J. Physiol. (1981), **321**, pp. 211–223
With 2 text-figures
Printed in Great Britain


# DO IDENTICAL ACTIVITY PATTERNS IN FAST AND SLOW MOTOR AXONS EXERT THE SAME INFLUENCE ON THE TWITCH TIME OF CAT SKELETAL MUSCLE?

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(*Received 22 August 1980*)

SUMMARY

1. The fast-twitch flexor digitorum (the lateral head; equivalent to the flexor hallucis longus) and slow-twitch soleus muscles of the cat were denervated, and the two nerves immediately reunited to one or the other muscle. Contraction times of the dually reinnervated muscle were examined 9 weeks post-operatively in response to separate stimulation of its own and the foreign nerve. Over a 5 week period before the terminal experiment, a variety of artificial activity patterns was imposed on the two nerves.

2. Following the dual-union operation, the flexor digitorum muscle was preferentially reinnervated by its own nerve. In contrast, the soleus muscle showed no evidence of preferential reinnervation.

3. When neural activity was not artificially modified, the dually reinnervated flexor digitorum or soleus muscle showed faster contractions in response to stimulation of the flexor digitorum nerve than to stimulation of the soleus nerve.

4. Following a 5 week period in which neural activity was virtually eliminated by cord transection or in which the two nerves were stimulated at the same frequency, the contraction times of the dually reinnervated soleus muscle were the same in response to stimulation of either nerve.

5. In contrast, under the experimental conditions described above (cord transection or nerve stimulation), the dually reinnervated flexor digitorum muscle showed a significantly faster contraction in response to stimulation of its own nerve than to stimulation of the soleus nerve.

6. It is concluded that, when neural activity is absent or identical in pattern, motoneurones normally innervating the fast- or slow-twitch muscles exert the same influence on contraction times of the soleus muscle.

7. The dependence of contraction times of the dually reinnervated flexor digitorum muscle upon the type of the innervating motoneurone may be explained either by selective reinnervation of a particular group of muscle fibres or by different trophic substances emanating from the motoneurones.

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## INTRODUCTION

The cross-reinnervation experiments originally reported by Buller, Eccles & Eccles (1960*b*) leave little doubt that the speed of contraction of mammalian skeletal muscle depends, at least in part, upon the type of the innervating motoneurone. The neural influence upon muscle contraction was then suggested to be due to one or a combination of two factors (Buller *et al.* 1960*b*): (1) the presence of a specific trophic substance emanating from the innervating motoneurones; (2) the discharge frequency of the innervating motoneurones, which in turn determines the pattern of muscle activity.

It is known that the discharge frequency of the motoneurones innervating slow-twitch muscles is lower than that of those subserving fast-twitch muscles (Granit, Henatsch & Steg, 1956; Granit, Phillips, Skoglund & Steg, 1957). The speed of contraction of fast-twitch muscle is reduced following daily stimulation of the nerve at relatively low (5–10/sec) frequencies (Eccles, Eccles & Kozak, 1962; Salmons & Vrbová, 1962; Pette, Smith, Staudte & Vrbová, 1973; Salmons & Sréter, 1976; Buller & Pope, 1977). Conversely, daily nerve stimulation at a high frequency (100/sec) increases the speed of contraction of the slow-twitch soleus muscle (Smith, 1978). Also, a fast-twitch muscle, cross-reinnervated by the soleus nerve, fails to transform its speed of contraction if motoneurone activity is minimized by transection of the spinal cord at the thoracic level (Buller *et al.*, 1960*b*). Furthermore, the soleus muscle cross-reinnervated by the nerve originally innervating a fast-twitch muscle can still maintain its slow contraction as long as the foreign nerve is stimulated at a low frequency (Salmons & Sréter, 1976). Therefore, the contractile properties of a muscle are undoubtedly influenced by the presence of a certain pattern of motoneurone activity, regardless of the type of the innervating motoneurone.

Lømo, Westgaard & Dahl (1974) have shown that the contraction time of the denervated soleus muscle may be altered, depending upon the frequency of stimuli applied directly to the muscle. Thus, the contractile properties can be transformed by a certain pattern of muscle activity even in the absence of any neural trophic factor. The possibility remains, however, that under normal conditions a neurotrophic factor might control muscle contraction. When muscle activity is practically eliminated, either by immobilization of the leg (Fischbach & Robbins, 1969; also see Mann & Salafsky, 1970) or by cord transection (Buller, Eccles & Eccles, 1960*a*; Eccles *et al.* 1962; Salmons & Vrbová, 1969; Hoh & Dunlop, 1975; Gallego, Huizar, Kudo & Kuno, 1978; but cf. Davis & Montgomery, 1977), the soleus muscle is no longer capable of maintaining its slow contraction; yet, contraction time of the soleus muscle becomes slower than normal if muscle activity is eliminated by denervation (Eccles *et al.* 1962; Lewis, 1972, 1973; Lømo *et al.* 1974). Evidently, muscle inactivity exerts different effects on the contractile properties, depending upon the presence or absence of innervation. This suggests that the speed of muscle contraction is subject to a trophic influence from the innervating motoneurones, in addition to being affected by their discharge pattern (see Gallego *et al.* 1978).

The principal question to be examined in the present study is whether the two types of motoneurones normally innervating the fast- and slow-twitch muscles exert the same trophic influence on the innervated muscle if their activity is absent or identical in pattern. To answer this question, a given fast- or slow-twitch muscle was dually reinnervated by its own and foreign nerves, and the same pattern of activity was artificially imposed on the two nerves. Following this procedure, the speed of contraction was examined in the same muscle in response to separate stimulation of its own and foreign nerves.

## METHODS

*Preparation*. Adult cats (1·9–2·9 kg in weight) were anaesthetized by an I.P. injection of sodium pentobarbitone (35 mg/kg). Under aseptic conditions, the nerves to the soleus muscle and the lateral head of the flexor digitorum longus muscle were exposed in the left hind leg. *The lateral head of the flexor digitorum longus is equivalent to the flexor hallucis longus muscle* (see Mommaerts, Seraydarian, Suh, Kean & Buller, 1977), and *this muscle or its nerve is referred to below as the flexor digitorum throughout the paper*. These nerves were sectioned near the muscles, and both their central stumps were either sewn directly into the soleus muscle or reunited to the peripheral stump of the cut flexor digitorum nerve. In the rat, the fast-twitch muscle is known to be reinnervated almost exclusively by its own nerve when this nerve and the soleus nerve compete for reinnervation of the muscle (Hoh, 1975). It is not known whether selective reinnervation of the fast-twitch muscle occurs also in the cat (see Results). However, to minimize the possibility of selective reinnervation, the flexor digitorum nerve was crushed about 10 mm central to the site of the reunion when the flexor digitorum muscle was dually reinnervated. Since the growth rate of injured nerve is 2·8–4·4 mm/day (Gutmann, 1942; Gutmann, Guttmann, Medawar & Young, 1942), regeneration of the flexor digitorum nerve into the muscle might be assumed to lag about 3 days behind that of the soleus nerve under such conditions. In the contralateral leg, the soleus and flexor digitorum nerves were cut and reunited to their own muscles, which then served as self-reinnervated controls. At the time of the reunion operation, the dorsal roots from the sixth lumbar through the first sacral segments were bilaterally sectioned, except in control animals with intact neural activity (see below).

*Neural activity*. About 30 days after reunion of a cut nerve to its muscle, nerve stimulation begins to elicit muscle contraction (Eccles *et al.* 1962; Kuno, Miyata & Muñoz-Martinez, 1974). Therefore, 4 weeks after the reunion operation various patterns of activity were imposed on soleus and flexor digitorum nerves over a further 5 week period. In three groups of animals described below, the spinal cord was transected at the twelfth thoracic level 4 weeks after bilateral dorsal root section and nerve reunion (see above). In the first group of animals, bipolar stimulating electrodes embedded in a hollow silicone cuff were implanted around the sciatic nerve on the left (dual reinnervation) side at the time of cord transection. The sciatic nerve was stimulated continuously every 12 sec at a frequency of 10/sec for 1 sec (mean frequency, 50 pulses/min) through a miniature stimulator mounted on the animals (Smith, 1978). Details of the procedure for chronic nerve stimulation have been described previously (Gallego, Kuno, Núñez & Snider, 1979). The experimental conditions were essentially the same for the second group of animals, except that the sciatic nerve was stimulated every 60 sec at a frequency of 50/sec for 1 sec (mean frequency, 50 pulses/min). Thus, while the two groups received different stimulus patterns (10 or 50/sec), the total number of stimuli applied (50 pulses/min) and the train duration (1 sec) were identical. The stimulus intensity was adjusted daily above twice the threshold for initiation of contractions of the hind leg muscles. The third group did not receive chronic stimulation, and it was assumed that activity of soleus and flexor digitorum motoneurones was virtually, if not competely, absent. The urinary bladder of cord-transected animals was emptied manually every day.

The results obtained from these three groups of animals were compared with those from a control group in which only the dual- (in the left hind leg) and self-union (in the right hind leg) operations were performed, leaving the spinal cord and dorsal roots intact (intact neural activity). Each group consisted of four or five animals. However, in one animal the nerves united to the soleus muscle also reinnervated the flexor digitorum muscle, and in another animal the soleus nerve was partially damaged during dissection in the terminal experiment. The results from these animals were included in the observations on contraction times (Table 2*C*) but excluded from the evaluation of the maximum twitch tensions (Table 1*B*). The mean values of the results obtained under different experimental conditions were examined by two-tailed $t$ tests with the significance limit of $P < 0·05$.

*Experimental procedure.* The terminal experiment was performed 9 weeks after the nerve-union operation in the hind legs (a 4 week period after the nerve suture plus a 5 week exposure to a given pattern of neural activity). The cats were anaesthetized I.P. with sodium pentobarbitone (35 mg/kg). Both common carotid arteries were ligated, the trachea cannulated and the vertebral arteries permanently clamped. The loss of the brain function due to ischaemia was shown to be complete.

TABLE 1. Twitch tensions of dual-and self-reinnervated muscles in intact and cord-transected animals. F, flexor digitorum; S, soleus. Small letter preceding each arrow indicate the nerves used for stimulation. Capital letters following each arrow indicate the muscles in which contractions were observed. All values give the mean ± S.D. All tensions (*a–d*) in g. *n*, numbers of muscles examined

| | Dual reinnervation side (left) | | Self reinnervation side (right) | | Ratio |
|---|---|---|---|---|---|
| | *a* | *b* | *c* | *d* | *e* |
| Dual reinnervation of the left soleus muscle | s → S | f → S | s → S | f → F | a/c |
| *A* Cord intact | 122 ± 83 (*n* = 5) | 39·4 ± 23 (*n* = 5) | 214 ± 49 (*n* = 5) | 514 ± 207 (*n* = 5) | 0·557 ± 0·306 (*n* = 5) |
| *B* Cord transection | 28·8 ± 8·9* (*n* = 3) | 77·2 ± 18* (*n* = 4) | 61·3 ± 38* (*n* = 5) | 325 ± 164 (*n* = 5) | 0·428 ± 0·188 (*n* = 3) |
| Dual reinnervation of the left flexor digitorum muscle | f → F | s → F | s → S | f → F | a/d |
| *C* Cord intact | 505 ± 39 (*n* = 4) | 106 ± 41 (*n* = 4) | 198 ± 50 (*n* = 4) | 567 ± 70 (*n* = 4) | 0·901 ± 0·131· (*n* = 4) |
| *D* Cord transection | 455 ± 194 (*n* = 5) | 121 ± 96 (*n* = 5) | 84·4 ± 41* (*n* = 5) | 625 ± 257 (*n* = 5) | 0·757 ± 0·299 (*n* = 5) |

* Significant difference between intact and cord-transected animals.

† Significant difference from 0·60.

by total cessation of respiratory movements and by dilation of the pupils. The spinal cord was then divided at the atlanto-occipital membrane, and in addition, the pontobulbar region was permanently destroyed mechanically, using a haemostat inserted through the atlanto-occipital foramen. The animal was then maintained on artificial respiration with end-tidal $CO_2$ levels held at 2·5–3·5 %. External heat kept the rectal temperature at 36–38 °C.

In the left hind leg, the distal tendon of the dually reinnervated muscle was cut and attached to a force displacement transducer (Grass FT 10). The soleus and flexor digitorum nerves were dissected and cut, and their distal stumps were prepared for separate stimulation. Isometric contractions of the muscle were measured at an initial tension level of 100 g in a pool of paraffin oil maintained at 37 °C. On the contralateral (self-reinnervation) side, isometric twitches were similarly examined for the soleus and flexor digitorum muscles. In the majority of experiments, isometric contractions of the intact medial gastrocnemius muscle in each leg were also measured.

## RESULTS

*Selective reinnervation of the fast-twitch muscle.* Table 1 *A* shows the maximum twitch tensions of the soleus muscle dually reinnervated by the soleus and flexor digitorum nerves in the animals whose spinal cords and dorsal roots were left intact. The mean maximum twitch tension of the soleus muscle in response to stimulation of the soleus nerve was 122 g (Table 1 *Aa*) which was not significantly different from that obtained by stimulation of the foreign, flexor digitorum nerve (39 g; Table 1 *Ab*). The lack of significant difference might have been due to an insufficiency in the number of observations. Also, individual motor units reinnervated by the flexor digitorum nerve might produce different twitch tensions from those reinnervated by the soleus nerve. Therefore, the degree of reinnervation by the two nerves, estimated by comparison of their twitch tensions, might be misleading. Thus, comparison was made between the maximum twitch tension of the dually reinnervated soleus muscle in response to stimulation of the soleus nerve and that of the contralateral self-reinnervated soleus muscle. Boyd & Davey (1966) have reported that the number of alpha motoneurones innervating the lateral flexor digitorum muscle is about 50% larger than that supplying the soleus muscle. Therefore, if the soleus muscle were preferentially reinnervated by its own nerve, one would expect the maximum twitch tension evoked by stimulation of soleus nerve to exceed significantly 40% of that observed in the self-reinnervated soleus muscle on the contralateral side (214 g; Table 1 *Ac*). The mean ratio was 0·56 (Table 1 *Ae*) which was not significantly different from 0·40. Thus, there was no evidence that the soleus muscle is preferentially innervated by its own nerve following the dual reinnervation.

In contrast with the soleus muscle, the maximum twitch tension of the dually reinnervated flexor digitorum muscle (505 g; Table 1 *Ca*), elicited by its own nerve, was significantly greater than 0·6 of that (567 g; Table 1 *Cd*) observed in the self-innervated flexor digitorum muscle on the contraleral side (0·90; Table 1 *Ce*). Apparently, the flexor digitorum muscle is preferentially reinnervated by its own nerve when given a choice between the two nerves. It should be noted that this selective reinnervation by the flexor digitorum nerve occurred even under the conditions which were deliberately biased in favour of reinnervation by the soleus nerve (see Methods).

In cord-transected animals, however, the twitch tension of the dually reinnervated flexor digitorum muscle produced by stimulation of its own nerve did not signicantly exceed 60% of that of the self-united flexor digitorum muscle (0·76; Table 1 *De*). Thus, preferential reinnervation by the native nerve was no longer evident following cord transection. It is possible that selective reinnervation of the fast-twitch muscle may partly depend upon activity of the motoneurones and/or the muscle. This possibility, however, must be regarded with some caution since the number of animals examined is limited.

Another puzzling phenomenon was the differential response of twitch tension to cord transection. In cord-transected animals, there was a significant decrease in the maximum twitch tension of the self-united soleus muscles (Table 1 *Bc*, 1 *Dc*; also, see 1 *Ba*), but not in the self-united flexor digitorum muscle (Table 1 *Bd*, 1 *Dd*; also, see 1 *Da*). One might argue that the loss of activity following cord transection is greater in soleus than in flexor digitorum motoneurones. However, the twitch tension of the flexor digitorum muscle produced by stimulation of the soleus nerve was not significantly decreased (Table 1 *Db*). Another possibility is that cord transection may result in greater atrophy of the soleus muscle than of the flexor digitorum muscle. This was also unlikely since the twitch tension of the soleus muscle elicited by the flexor digitorum nerve was increased rather than decreased (Table 1 Bb). The explanation of these results remains unclear.

*Twitch times of the dually reinnervated soleus muscle.* Fig. 1 *Ba* illustrates contractions of a dually reinnervated soleus muscle in response to stimulation of the soleus nerve (single arrow) and the foreign, flexor digitorum nerve (double arrow) in an animal whose spinal cord and dorsal roots were left intact (intact neural activity). In agreement with previous observations (Buller & Lewis, 1965; also, see Buller *et al.* 1960*b*; Kuno *et al.* 1974), stimulation of the soleus nerve resulted in consistently longer contraction times than did stimulation of flexor digitorum nerve in each animal examined. However, this difference in contraction time was not statistically different (73 and 59 msec; Table 2Aa, 2Ab), presumably because of the large variability of contraction times among different animals. On the other hand, the ratio of the twitch times observed for the same muscle in response to stimulation of the native and foreign nerves was, on the average, 1·23 (Table 2*Ae*) which was significantly larger than 1·00. In these animals, the soleus nerve evoked contractions with similar twitch

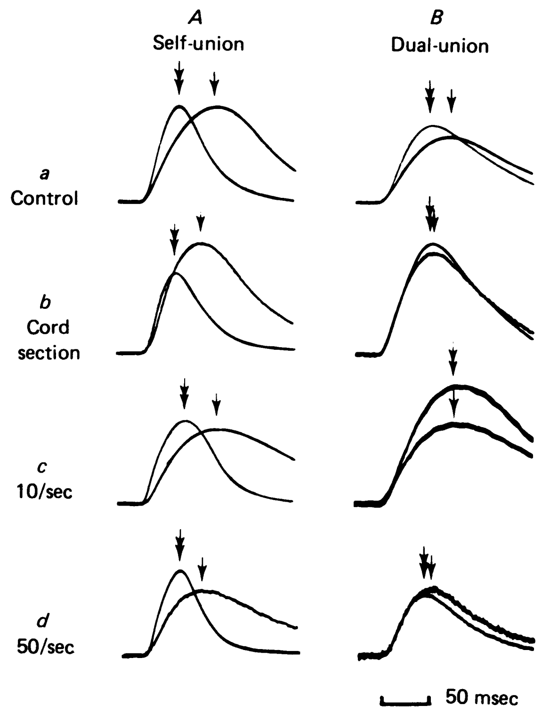


Fig. 1. *A*, isometric contractions of self-reinnervated flexor digitorum (double arrows) and soleus (single arrows) muscles in the right hind legs. *B*, isometric contractions of dually reinnervated soleus muscles in the left hind legs of the same animals in response to stimulation of the flexor digitorum (double arrows) and soleus (single arrows) nerves. Arrows indicate the peaks of twitches. *a*, from an animal with intact neural activity. *b*, from a cord-transected animal. *c* and *d*, from animals whose left sciatic nerves were stimulated at 10/sec and 50/sec, respectively.

times in the dual- (left side) and self-united (right side) soleus muscles; the ratio of the twitch times (0·94; Table 2*Af*) did not significantly differ from 1·00 (also see Fig. 1*Aa*, 1*Ba*, single arrows).

In four animals, the lumbosacral dorsal roots were bilaterally sectioned at the time of the nerve reunion operation. The results obtained from these animals 9 weeks later were essentially the same as those observed in animals with intact neural activity: the mean ratio of the twitch times obtained by stimulation of the soleus and flexor digitorum nerves was significantly larger than 1·00 (1·39; Table 2*Be*), while that of the dual- and self-reinnervated soleus muscles in response to stimulation of the soleus nerves was not significantly different from 1·00 (1·06; Table 2*Bf*).

Illustrated in Fig. 1*Bb* are contractions of the dually reinnervated soleus muscle

TABLE 2. Contraction times following dual reinnervation of the soleus muscle (left leg) and self-reinnervation of the soleus and flexor digitorum muscles (right leg). F, flexor digitorum; S, soleus. Small letters preceding each arrow indicate the nerves used for stimulation. Capital letters following each arrow indicate the muscles in which contractions were observed. All values give the mean ± S.D. All twitch times (*a–d*) in msec. *n*, numbers of muscles examined

| Conditions for neural activity | Dual reinnervation side (left) | | Self reinnervation side (right) | | Ratio | |
|---|---|---|---|---|---|---|
| | *a* s → S | *b* f → S | *c* s → S | *d* f → F | *e* a/b | *f* a/c |
| *A* Cord intact | 72·5 ± 12 ($n = 5$) | 58·7 ± 9·7 ($n = 5$) | 76·6 ± 7·5 ($n = 5$) | 38·0 ± 4·3 ($n = 5$) | 1·23 ± 0·075* ($n = 5$) | 0·940 ± 0·086 ($n = 5$) |
| *B* Dorsal root section | 77·7 ± 15 ($n = 4$) | 55·6 ± 8·9 ($n = 4$) | 73·5 ± 15 ($n = 4$) | 35·1 ± 2·4 ($n = 4$) | 1·39 ± 0·050* ($n = 4$) | 1·06 ± 0·068 ($n = 4$) |
| *C* Cord transection | 64·8 ± 8·7 ($n = 5$) | 62·5 ± 8·9 ($n = 5$) | 72·9 ± 7·6 ($n = 5$) | 31·6 ± 2·6 ($n = 5$) | 1·05 ± 0·158 ($n = 5$) | 0·890 ± 0·087 ($n = 5$) |
| *D* Stimulation at 10/sec | 83·6 ± 5·1 ($n = 4$) | 72·9 ± 8·2 ($n = 4$) | 75·8 ± 5·5 ($n = 4$) | 34·7 ± 4·8 ($n = 4$) | 1·16 ± 0·162 ($n = 4$) | 1·11 ± 0·079 ($n = 4$) |
| *E* Stimulation at 50/sec | 53·8 ± 10 ($n = 4$) | 52·1 ± 12 ($n = 4$) | 79·1 ± 18 ($n = 4$) | 31·4 ± 1·9 ($n = 4$) | 1·05 ± 0·149 ($n = 4$) | 0·688 ± 0·073* ($n = 4$) |

* Significant difference from 1·00.

evoked by separate stimulation of the two nerves in a cord-transected animal. In these animals, the contraction times of the dually reinnervated soleus muscle, elicited by separate stimulation of the soleus (65 msec; Table 2 *Ca*) and flexor digitorum nerves (63 msec; Table 2 *Cb*) were not significantly different. The mean ratio of the twitch times was 1·05 (Table 2 *Ce*) which was not significantly different from 1·00. Thus, when neural activity was virtually eliminated by cord transection, the influence of the soleus nerve upon contraction times of its own muscle was indistinguishable from that of the foreign, flexor digitorum nerve. Again, the dual- and self-reinnervated soleus muscles showed no significant difference in contraction time in response to stimulation of the soleus nerves (0·89; Table 2 *Cf*).

Fig. 1 *Bc* shows the effects of chronic stimulation of the left sciatic nerve at 10/sec on contraction times of the dually reinnervated soleus muscle in a cord-transected animal. In every animal examined, the soleus nerve elicited a slightly slower contraction on the stimulated side (Fig. 1 *Bc*, single arrow) than on the unstimulated side (Fig. 1 *Ac*, single arrow). However, the mean ratio of their contraction times observed in the same animal (1·11; Table 2 *Df*) was not significantly different from 1·00. Separate stimulation of the soleus and flexor digitorum nerves resulted in similar twitch times of the dually reinnervated soleus muscle (Fig. 1 Bc, single and double arrows): the mean ratio of the contraction times (1·16; Table 2 *De*) was not significantly different from 1·00. Thus, reaction of the soleus muscle to chronic nerve stimulation at a low frequency was independent of the type of the innervating motoneurone.

Following chronic stimulation of the left sciatic nerve at 50/sec, the dually reinnervated soleus muscle contracted faster upon stimulation of the soleus nerve (Fig. 1 *Bd*, single arrow) than did the contralateral self-united soleus muscle (Fig. 1 *Ad*, single arrow). The mean ratio of their contraction times (0·69; Table 2 *Ef*) was significantly different from 1·00. Once again, the dually reinnervated soleus muscle showed essentially the same contraction time in response to stimulation of its own or the foreign nerve (Fig. 1 *Bd*; Table 2 *Ee*).

From these results, summarized in Table 2 *e*, it seems reasonable to conclude that the difference in contraction time observed in the dually reinnervated soleus muscle following stimulation of its own and the foreign nerve in the animals with intact neural activity (*A*) is no longer discernible if activity of the two nerves is virtually eliminated (*C*) or is made identical in pattern (*D*, *E*).

*Twitch times of the dually reinnervated flexor digitorum muscle.* Fig. 2 shows sample records of contractions of the self-reinnervated flexor digitorum (*A*, double arrows) and soleus muscles (*A*, single arrows) as well as those observed in the dually reinnervated flexor digitorum muscles (*B*) under different experimental conditions. The numerical data from these experiments are summarized in Table 3. With intact neural activity (Fig. 2 *Ba*), the flexor digitorum nerve evoked a significantly faster contraction (30 msec; Table 3 *Aa*) in the dually reinnervated flexor digitorum muscle than did the soleus nerve (45 msec; Table 3 *Ab*): the ratio of these contraction times observed in the same muscle averaged 0·67 (Table 3 *Ae*) which was significantly different from 1·00. Unexpectedly, the contraction time of the dually reinnervated flexor digitorum muscle evoked by its own nerve was significantly faster than that of the contralateral self-reinnervated flexor digitorum muscle (Table 3 *Af*). This difference might be explained by assuming that, when placed in competition with the soleus nerve, the left flexor digitorum nerve preferentially reinnervates fast-contracting muscle fibres, whereas the contralateral self-united nerve may reinnervate the majority of the flexor digitorum muscle fibres uniformly, including relatively slow-contracting fibres (see Discussion).

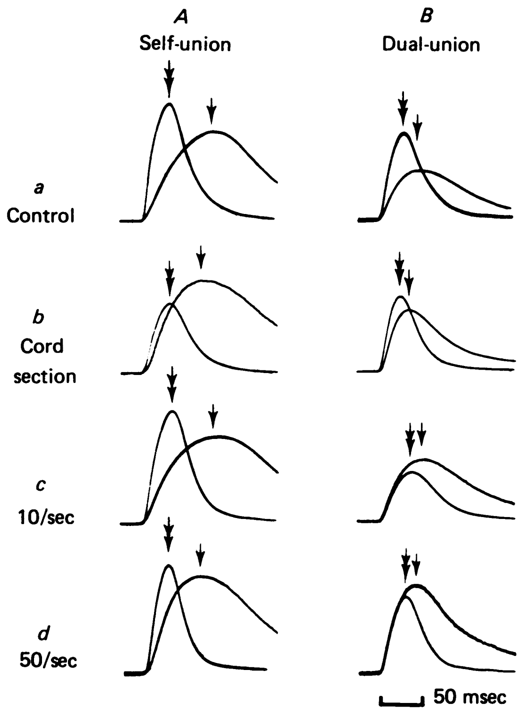


Fig. 2. *A*, isometric contractions of self-reinnervated flexor digitorum (double arrows) and soleus (single arrows) muscles in the right hind legs. *B*, isometric contractions of dually reinnervated flexor digitorum muscles in the left hind legs of the same animals in response to stimulation of the flexor digitorum (double arrows) and soleus (single arrows) nerves. Arrows indicate the peaks of twitches. *a*, from an animal with intact, neural activity. *b*, from a cord-transected animal. *c* and *d*, from animals whose left sciatic nerves were stimulated at 10/sec and at 50/sec, respectively.

Unlike the dualy reinnervated soleus muscle, the dually reinnervated flexor digitorum muscle responded to stimulation of its own nerve and the soleus nerve with twitch times whose ratio consistently differed from 1·00, even in cord-transected animals (Table 3*Ce*) or when the two nerves had been exposed to the same pattern of activity (Table 3*De*, 3*Ee*). Evidently, the difference in contraction time of the dually reinnervated flexor digitorum muscle in response to stimulation of the native and foreign nerves seen in animals with intact neural activity (Table 3*Ae*) cannot entirely be attributed to the difference in discharge frequency between the two groups of motoneurones (see Discussion).

Chronic stimulation of the left sciatic nerve at 10/sec significantly increased the contraction time of the flexor digitorum muscle compared with that of the self-reinnervated muscle on the unstimulated side (Table 3*Df*). Also, confirming previous observations (see Introduction), twitch

TABLE 3. Contraction times following dual reinnervation of the flexor digitorum muscle (left leg) and self-reinnervation of the soleus and flexor digitorum muscles (right leg). F, flexor digitorum; S, soleus. Small letters preceding each arrow indicate the nerves used for stimulation. Capital letters following each arrow indicate the muscles in which contractions were observed. All values give the mean ± S.D. All twitch times (*a–d*) in msec. *n*, numbers of muscles examined

| Conditions for neural activity | Dual reinnervation side (left) | | Self reinnervation side (right) | | Ratio | |
|---|---|---|---|---|---|---|
| | *a* f → F | *b* s → F | *c* s → S | *d* f → F | *e* a/b | *f* a/d |
| *A* Cord intact | 30·0 ± 1·3 ($n = 4$) | 44·8 ± 5·1† ($n = 4$) | 76·8 ± 10 ($n = 4$) | 34·8 ± 1·7 ($n = 4$) | 0·669 ± 0·049* ($n = 4$) | 0·858 ± 0·050* ($n = 4$) |
| *B* Dorsal root section | — | — | — | — | — | — |
| *C* Cord transection | 32·2 ± 5·8 ($n = 5$) | 40·9 ± 3·5† ($n = 5$) | 77·8 ± 8·2 ($n = 5$) | 35·4 ± 3·2 ($n = 5$) | 0·788 ± 0·123* ($n = 5$) | 0·906 ± 0·118 ($n = 5$) |
| *D* Stimulation at 10/sec | 39·5 ± 3·0 ($n = 4$) | 46·8 ± 7·1 ($n = 4$) | 80·0 ± 22·5 ($n = 4$) | 30·8 ± 5·2 ($n = 4$) | 0·854 ± 0·084* ($n = 4$) | 1·30 ± 0·136* ($n = 4$) |
| *E* Stimulation at 50/sec | 35·0 ± 5·0 ($n = 5$) | 42·2 ± 6·2 ($n = 5$) | 73·0 ± 19·6 ($n = 5$) | 30·0 ± 5·1 ($n = 5$) | 0·834 ± 0·110* ($n = 5$) | 1·17 ± 0·130* ($n = 5$) |

* Significant difference from 1·00.

† Significant difference in twitch time between responses to stimulation of the native and foreign nerves.

time of the intact, medial gastrocnemius muscle on the stimulated side was significantly longer than that on the contralateral side (data not shown). Surprisingly, chronic nerve stimulation at 50/sec likewise slowed contraction of the flexor digitorum muscle significantly (Table 3*Ef*). This was in contrast with the behaviour of the dually reinnervated soleus muscle whose contraction time was significantly reduced following stimulation at 50/sec (Table 2*Ef*). Contraction of the intact medial gastrocnemius muscle on the side stimulated at 50/sec was also significantly slower than that on the contralateral side. It has recently been reported that contraction of the rabbit fast-twitch muscle is also slightly prolonged following chronic nerve stimulation at 40/sec (Hudlicka & Tyler, 1980). A possible implication of these results is that a particular activity pattern may exert qualitatively different effects on the contraction times of fast and slow muscles.

## DISCUSSION

The dually reinnervated soleus or flexor digitorum muscle shows consistently slower contractions in response to stimulation of the soleus nerve than to stimulation of the flexor digitorum nerve if intact neural activity is maintained (Buller *et al.* 1960*b*; Buller & Lewis, 1965; Kuno *et al.* 1974). These differential neural effects may be attributed to different trophic factors and/or different discharge patterns associated with the two groups of innervating motoneurones. When activity of the two groups of motoneurones was minimized by cord transection or when the same pattern of activity was imposed on the two nerves, the dually reinnervated soleus muscle no longer showed a significant difference in twitch time in response to stimulation of the native and foreign nerves. Thus it seems clear that the differential neural effects on contractile properties of the soleus muscle observed with intact neural activity are related to the motoneurone discharge patterns rather than to the type of the motoneurone *per se*. This does not preclude the involvement of a neurotrophic factor in regulating the speed of muscle contraction. It is known that transection of the spinal cord at thoracic levels leads to an increase in the speed of contraction of the soleus muscle in both kittens and adult cats without affecting the contractile properties of the fast-twitch muscle of the hind limb (Buller *et al.* 1960*a*, *b*; Gallego *et al.* 1978; but cf. Davis & Montgomery, 1977). However, if muscle activity is abolished by denervation, both the slow- and fast-twitch muscles contract more slowly (Eccles *et al.* 1962; Lewis, 1972, 1973). The observation that the effects of muscle inactivity differ from those of denervation strongly suggests that the presence of innervation maintains some influence upon contractile properties of the muscle even in the absence of impulse activity. From these results, two alternative conclusions may be inferred: (1) the trophic factors which control the speed of muscle contractions are identical in soleus and flexor digitorum motoneurones or (2) the soleus muscle is not able to distinguish a difference between the trophic factors (see below).

The behaviour of the dually reinnervated flexor digitorum muscle was apparently different from that of the soleus muscle. Even when activity of the two groups of motoneurones was virtually eliminated by cord transection or when the same pattern of activity was imposed on the two nerves, contractions of the flexor digitorum muscle evoked by its own nerve were consistently faster than those produced by the soleus nerve. To explain this phenomenon, two possibilities may be considered. First, the soleus and flexor digitorum motoneurones possess different trophic factors whose differential effect may be manifested in the flexor digitorum muscle but not in the soleus muscle (see above). It is not clear how the two muscles can differ in responsiveness to these trophic factors, but this possibility cannot be excluded. The second possibility, suggested by Dr R. Sealock, is that upon dual reinnervation flexor digitorum motoneurones preferentially supply fast-contracting muscle fibres, while soleus motoneurones may tend to innervate relatively slow-contracting fibres. It is known that about 11 % of flexor digitorum muscle fibres have the histochemical profile characteristic of the 'slow' type (Dum, Burke & Hodgson, 1978) and that about 12 % of the motor unit population show relatively slow (> 40 msec in twitch time) contractions (Dum *et al.* 1978; also see Bagust, Knott, Lewis, Luck & Westerman, 1973). Thus, the consistently faster contractions of the dually reinnervated flexor digitorum muscle evoked by its own nerve under a variety of experimental conditions (Table 3*e*) may be adequately explained by assuming that flexor digitorum motoneurones preferentially reinnervate the 'fast' subgroup of muscle fibres. Consistent with this assumption, about 90 % of the flexor digitorum muscle fibres are selectively reinnervated by their own nerve when competing with the soleus nerve (Table 1 *Ce*); moreover, when neural activity was left intact, contractions of the dually reinnervated flexor digitorum muscle, evoked by its own nerve were significantly faster than those of the self-reinnervated flexor digitorum muscle on the contralateral side (Table 3 *Af*). Also, Buller *et al.* (1960*b*) observed that the contraction time of the flexor digitorum muscle, which had been cross-innervated by the soleus nerve (i.e. no competition with the native nerve), was the same as that obtained from self-innervated control flexor digitorum muscles in cord-transected kittens. This would be expected if the influence of soleus and flexor digitorum motoneurones on muscle contractile properties were similar and the soleus motoneurones were able to uniformly reinnervate the fast-twitch muscle. If the different contraction times of the dually reinnervated flexor digitorum muscle in response to stimulation of the native and foreign nerves were indeed due to selective reinnervation of a particular group of muscle fibres, there would be no need to postulate that soleus and flexor digitorum motoneurones emanate different trophic factors which regulate the speed of muscle contractions.

We wish to thank Dr R. Sealock for his valuable discussion, Mrs Ellen Z. Curtin for her editorial comments and Miss Cynthia V. Taylor for her technical assistance. This work was supported by project (NS 1132) and research (NS 10319) grants from the U.S. Public Health Service and was carried out during the tenure of a Postdoctoral Fellowship (to J.M.G.) from the Muscular Dystrophy Association.

## REFERENCES

Bagust, J., Knott, S., Lewis, D. M., Luck, J. C. & Westerman, R. A. (1973). Isometric contractions of motor units in a fast twitch muscle of the cat. *J. Physiol.* **231**, 87–104.

Boyd, I. A. & Davey, M. R. (1966). The composition of peripheral nerve. In *Control and Innervation of Skeletal Muscle*, ed. Andrew, B L., pp. 35–52. Dundee: University of St. Andrew Press.

Buller, A. J., Eccles, J. C. & Eccles, R. M. (1960*a*). Differentiation of fast and slow muscles in the cat hind limb. *J. Physiol.* **150**, 399–416.

Buller, A. J., Eccles, J. C. & Eccles, R. M. (1960*b*). Interactions between motoneurones and muscles in the cat hind limb. *J. Physiol.* **150**, 417–439.

Buller, A. J. & Lewis, D. M. (1965). Further observations on mammalian cross-innervated skeletal muscle. *J. Physiol.* **178**, 343–358.

Buller, A. J. & Pope, R. (1977). Plasticity in mammalian skeletal muscle. *Phil. Trans. R. Soc.* B **278**, 295–305.

Davis, C. J. F. & Montgomery, A. (1977). The effect of prolonged inactivity upon the contraction characteristics of fast and slow mammalian twitch muscle. *J. Physiol.* **270**, 581–594.

Dum, R. P., Burke, R. E. & Hodgson, J. A. (1978). Analysis of the motor unit population in cat flexor digitorum longus (FDL) muscle. *Soc. Neurosci. Abstr.* **4**, 294.

Eccles, J. C., Eccles, R. M. & Kozak, W. (1962). Further investigations on the influence of motoneurones on the speed of muscle contraction. *J. Physiol.* **163**, 324–339.

Fischbach, G. D. & Robbins, N. (1969). Changes in contractile properties of disused soleus muscle. *J. Physiol.* **201**, 305–320.

Gallego, R., Huizar, P., Kudo, N. & Kuno, M. (1978). Disparity of motoneurone and muscle differentiation following spinal transection in the kitten. *J. Physiol.* **281**, 253–265.

Gallego, R., Kuno, M., Núñez, R. & Snider, W. D. (1979). Disuse enhances synaptic efficacy in spinal motoneurones. *J. Physiol.* **291**, 191–205.

Granit, R., Henatsch, H. D. & Steg, G. (1956). Tonic and phasic ventral horn cells differentiated by post-tetanic potentiation in cat extensors. *Acta physiol. scand.* **37**, 114–126.

Granit, R., Phillips, C. G., Skoglund, S. & Steg, G. (1957). Differentiation of tonic from phasic alpha ventral horn cells by stretch, pinna and crossed extensor reflexes. *J. Neurophysiol.* **20**, 470–481.

Gutmann, E. (1942). Factors affecting recovery of motor function after nerve lesions. *J. Neurol. Neurosurg. Psychiat.* **5**, 81–95.

Gutmann, E., Guttmann, L., Medawar, P. B. & Young, J. Z. (1942). The rate of regeneration of nerve. *J. exp. Biol.* **19**, 14–44.

Hoh, J. F. Y. (1975). Selective and non-selective reinnervation of fast-twitch and slow-twitch rat skeletal muscle. *J. Physiol.* **251**, 791–801.

Hoh, J. F. Y. & Dunlop, C. (1975). Transformation of slow-twitch muscle to fast-twitch muscle in paraplegic rat. *Proc. IIIrd International Congress on Muscle Diseases. International Congress Series*, no. 334, p. 50, Amsterdam: Excerpta Medica.

Hudlicka, O. & Tyler, K. R. (1980). Importance of different patterns of frequency in the development of contractile properties and histochemical characteristics of fast skeletal muscle. *J. Physiol.* **301**, 10–11*P*.

Kuno, M., Miyata, Y. & Muñoz-Martinez, E. J. (1974). Properties of fast and slow alpha motoneurones following motor reinnervation. *J. Physiol.* **242**, 273–288.

Lewis, D. M. (1972). The effect of denervation on the mechanical and electrical responses of fast and slow mammalian twitch muscle. *J. Physiol.* **222**, 51–75.

Lewis, D. M. (1973). Effect of denervation on the differentiation of twitch muscles in the kitten hind limb. *Nature, New Biol.* **241**, 285–286.

Lømo, T., Westgaard, R. H. & Dahl, H. A. (1974). Contractile properties of muscle: control by pattern of muscle activity in the rat *Proc. R. Soc.* B. **187**, 99–103.

Mann, W. S. & Salafsky, B. (1970). Enzymic and physiological studies on normal and disused developing fast and slow cat muscles. *J. Physiol.* **208**, 33–47.

Mommaerts, W. F. H. M., Seraydarian, K., Suh, M., Kean, C. J. C. & Buller, A. J. (1977). The conversion of some biochemical properties of mammalian skeletal muscles following cross-reinnervation. *Expl Neurol.* **55**, 637–653.

Pette, D., Smith, M. E., Staudte, H. W. & Vrbová, G. (1973). Effects of long term electrical stimulation on some contractile and metabolic characteristics of fast rabbit muscles. *Pflügers Arch.* **338**, 257–272.

Salmons, S. & Sréter, F. A. (1976). Significance of impulse activity in the transformation of skeletal muscle type. *Nature, Lond.* **263**, 30–34.

Salmons, S. & Vrbová, G. (1969). The influence of activity on some contractile characteristics of mammalian fast and slow muscles. *J. Physiol.* **201**, 535–549.

Smith, D. (1978). Miniature stimulator for chronic animals. *Pflügers Arch.* **376**, 93–95.