2005).

By yet another mechanism, stretch reflex recovery might be augmented by the process in which motoneurons transform synaptic input into motor output (motor unit firing and force generation). This transform, i.e. input/output gain, is not static but can change several-fold in response to the activity level of persistent inward currents (PICs) that are intrinsic to motoneurons and regulated by descending monoaminergic input to motoneurons (Heckman *et al.* 2005). An increase in motoneuron gain occurring after nerve crush could explain how diminished input might yield supranormal output. While it is unknown whether the contribution of PIC to motoneuron excitability changes after nerve injury and regeneration, PIC does exhibit plasticity after spinal cord injury and may be responsible for the muscle spasms observed in the spasticity syndrome (Li & Bennett, 2003; Norton *et al.* 2008). Similarly, an increase in PIC and the associated increase in input/output gain could compensate for diminished sensory feedback to restore the stretch reflex.

In sum, our findings strongly suggest that the degree of successful reconnection between regenerating spindle afferents and their receptors following nerve crush is the sole determinant in restoring monosynaptic input from spindle afferents to motoneurons. The degree of partial restoration of monosynaptic input following crush-regenerated afferents was not sufficient, however, to account for greater-than-normal recovery of the reinnervated muscle's reflex contraction in response to stretch. These findings, as well as our recent studies of regenerated-cut nerves in rat (Alvarez *et al.* 2010), suggest that mechanisms within the spinal cord contribute substantially beyond peripheral mechanisms in determining recovery of the stretch reflex.

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### Author contributions

Conception and design of the experiments: T.C.C. Collection, analysis and interpretation of data: J.F.P., P.N., S.T.N., K.T.R., T.R.N., M.J.P. and T.C.C. Drafting the article or revising it critically for important intellectual content: J.F.P., S.T.N., T.R.N., M.J.P. and T.C.C. All experiments were performed in the Cope laboratory except two, which were performed in the Nichols laboratory. All authors approved the final version of the manuscript.

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