REPRESSION OF INACTIVE MOTOR NERVE TERMINALS IN PARTIALLY DENERVATED RAT MUSCLE AFTER REGENERATION OF ACTIVE MOTOR AXONS

By RICHARD R. RIBCHESTER* AND TORFINN TAXT

*From the Institute of Physiology, Karl Johans Gate 47, Oslo, Norway and the
*Department of Physiology, University Medical School, Teviot Place,
Edinburgh EH8 9AG*

SUMMARY

1. The fourth deep lumbrical muscle in the hind foot of adult rats was partially denervated by crushing the sural nerve (s.n.). The denervated muscle fibres became completely reinnervated by sprouts from lateral plantar nerve (l.p.n.) motor axons. By about 20 days after the nerve crush, s.n. motor axons started to reinnervate the muscle.

2. In control muscles, a small proportion of the muscle fibres – about 2·5% of the muscle per motor unit – was reinnervated by s.n. motor axons over the following 20 days. Hence the regenerating terminals were able to re-establish functional synapses, despite the fact that all the muscle fibres were functionally innervated by l.p.n. terminals.

3. When nerve impulse conduction in the l.p.n. was blocked with tetrodotoxin for up to 2 weeks, starting from the time when s.n. axons returned to the muscle, s.n. motor axons retrieved a much larger proportion of the muscle fibres – about 6·5% of the muscle per motor unit. There was a concomitant decrease in the tension produced by the sprouted l.p.n. motor axons. Intracellular recordings showed that many muscle fibres became innervated exclusively by regenerated s.n. motor nerve terminals. Measurements of end-plate potentials suggested that l.p.n. sprouts and the original nerve terminals were eliminated non-selectively. These results suggest that regenerating, active motor nerve terminals have an additional competitive advantage in reinnervating innervated muscles, if the intact terminals are inactive.

4. When the l.p.n. was cut, rather than blocked, extensive reinnervation by the s.n. occurred – about 30% of the muscle per motor unit. This suggests that the absence of an intact nerve terminal in the motor end-plate provides a stronger stimulus than inactivity for synapse formation by regenerating motor axons.

INTRODUCTION

Neuronal connexions are not immutable; they can be modified by external stimuli. The most effective stimulus is damage to connexions made on nearby cells. This may cause sprouting of the intact innervation and replacement of the damaged synapses (reviewed by Cotman, Nieto-Sampedro & Harris, 1981). Connectivity also changes

---

* Address for all correspondence and reprint requests.

in response to non-destructive cues in the environment. For instance, a restricted visual experience during early development leads to permanent changes in the synaptic organization of the visual cortex (Hubel, Wiesel & LeVay, 1977; Rauschecker & Singer, 1981). It is an intriguing possibility that these kinds of effects share common cellular mechanisms.

It is now well known that partial denervation of skeletal muscle causes intact motor axons to sprout and reinnervate denervated muscle fibres (Van Harreveld, 1945; Edds, 1950; Brown, Holland & Hopkins, 1982). If the damaged motor axons are allowed to regenerate, re-formation of neuromuscular junctions occurs. In lower vertebrates, the regenerating motor axons may retrieve all of their original muscle fibres, displacing the sprouted nerve terminals (Bennett & Raftos, 1977; Wigston, 1980). In mammals, however, fewer muscle fibres are reinnervated by the regenerating axons, especially if the nerve injury is restricted to only a few of the axons innervating the muscle (Guth, 1962; Brown & Ironton, 1978) or if the reinnervation is delayed by cutting or resecting the nerve (Frank, Jansen, Lømo & Westgaard, 1975; Thompson, 1978). What is clear from these studies is that regenerating motor axons are able to compete with terminals on innervated muscle fibres. The outcome can be resolved in favour of the regenerating terminals, causing complete regression of intact terminals. In the extreme case, when a foreign nerve is implanted into the end-plate region of a normal innervated muscle, some of the intact terminals are displaced (Bixby & Van Essen, 1979).

One explanation of these effects is that the large (intact) motor units are at a competitive disadvantage because their cell bodies must support many terminals compared with the regenerated motoneurones, which have few or no terminals (Brown & Ironton, 1978; Jansen, Thompson & Kuffler, 1978). It is not known to what extent other factors, such as activity, might influence the attempted reinnervation of innervated muscle fibres by regenerating motor axons.

The results of a previous paper (Ribchester & Taxt, 1983*b*) suggest that the level of activity of a motoneurone influences the outcome of competition between regenerating terminals during reinnervation of a completely *denervated* muscle. The aim of the present study was to determine whether regenerating motor axons were able to reinnervate more muscle fibres when a completely *innervated* muscle was made inactive. We attempted this by making a minor partial denervation of a muscle. The denervated muscle fibres quickly became innervated by sprouts from the remaining intact motor axons. Later, when the regenerating motor axons returned to the muscle, the activity of the intact motor units was blocked selectively with tetrodotoxin (Betz, Caldwell & Ribchester, 1980; Ribchester & Taxt, 1983*b*). The results suggest that in these muscles more muscle fibres were innervated by regenerating motor axons than was the case in completely innervated muscles in which the intact nerve was not blocked.

Preliminary results have been presented to the Physiological Society (Ribchester & Taxt, 1983*a*).

METHODS

The experiments exploited the dual innervation of the fourth deep lumbrical muscle in the rat hind foot (Fig. 1). The procedures for blocking activity in the lateral plantar nerve (l.p.n.) and for measuring tension, making intracellular recordings and staining with zinc iodide–osmium (ZIO) have been described previously (Betz *et al.* 1980; Ribchester & Taxt, 1983*b*). The difference in the present experiments was the location of the initial nerve crush.
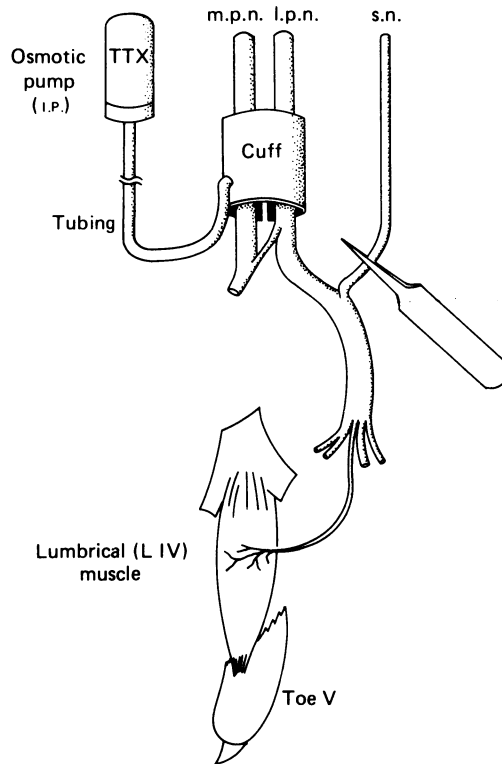

Fig. 1. Diagram showing the experimental design. Bilateral partial denervation of the fourth deep lumbrical muscle was produced by crushing the sural nerve (s.n.). After 21–28 days activity in the medial plantar nerve (m.p.n.) and the lateral plantar nerve (l.p.n.) on one side was blocked by chronic superfusion with tetrodotoxin (TTX) from an osmotic minipump, which was implanted intraperitoneally. Note that this procedure produced a complete block of nerve impulse conduction in the intact nerve supply to the muscle during regeneration of the crushed (s.n.) motor axons (which were not blocked).

The hind foot was partially denervated by crushing the branch of the sural nerve (s.n.) which joins by anastomosis with the l.p.n. in the region of the ankle. The operations were performed bilaterally in animals anaesthetized with sodium pentobarbitone (Mebumal, 60 mg/kg). This operation produced a minor partial denervation of the muscle, because the s.n. is usually the source of only one or two of the motor axons which innervate the lumbrical muscle and the l.p.n. supplies about eight motor axons. Therefore, only a small amount of sprouting of each remaining l.p.n. axon was required for the muscle to become completely reinnervated. Three to four weeks after the operation, when regenerating s.n. axons had just returned to the muscle (see Results), activity in the l.p.n. and medial plantar nerve (m.p.n.) on one side was blocked by chronic superfusion of the nerves with tetrodotoxin (TTX, Sigma; 0·25 μg/h). The quality of the nerve block was assessed

daily by brief pinching of the toe pads with forceps (Ribchester & Taxt, 1983*b*). In another group of rats the l.p.n. was cut at this time rather than blocked. This permitted a comparison of reinnervation of muscles by the s.n. when its axons returned either to control muscles, to completely innervated but inactive (l.p.n.-blocked) muscles, or to denervated (l.p.n.-cut) muscles. After an interval of up to 2 weeks the nerve block was tested in the anaesthetized animals by stimulating above and below the nerve cuff. The lumbrical muscles and the attached nerves were then isolated for tension measurements and intracellular recording (Ribchester & Taxt, 1983*b*; Taxt, 1984).

One additional pair of muscles was operated on for l.p.n. block in the same way as the others, but after 11 days the l.p.n. and m.p.n. were cut. Two days later the muscles were isolated, tension measurements were made and the nerve terminals were stained with ZIO (Betz *et al.* 1980). Neither muscle responded to stimulation of the distal stump of the l.p.n.

All the experiments were done in Oslo.

<div align="center">RESULTS</div>

Sprouting and reinnervation

Up to 16 days after the s.n. crush, stimulation of the s.n. produced no tension response. Tension measurements were made in a group of nine muscles with no s.n. input, 14–20 days after s.n. crush. The ratio of the twitch tension obtained by l.p.n. stimulation to that obtained by direct muscle stimulation was 0.99 ± 0.03 (mean $\pm$ S.D.). Intracellular recordings were made from 103 muscle fibres in seven of the muscles. No muscle fibre in these muscles was innervated by the s.n. and all but one produced an end-plate potential (e.p.p.) to l.p.n. stimulation. These measurements suggest that by the time s.n. motor axons returned, the intact l.p.n. motor axons had sprouted and reinnervated all the muscle fibres (cf. Brown & Ironton, 1978; Thompson, 1978; Betz *et al.* 1980).

In some muscles, reinnervation by s.n. motor axons occurred as early as 16 days after the nerve crush. Examples of twitch tension recordings made from control, l.p.n.-blocked, and l.p.n.-cut muscles more than 35 days after s.n. crush are shown in Fig. 2. These recordings show first, that the s.n. tension response occurred with a much longer latency than the l.p.n. tension response (Fig. 2*A*, *B*). This is presumably because the conduction velocity of action potentials in the regenerated motor axons was lower than in unoperated muscles (Sanders & Whitteridge, 1946). Second, the s.n. twitch responses were larger in the l.p.n.-blocked muscles than in the reinnervated controls. Third, there was significant summation of the l.p.n. and s.n. twitch responses when both nerves were stimulated simultaneously in normal (Fig. 2*C*) and l.p.n.-blocked muscles, but not in the reinnervated controls (Fig. 2*B*). This is important because it suggests that a significant fraction of the l.p.n.-blocked muscles became innervated only by s.n. motor axons. Finally, the s.n. tension in muscles with the l.p.n. cut, rather than blocked, was much larger than in the other cases (Fig. 2*D*).

Motor unit sizes

L.p.n.-blocked and control muscles. The difference between s.n. tensions in the l.p.n.-blocked and control muscles was evident at the level of individual motor units. Examples of twitch tension recordings to graded nerve stimulation are shown in Fig. 3. These kinds of recordings allowed the number of l.p.n. and s.n. motor units and
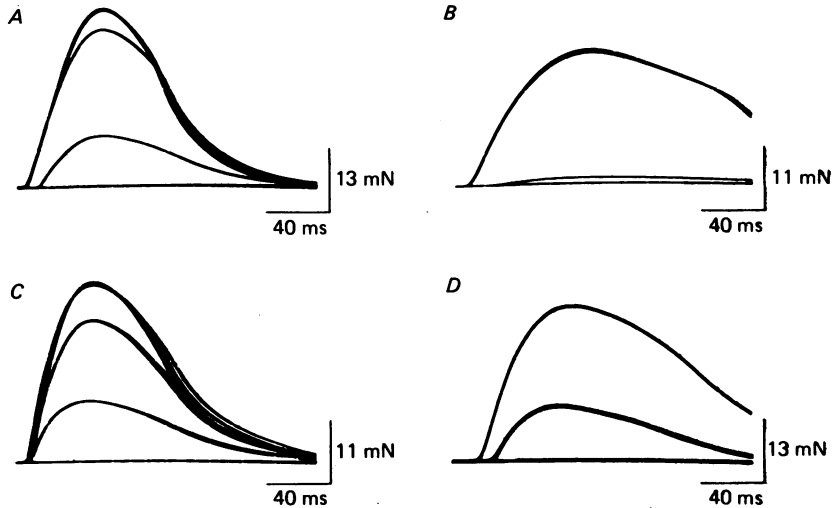
Fig. 2. Superimposed twitch tension recordings from a partially denervated and s.n.-reinnervated, l.p.n.-blocked muscle (*A*), a contralateral s.n.-reinnervated control muscle (*B*), an unoperated lumbrical muscle (*C*) and an s.n.-reinnervated muscle to which the l.p.n. was cut 21 days after the s.n. crush (*D*). In *A–C*, three traces are superimposed in addition to the base line; the lower trace is the response to s.n. stimulation, the middle trace l.p.n. stimulation and the upper trace combined nerve stimulation. In *B*, combined nerve stimulation resulted in no more tension than stimulating the l.p.n. alone, producing completely superimposed traces. In *D*, the lower trace is the response to s.n. stimulation and the upper trace is the response to direct (bath-applied) muscle stimulation. The numbers of s.n. motor units in these muscles were as follows: *A*, 3; *B*, 1; *C*, 3; *D*, 1.
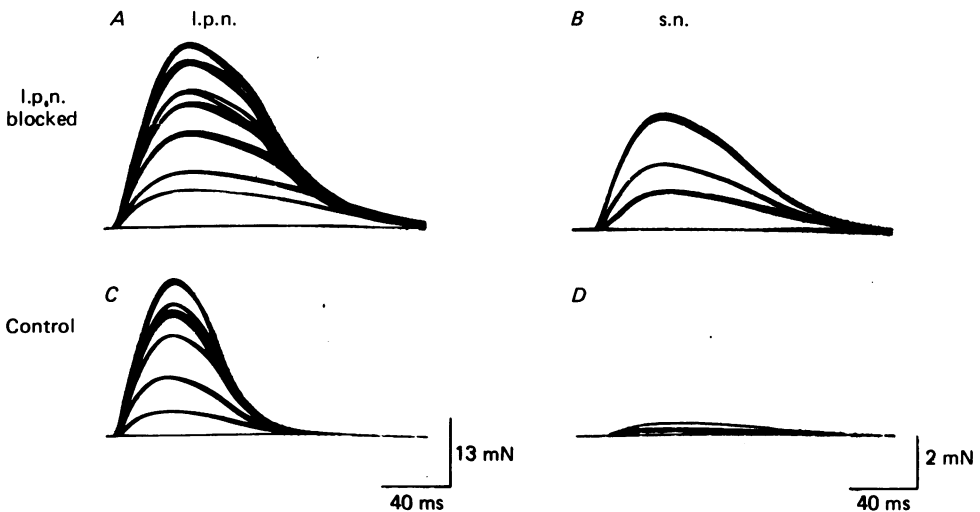

Fig. 3. Responses of s.n.-reinnervated lumbrical muscles to graded stimulation of the l.p.n. and the s.n. in an l.p.n.-blocked muscle (*A*, *B*) and the contralateral control (*C*, *D*). The l.p.n. was blocked for 10 days. In *A*, the time course of the l.p.n. twitch was prolonged but there was a normal number of motor units. Both muscles contained three s.n. motor units. Note the difference in the tension calibrations.

the average size of s.n. motor units to be determined (Fig. 4). Both sets of recordings in Fig. 3 are from one animal, whose right l.p.n. was blocked for 11 days, starting from 28 days after s.n. crush. There were three s.n. motor units on both the l.p.n.-blocked and control sides. The total tension produced by the three units on the l.p.n.-blocked side was about 10 times that on the control side. Each of the s.n. units on the l.p.n.-blocked side was larger than the combined tension response of the s.n. units on the control side. In spite of this difference, the s.n. units of the l.p.n.-blocked side were usually not larger than the l.p.n. units on either side.
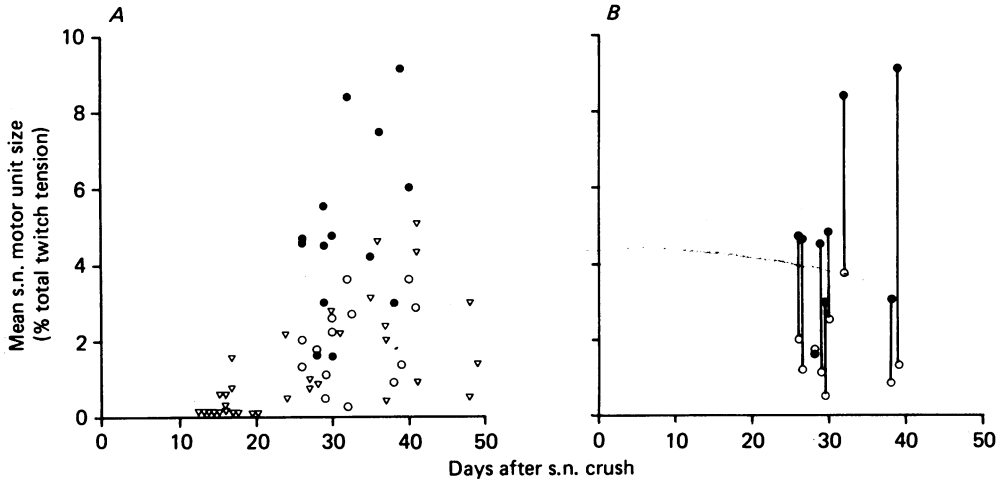

Fig. 4. Reinnervation of lumbrical muscles after crushing the s.n. Each point is the mean tension produced by stimulation of the s.n. divided by the number of s.n. motor units and expressed as a percentage of the total muscle tension. A, data are shown for l.p.n.-blocked muscles (●), contralateral controls (○) and muscles from animals which received no TTX (▽). B, data from nine pairs of the muscles shown in A, in which both the l.p.n.-blocked muscles and the contralateral controls received innervation from the s.n.

In normal lumbrical muscles, each s.n. motor axon innervates about 8 % of the muscle (Betz et al. 1979; see also Fig. 5A). Thirty-five days or more after s.n. crush the mean tension produced by the regenerated motor axons was only 2.5 ± 1.6 % of the total tension (Fig. 4A, Table 1). This suggests that each s.n. motor axon reinnervated only about one-third of the number of muscle fibres innervated by s.n. axons in normal, unoperated muscles.

In l.p.n.-blocked muscles the percentage of the total tension obtained by stimulating the s.n. increased with time. After 35 days post-crush, stimulation of the s.n. produced 6.4 ± 2.3 % of the muscle tension per motor unit. This was not significantly different from the s.n. motor unit size in unoperated muscles (Fig. 5A; $P < 0.05$, Wilcoxon test). The l.p.n. tetanic tension decreased to about 89 % of the total muscle tension. Even before 36 days post-crush, the s.n. motor units were significantly larger (Table 1) but the l.p.n. tension was not significantly different from controls.

A paired comparison of the s.n. motor unit sizes from l.p.n.-blocked and contralateral control muscles was possible in nine animals (Fig. 4B). In the remainder, one or other of the muscles on the two sides contained no s.n. input. The s.n. motor units on the

TABLE 1. Summary of twitch and tetanic tension data from l.p.n.-blocked and control muscles following s.n. reinnervation. Data from contralateral control muscles were combined with data from animals which received no TTX, as there was no significant difference between them. N = number of muscles

	Days post-crush			
	16–35 days		36–52 days	
	Control	L.p.n.-blocked	Control	L.p.n.-blocked
Number of motor units				
S.n.	2·0±1·1	1·4±0.5	2·0±1·1	3·0±0·7
L.p.n.	8·2±1·5	8·6±2·0	8·3±1·8	6·5±1·8
S.n. motor unit size (% twitch tension)	1·4±1·0	4·3±1·9*	2·5±1·6	6·4±2·3*
L.p.n. tension (% total tetanic tension)	97·1±2·9	97·5±2·9	97·9±2·4	89·2±4·5*
N	25	10	13	4

* Significantly different from control ($P < 0.01$, t test).

l.p.n.-blocked side were larger in all but one of the nine cases by factors of between 3 and 10.

The difference in s.n. motor unit size between l.p.n.-blocked and control muscles cannot be explained by any difference in the number of axons innervating the muscles as there was no such difference (Table 1). Neither can it be explained by any systemic effect of TTX (Taxt, 1984), as there was no difference in the mean size of s.n. motor units between contralateral reinnervated control muscles and s.n.-reinnervated muscles from animals which were not subsequently operated for l.p.n. block and which therefore received no TTX (Fig. 4*A*).

In two partially paralysed and one control muscle, very small motor units (0·5–1·0 % of the total tension) were the only ones found in the s.n., up to 50 days after s.n. crush. In these cases the interval between the l.p.n. and s.n. twitches was about 60 ms and the rise time of the s.n.-evoked twitches was also approximately 60 ms. Normally, these values were no more than 20 ms and 40 ms respectively. The responses were obtained at stimulus intensities between 3 and 5 V, while the s.n. motor axons in other preparations had threshold of 2–3 V. Because these were unusual responses, we excluded them from the data. We do not know the explanation of these small twitch contractions. Perhaps they were due to reinnervation of extrafusal fibres by γ-efferents, or autonomic motor nerve fibres, or perhaps they represented sprouts from s.n. motor axons which innervate other muscles in the foot (cf. Emonet-Denand, Laporte & Proske, 1971; Landmesser, 1971; Bennett, McLachlan & Taylor, 1973; Grinnell, Letinsky & Rheuben, 1979; Brown, Hopkins & Keynes, 1982).

L.p.n.-cut muscles. In one group of animals the l.p.n. was cut 20–25 days after the s.n. crush, so that the regenerating s.n. motor axons could be confronted with a completely denervated target. The aim was to determine the maximum capability of s.n. axons to re-form synapses in the muscle. The s.n. motor axons quickly reinnervated a larger fraction of the muscles than in any of the other cases. Within 7 days, stimulation of the s.n. motor units produced 31·2±22·1 % of the total tension and no motor unit produced less than 10 %.

The distributions of the s.n. motor unit twitch tensions in all three kinds of
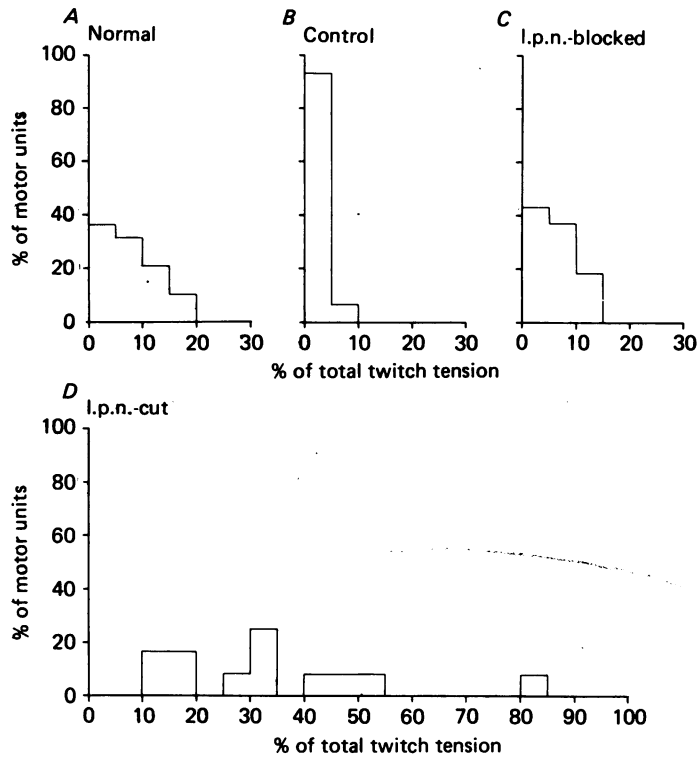
Fig. 5. Distribution of s.n. motor unit sizes in unoperated (A), s.n.-reinnervated (B), s.n.-reinnervated, l.p.n.-blocked (C) and s.n.-reinnervated, l.p.n.-cut muscles (D) 32 days or more (B, C) and 28 days or more (D) after s.n. crush. Numbers of muscles (N) and numbers of motor units (n) were as follows: A, N = 7, n = 19; B, N = 7, n = 15; C, N = 6, n = 16; D, N = 7, n = 12. The mean motor unit size was not significantly different between A and C and both were significantly different from that in B (P < 0·01, Wilcoxon test).

experimental muscle and in normal, unoperated muscles are summarized in Fig. 5. S.n. motor units in normal muscles produced $7·7 \pm 4·6 \%$ of the total tension (cf. Betz et al. 1979).

Single and polyneuronal innervation of l.p.n.-blocked muscles

It was important to determine whether the regenerating axons innervated any muscle fibres exclusively, because this would show whether the active motor nerve terminals were able to displace the inactive terminals that were already present. We therefore measured the amount of dual and single innervation of muscle fibres, using tension overlap measurements and intracellular recording.

Tension overlap. Measurement of the deficit in tetanic tension obtained by separate and combined stimulation of the l.p.n. and s.n. suggested that about 50–75 % of the muscle fibres innervated by regenerated s.n. motor axons were also innervated by l.p.n. axons at all times after reinnervation (Table 2). This suggests that a substantial number of fibres were innervated only by s.n. motor axons. The difficulty with these

TABLE 2. Dual innervation of muscle fibres in l.p.n.-blocked muscles studied with both tension measurements and intracellular recording. The fraction of the muscle innervated by s.n. motor axons also innervated by l.p.n. motor axons is expressed as a percentage of the s.n. innervation. Numbers in parentheses below the intracellular recording data are total numbers of muscle fibres impaled. N = number of muscles

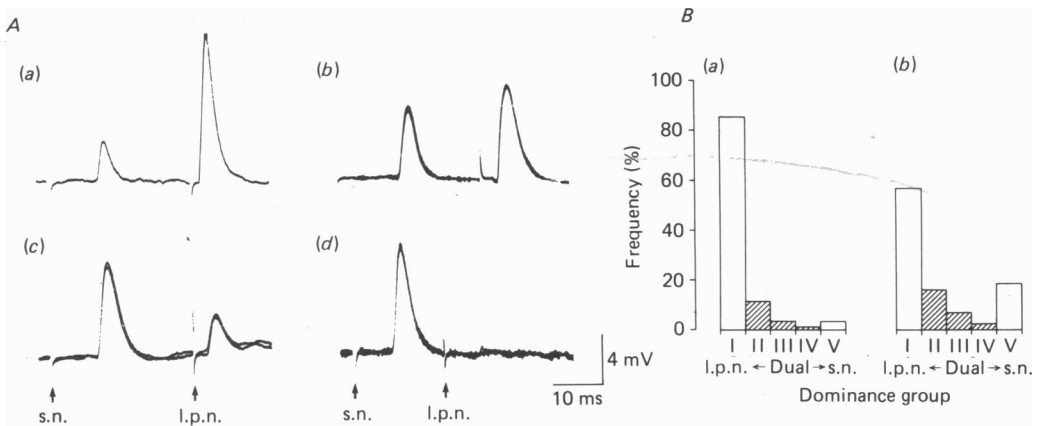|	Dual innervation (% s.n. innervation)	
Days post s.n. crush	Tetanic tension overlap	Intracellular recording
16–35 (N = 4)	62·1 ± 7·0	62·5 ± 25·0 (99)
36–52 (N = 3)	50·6 ± 20·6	56·4 ± 19·3 (138)

Fig. 6. *A*, examples of intracellular recordings from cut muscle fibre preparations of l.p.n.-blocked muscles between 30 and 40 days after s.n. crush. The s.n. was stimulated first and the l.p.n. about 20 ms later. Most fibres with s.n. input were also innervated by l.p.n. motor nerve terminals (*a–c*) – hence the appearance of two e.p.p.s. A significant number of muscle fibres was also innervated only by s.n. terminals (*d*). *B*, nerve dominance histograms (Ribchester & Taxt, 1983*b*) from two l.p.n.-blocked muscles 36 and 39 days after s.n. crush. Open bars, fibres innervated exclusively by the l.p.n. (group I) or by the s.n. (group V). Hatched bars, fibres innervated by both l.p.n. and s.n. terminals. Group II, l.p.n.-e.p.p. more than twice the size of the s.n.-e.p.p.; Group III, l.p.n.-e.p.p. one-half to twice the size of the s.n.-e.p.p.; Group IV, s.n.-e.p.p. more than twice the size of the l.p.n.-e.p.p. (*a*) 10 days blocked, nine l.p.n. motor units, three s.n. motor units, eighty-two fibres impaled; (*b*) 11 days blocked, seven l.p.n. motor units, three s.n. motor units, forty-four fibres impaled.

data is that polyneuronally innervated muscle fibres with a subthreshold input from either nerve would be overlooked by a tension overlap measurement.

Intracellular recording. In all, fifty-nine out of 237 fibres recorded from seven muscles were innervated by s.n. motor axons (Table 2, Fig. 6*A*). Most of these fibres were also innervated by axons in the l.p.n. There was no difference in the rise time of e.p.p.s from either nerve in dual-innervated muscle fibres, which suggests that the terminals were located on the same or closely adjacent motor end-plates. Significant numbers of fibres were innervated only by s.n. axons. Nerve dominance histograms (Ribchester & Taxt, 1983*b*) from two muscles are shown in Fig. 6*B*. In these muscles

thirty-two of the 139 muscle fibres impaled (i.e. about 23 %) were innervated by the s.n. and twelve of these were innervated only by the s.n. The amount of dual innervation (63 % of the fibres innervated by the s.n.) was similar to that calculated from the tetanic tension overlap measurements. Thus, although some subthreshold synaptic inputs were present in these muscles (Fig. 6 *Aa,c*; dominance groups II and IV in Fig. 6 *B*), most inputs were suprathreshold.
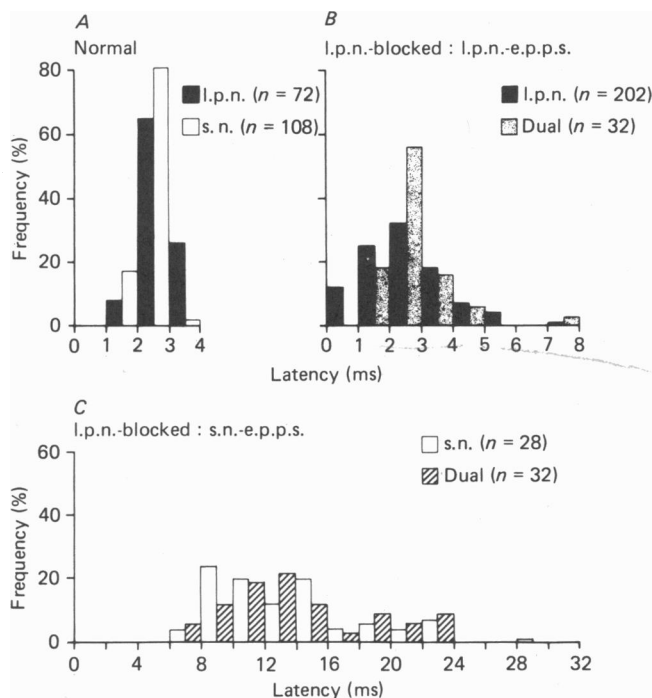

Fig. 7. Distribution of e.p.p. latency in unoperated (*A*) and l.p.n.-blocked muscles after s.n. crush (*B, C*). The mean latencies of s.n.-e.p.p.s and l.p.n.-e.p.p.s in unoperated muscles were not significantly different. In the l.p.n.-blocked muscles the latency of the l.p.n.-e.p.p.s in fibres innervated exclusively by l.p.n. motor axons was not significantly different from that of dual-innervated muscle fibres (*B*). The mean latencies were significantly longer than in unoperated muscles (see text). The mean latency of s.n.-e.p.p.s (*C*) was significantly greater than that of the l.p.n.-e.p.p.s in both dual-innervated muscle fibres and in fibres innervated only by the s.n.

The possibility that the regenerating s.n. terminals only competed for innervation of the muscle fibres which they innervated originally was investigated by studying the latencies of the e.p.p.s. Evidence that s.n. terminals also competed for innervation of muscle fibres innervated by the original l.p.n. terminals (rather than by sprouts) was obtained. Histograms of e.p.p. latency in four normal unoperated and six l.p.n.-blocked muscles are shown in Fig. 7.

In the normal muscles, stimulation of the l.p.n. or the s.n. always produced an e.p.p. with a latency of less than 4 ms. In l.p.n.-blocked muscles about 5 % of the muscle fibres produced e.p.p.s to l.p.n. stimulation (l.p.n.-e.p.p.s) with latencies greater than 4 ms. These long-latency responses were probably due to slowly conducting non-

myelinated terminal sprouts. The latencies of l.p.n.-e.p.p.s in dual-innervated fibres fell within the same range as in singly innervated fibres; thus twenty-nine out of thirty-two of the dual-innervated fibres had l.p.n.-e.p.p.s with a latency of less than 4 ms.

The latency of e.p.p.s to s.n. stimulation (s.n.-e.p.p.s) in these muscles was greater than that of l.p.n.-e.p.p.s (Fig. 6), presumably because of a lower conduction velocity in the regenerated motor axons. There was no difference in the distribution of s.n.-e.p.p. latencies between dual-innervated muscle fibres and fibres innervated only by the s.n.
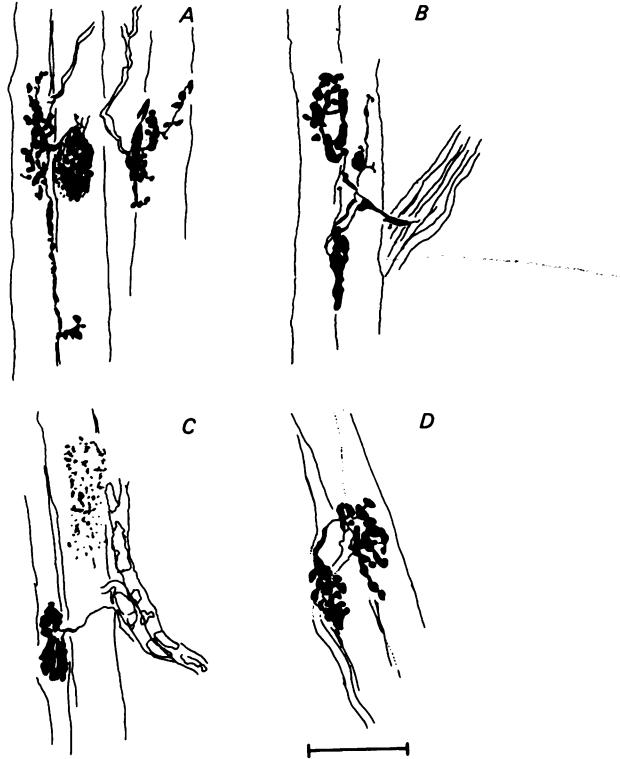

Fig. 8. Camera lucida drawings of s.n. terminals stained with zinc iodide–osmium from an l.p.n.-blocked muscle (*A, B*) and its contralateral control (*C, D*). The l.p.n. on the right side was blocked for 11 days and then the l.p.n. on both sides was cut 2 days before isolating the muscles. Calibration, 40 *μ*m.

The tensions produced by s.n. stimulation in most control muscles were so small that it was impossible to measure tension overlap reliably (Table 1, Fig. 2*B*). For the same reason, we did not make intracellular recordings routinely from the control muscles. Two control muscles with reasonable tension responses to s.n. stimulation were studied. One of the muscles contained four s.n. motor units, which produced a total of 14 % of the total twitch tension. The amount of tetanic tension overlap suggested that 25 % of the muscle fibres innervated by the s.n. were also innervated by the l.p.n. Intracellular recordings were made from the region of the muscle which was seen to contract when the s.n. was stimulated. Recordings were obtained from eleven muscle fibres innervated by the s.n. and two of these fibres also produced an e.p.p. to l.p.n. stimulation. An extensive search of the rest of the muscle for s.n.-innervated fibres produced six more, of which only one was polyneuronally innervated.

In the other control muscle there were three s.n. motor units, which produced altogether 4·1 % of the total twitch tension. S.n. stimulation produced an e.p.p. in one muscle fibre out of twenty impaled, and this fibre also received an l.p.n. input.

Zinc iodide–osmium staining

Nerve terminals belonging to s.n. motor axons were examined in one pair of muscles stained with zinc iodide–osmium (ZIO). There were three s.n. motor units on the l.p.n.-blocked side and one on the control side, but the units on the blocked side produced about five times the tension of the unit on the control side.

Camera lucida drawings of motor nerve terminals are shown in Fig. 8. Most of the muscle fibres in the ZIO-stained preparations had no nerve terminal. Those terminals that were stained belonged to the s.n. motor axons. There were 151 terminals in the l.p.n.-blocked muscle and thirty-eight in the control muscle. The ratio of the numbers of terminals in the two muscles was therefore similar to the ratio of the nerve-evoked tensions. In the l.p.n.-blocked muscle, many teased bundles of fibres contained nerve terminals. Many had long terminal sprouts and terminals on adjacent muscle fibres were joined by sprouts (Fig. 8*A*, *B*). In the control muscle the terminals were scattered throughout. Fig. 8 *D* shows one of the few instances of terminals on adjacent muscle fibres. The terminals appeared to be located at the original motor end-plates, judging by the distance of stained terminals from the degeneration debris of l.p.n. terminals on adjacent fibres (Fig. 8*C*). The s.n. terminals were of similar appearance to terminals in unoperated muscles, but the preterminal axons were rather narrow.

DISCUSSION

The present results confirm that when motor axons regenerate into the end-plate region of a completely innervated muscle, some intact nerve terminals regress and permit the formation of new synapses by the regenerating axons (Brown & Ironton, 1978; Thompson, 1978; Bixby & Van Essen, 1979). The results also show that when the muscles are completely inactive because of nerve impulse conduction block, then more terminals are displaced by the regenerating active synapses. These observations therefore support and extend the conclusions of the previous study (Ribchester & Taxt, 1983*b*), in which inactive and active terminals were allowed to compete for synaptic sites in completely denervated muscle. Since the selective regression of the inactive terminals in both cases was entirely dependent on the presence of the active nerve terminals, the inactive terminals must have been repressed through interaction with the active terminals. We do not know whether the repression of the inactive terminals involves a gradual reduction in the quantum content of e.p.p.s, as in reinnervated amphibian muscle (Dennis & Yip, 1978; Wigston, 1980), or whether it occurs more suddenly. Continuous intracellular recording during synapse elimination would settle this point.

The outcome of the present experiments can be compared in more detail with that of our previous experiments (Ribchester & Taxt, 1983*b*). In those experiments, lumbrical muscles were completely denervated by crushing the muscle nerve. Both l.p.n. and s.n. motor axons returned to the muscle at the same time. Upon

reinnervation, nerve impulse conduction was blocked in the l.p.n. for up to 2 weeks, in the same way as described in the present experiments. Thus, apart from the inactivity of the l.p.n. axons, s.n. and l.p.n. terminals competed on an equal footing for reinnervation of muscle fibres. The outcome was that the active terminals obtained exclusive innervation of about twice as many muscle fibres as they innervated before the complete denervation. In the present experiments, most muscle fibres were not deprived of their original innervation: the partial denervation produced by s.n. crush removing terminals from only 10–20 % of the muscle fibres. Sprouting of the l.p.n. axons quickly produced reoccupation of the denervated end-plates. Subsequent l.p.n. block produced completely inactive, innervated muscles. Upon regeneration, the active motor axons reinnervated, at most, no more muscle fibres than they innervated before the partial denervation.

The results from both kinds of experiment are compatible with the same cellular mechanism. The discrepancy in the sizes of the motor units could be explained if the rate of expansion of motor units by new synapse formation depended upon the number of synapses formed initially (Cotman *et al.* 1981). In the completely denervated muscles (Ribchester & Taxt, 1983*b*), large s.n. motor units were established at the outset. This is presumably because the regenerating l.p.n. and s.n. motor axons had equal access to vacant motor end-plates. In the partially denervated muscles (present study), all the motor end-plates were occupied and this constituted an additional barrier to synapse formation by the regenerating s.n. motor axons (see also Brown & Ironton, 1978). This conclusion is supported by the observation that when the l.p.n. was cut, rather than blocked, the regenerating s.n. axons rapidly established synaptic contact with large numbers of muscle fibres.

In all the muscles described in the present and previous experiments, inactive muscle fibres became polyneuronally innervated by active and inactive motor axons. This contrasts with the failure to find polyneuronal innervation in partially inactive muscles which were not surgically denervated (Betz *et al.* 1980). Blocking activity in part of the preganglionic nerve supply to the cardiac ganglion of the frog (Roper & Ko, 1978), or blocking activity in the ganglion cells of one eye in the cat (Archer, Dubin & Stark, 1982) or goldfish (Meyer, 1982), also produces only limited increases in the convergence of synaptic input to the respective post-synaptic cells. The amount of nerve sprouting in all these cases appears to be greater after partial denervation (Ko & Roper, 1978; Schmidt, Cicerone & Easter, 1978; Betz *et al.* 1980; Robson, 1981). The differences between these observations and the present results suggest that inactivity alone provides only a weak stimulus for the growth of nerve processes and the formation of new synapses, at least in adult skeletal muscle. Once new connexions are permitted, interactions whose outcome is strongly dependent on the activity of the synaptic inputs may occur.

The situation may be somewhat different during ectopic synapse formation. Inactivity in muscle produced by blocking nerve impulse conduction or synaptic transmission causes the axons of an ectopically implanted nerve to make many synapses (Jansen, Lømo, Nicolaysen & Westgaard, 1973; Tonge, 1977).

assistance in all technical matters. We thank the European Science Foundation for a Twinning Grant and the Nordic Insulin Fund for a grant to purchase osmotic minipumps and TTX.

REFERENCES

ARCHER, M. R., DUBIN, M. W. & STARK, L. A. (1982). Abnormal development of retino-geniculate connectivity in the absence of action potentials. *Science, N.Y.* **217**, 743–745.

BENNETT, M. R., McLACHLAN, E. M. & TAYLOR, R. S. (1973). The formation of synapses in mammalian striated muscle reinnervated with autonomic preganglionic nerves. *J. Physiol.* **233**, 501–517.

BENNETT, M. R. & RAFTOS, J. (1977). The formation and regression of synapses during the reinnervation of axolotl striated muscles. *J. Physiol.* **265**, 261–293.

BETZ, W. J., CALDWELL, J. H. & RIBCHESTER, R. R. (1979). The size of motor units during post-natal development of rat lumbrical muscle. *J. Physiol.* **297**, 463–478.

BETZ, W. J., CALDWELL, J. H. & RIBCHESTER, R. R. (1980). Sprouting of active nerve terminals in partially inactive muscles of the rat. *J. Physiol.* **303**, 265–279.

BIXBY, J. L. & VAN ESSEN, D. C. (1979). Competition between foreign and original nerves in adult mammalian skeletal muscles. *Nature, Lond.* **282**, 726–728.

BROWN, M. C., HOLLAND, R. L. & HOPKINS, W. G. (1981). Motor nerve sprouting. *A. Rev. Neurosci.* **4**, 17–47.

BROWN, M. C., HOPKINS, W. G. & KEYNES, R. (1982). Short- and long-term effects of paralysis of the motor innervation of two different neonatal mouse muscles. *J. Physiol.* **329**, 439–450.

BROWN, M. C. & IRONTON, R. (1978). Sprouting and regression of neuromuscular synapses in partially denervated mammalian muscles. *J. Physiol.* **278**, 325–348.

COTMAN, C. W., NIETO-SAMPEDRO, H. & HARRIS, E. W. (1981). Synapse replacement in the nervous system of adult vertebrates. *Physiol. Rev.* **61**, 684–784.

DENNIS, M. J. & YIP, J. W. (1978). Formation and elimination of foreign synapses on adult salamander muscle. *J. Physiol.* **274**, 299–310.

EDDS, M. V. (1950). Collateral regeneration of residual motor axons in partially denervated muscles. *J. exp. Zool.* **113**, 517–551.

EMONET-DENAND, F., LAPORTE, Y. & PROSKE, U. (1971). Contraction of muscle fibres in two adjacent muscles innervated by branches of the same motor axon. *J. Neurophysiol.* **34**, 132–138.

FRANK, E., JANSEN, J. K. S., LØMO, T. & WESTGAARD, R. H. (1975). The interaction between foreign and original motor nerves innervating the soleus muscles of rats. *J. Physiol.* **247**, 725–743.

GRINNELL, A. D., LETINSKY, M. S. & RHEUBEN, M. A. (1979). Competitive interaction between foreign nerves innervating frog skeletal muscle. *J. Physiol.* **289**, 241–262.

GUTH, L. (1962). Neuromuscular function after regeneration of interrupted nerve fibres into partially denervated muscle. *Expl Neurol.* **6**, 129–141.

HUBEL, D. H., WIESEL, T. N. & LeVAY, S. (1977). Plasticity of ocular dominance columns in monkey striate cortex. *Phil. Trans. R. Soc. B* **278**, 377–409.

JANSEN, J. K. S., LØMO, T., NICOLAYSEN, K. & WESTGAARD, R. (1973). Hyperinnervation of skeletal muscle fibres: dependence on muscle activity. *Science, N.Y.* **181**, 559–561.

JANSEN, J. K. S., THOMPSON, W. & KUFFLER, D. P. (1978). The formation and maintenance of synaptic connections as illustrated by studies of the neuromuscular junction. *Prog. Brain Res.* **48**, 3–18.

Ko, C.-P. & ROPER, S. (1978). Disorganised and 'excessive' reinnervation of frog cardiac ganglia. *Nature, Lond.* **274**, 286–288.

LANDMESSER, L. (1971). Contractile and electrical responses of vagus-innervated frog sartorius muscle. *J. Physiol.* **213**, 707–725.

MEYER, R. L. (1982). Tetrodotoxin blocks the formation of ocular dominance columns in goldfish. *Science, N.Y.* **218**, 589–591.

RAUSCHECKER, J. P. & SINGER, W. (1981). The effects of early visual experience on the cat's visual cortex and their possible explanation by Hebb synapses. *J. Physiol.* **310**, 215–239.

RIBCHESTER, R. R. & TAXT, T. (1983*a*). Competition between active and inactive motor axons after reinnervation of partially denervated rat muscle. *J. Physiol.* **334**, 67–68*P*.

RIBCHESTER, R. R. & TAXT, T. (1983*b*). Motor unit size and synaptic competition in rat lumbrical muscles reinnervated by active and inactive motor axons. *J. Physiol.* **344**, 89–111.

ROBSON, J. A. (1981). Abnormal axonal growth in the dorsal lateral geniculate nucleus of the cat. *J. comp. Neurol.* **195**, 453–476.

ROPER, S. & KO, C.-P. (1978). Impulse blockade in frog cardiac ganglion does not resemble partial denervation in changing synaptic organization. *Science, N.Y.* **202**, 66–68.

SANDERS, F. K. & WHITTERIDGE, D. (1946). Conduction velocity and myelin thickness in regenerating nerve fibres. *J. Physiol.* **105**, 152–174.

SCHMIDT, J. T., CICERONE, C. M. & EASTER, S. S. (1978). Expansion of the half retinal projection to the tectum in goldfish: an electrophysiological and anatomical study. *J. comp. Neurol.* **177**, 257–278.

TAXT, T. (1984). Local and systemic effects of tetrodotoxin on synapse elimination in rat lumbrical muscle. *J. Physiol.* (in the Press).

THOMPSON, W. (1978). Reinnervation of partially denervated rat soleus muscle. *Acta physiol. scand.* **103**, 81–91.

TONGE, D. A. (1977). Effect of implantation of an extra nerve on the recovery of neuromuscular transmission from botulinum toxin. *J. Physiol.* **265**, 809–820.

VAN HARREVELD, A. (1945). Reinnervation of denervated muscle fibres by adjacent functioning motor units. *Am. J. Physiol.* **144**, 477–493.

WIGSTON, D. J. (1980). Suppression of sprouted synapses in axolotl muscle by transplanted foreign nerves. *J. Physiol.* **307**, 355–366.