

MOTOR UNITS IN CROSS-REINNERVATED FAST AND SLOW TWITCH MUSCLE OF THE CAT

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SUMMARY

1. Isometric contractile properties of motor units were measured in cross-reinnervated fast (flexor digitorum longus) and slow (soleus) twitch muscles of the cat. All but one cross was at least 95% pure.

2. There was a reduction in the number of motor units in all muscles, but totals remained about equal in cross-reinnervated soleus and flexor digitorum longus.

3. Motor unit tensions (mean and maximum values) were higher in cross-reinnervated soleus than in cross-reinnervated flexor digitorum longus, reversing the differences between normal muscles. This was due to increases in muscle mass and in the tension developed per unit cross-sectional area. There were motor unit tensions larger and smaller than those seen in normal muscle, but the range was comparable with that seen in self-reinnervated muscle.

4. The changes in twitch time to peak of whole muscle following cross-reinnervations resulted from a change over the whole range of motor units. The conversion of soleus was less complete than that of flexor digitorum longus, and the time to peak of its fastest motor unit was twice as long as any seen in normal flexor digitorum longus.

5. In neither of the cross-reinnervated muscles were the fast contracting motor units larger than the slow contracting ones, and in cross-reinnervated soleus they were smaller.

6. Axonal conduction velocity was correlated with motor unit tension in both muscles and with twitch time to peak in cross-reinnervated flexor digitorum longus, but in all cases less clearly than in normal muscles.

7. The ratio of twitch to tetanic tension increased with increasing twitch time to peak, as in normal muscles.

INTRODUCTION

Reinnervation of a fast twitch muscle by a nerve which normally supplies a slow twitch muscle results in a slowing of the contraction speed of the muscle (Buller, Eccles & Eccles, 1960). Cross-reinnervation of slow twitch muscle results in speeding of contraction. The interpretation of such experiments is complicated by the facts that most muscles have a heterogeneous mixture of histochemical fibre types and all

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consist of motor units with a range of contractile properties (Bessou, Emonet-Dénand & Laporte, 1963; Weurker, McPhedran & Henneman, 1965). At present, no species is known which possesses a pure fast twitch muscle and a pure slow twitch muscle in positions suitable for cross-union of their nerves. In the cat, the effects of cross-reinnervation have been studied on isometric contractions (Buller & Lewis, 1965*a*) and isotonic contractions (Buller & Kean, 1973). The two muscles used in many of these investigations have been subjected to motor unit studies: soleus by Weurker *et al.* (1965) and Bagust (1974) and flexor digitorum longus by Olson & Swett (1966) and Bagust, Knott, Lewis, Luck & Westerman (1973). Both muscles contain a wide range of motor units, although on grounds of histochemistry (Guth, Samaha & Albers, 1970; Edjehadi & Lewis, 1979) soleus contains only one fibre type, whereas flexor digitorum longus is a mixed muscle with three myosin ATPase fibre types.

It is known that the properties of the muscle fibres of a motor unit are related to those of its axonal component (Bessou *et al.* 1963). It is likely, therefore, that the changes in a cross-reinnervated muscle will be complex since the reinnervating nerve will not be pure in every type of cross-union. Even where the reinnervating nerve had formerly innervated a histochemically pure muscle (e.g. soleus) there is some evidence that the resulting muscle is not pure histochemically (Prewitt & Salafsky, 1970). Moreover, experiments on self-reinnervation (Bagust & Lewis, 1974) indicate that the normal relations between axons and muscle fibres may not be completely re-established.

In the present study, cross-reinnervated flexor digitorum longus and soleus muscles of the cat were examined to investigate the interaction of nerve and muscle fibres at the level of the single motor unit in order to determine the effects of the heterogeneity of nerves and muscles discussed above. A preliminary report of these experiments has been published (Bagust, Finol, Lewis, Webb & Westerman, 1974*a*), and the effects of cross-union on the conduction velocity in motor axons has been described in detail by Lewis, Bagust, Westerman, Webb & Finol (1978).

METHODS

The nerve cross-union operations were performed on eleven young adult cats of about 2.2 kg body weight, bred in a colony of animals maintained by the Animal Husbandry Department of Bristol University. Animals from the same source were also used by Bagust, Lewis & Westerman (1974). The animals were anaesthetized with halothane in 80% nitrous oxide and 20% oxygen and maintained with halothane and penthrane in 70% nitrous oxide and 30% oxygen. Aseptic techniques were used to expose and cross-union the nerves to flexor digitorum longus and soleus by the method described by Buller & Lewis (1965*a*).

The animals were housed in pens which allowed reasonable activity (Bagust & Lewis, 1974). The final experiments were performed about 6 months later (154–215 days, mean 186 days) under pentobarbitone anaesthesia (Nembutal 40 mg/kg) i.p. initially, supplemented i.v. when necessary. At the final experiment, body weight averaged 3.5 kg (s.d. = 0.79 kg). The methods used were generally those described by Bagust *et al.* (1973), Bagust *et al.* (1974*a*) and Bagust (1974), but some special points should be described.

Cross-reinnervation is not always pure in that some of the nerve axons may return to their own muscle despite careful nerve separation at the cross-union operation. Therefore it was necessary to prepare both nerves and both muscles at the final operation. In five animals it was clear from looking at the contractions that one muscle was innervated only by the foreign nerve, and this cross was chosen for recording. When there was some impurity, isometric contractions were recorded from both muscles, and the muscle was chosen which had the smaller amount of self-reinnervation as

judged by tetanic tensions. We were lucky in that ten muscles (all except one) had less than 5% of self-reinnervation tension (tetanic tension as a percentage of total muscle tension). The mean purity of those ten muscles was 98.8%, (s.d. = 1.8%). The eleventh muscle was a flexor digitorum longus which had 47% self-reinnervation, and the results of this one experiment are described separately when necessary.

The nerves were prepared for stimulation above the neuroma which indicated the site of cross-union. A length of nerve of 15–20 mm was cleared above the neuroma in order to minimise spread of the stimulus beyond the nerve under test. Dissection distal to the neuroma and around the muscle was kept minimal in order that axons which might run outside the main nerve trunks should not be damaged.

Some of the motor units were expected to be very small (Bagust & Lewis, 1974), and the splitting of ventral roots to isolate functionally single motor units was performed in a manner which was designed to reduce bias against small tension motor units. The roots were split into filaments of a size predicted to contain several axons from the nerve under test. These filaments were stimulated and if they evoked a muscle response they were split further using only the antidromic action potential as a guide to a unit. Once a filament was prepared which contained only one antidromic action potential, it was stimulated to test whether the muscle twitch and electromyogram was also all-or-none. At this stage a number of axons were found to produce no muscle response, and it was impossible to determine whether these axons were ones with an aberrant path to the original muscle, or whether they had innervated the muscle under test and either had been damaged or innervated too few muscle fibres to be detected. The last explanation is unlikely as the smallest twitch detected produced a myogram some five times greater than the noise of the recording system, and smaller contractions would have been detected.

Twitches of the whole muscle and all motor unit contractions were recorded with an unbonded wire strain gauge tension transducer with a load maximum of 700 g (Ether U2-16) and an unloaded resonant frequency of 750 Hz. Whole muscle tetani were recorded by clamping a cantilever beam to the transducer arm reducing the sensitivity. Experiments were abandoned when tetanic tension fell by more than 10%. This was tested every two hours or more often if twitch tension fell too rapidly. The final tetanic and twitch tensions were on average 92 and 76% of the initial values. All responses were analysed on line by a computer program which allowed a visual check of the validity of the analysis and provided a type-out of the analysis and a photograph of the myogram (Bagust *et al.* 1973; Bagust *et al.* 1974*b*; Bagust & Lewis, 1974).

In all except two experiments motor unit twitch tensions were recorded at a series of muscle lengths and the series was repeated first with twitches then tetani. In the two exceptions measurements were only made at the length at which whole muscle twitch tension was greatest. From three to forty units (mean sixteen) were examined in individual experiments, which lasted from 18 to 33 hr. The animals were given an initial dose of long acting penicillin and were fed via an indwelling gastric cannula with dried milk and sucrose mixed with a 0.18% NaCl, 4.3% glucose solution. The longest experiments were brought to an end by the growth of mould over the exposed cord and roots.

Apart from the one impure cross-reinnervated flexor digitorum longus muscle, two other exceptional experiments must be described. Inadvertently one soleus muscle had been reinnervated by the nerve from flexor hallucis longus rather than flexor digitorum longus. An effort was made to isolate as many motor units as possible from this muscle, and the results from forty-four are described separately where appropriate. In one flexor digitorum longus muscle there was an apparent block of the nerve conduction below the site of the neuroma after isolating only three motor units. Conduction above the nerve appeared normal as judged by the size, latency and duration of the compound action potential in the ventral roots, and this experiment was devoted to measuring as large a number as possible of α and γ conduction velocities in the cross-reinnervating soleus axons.

RESULTS

Axonal conduction velocities

The motor units were isolated from five flexor digitorum longus and six soleus muscles. Of the seventy-one flexor digitorum longus motor units, fifteen will be described separately in parts of this paper because they were isolated from a muscle

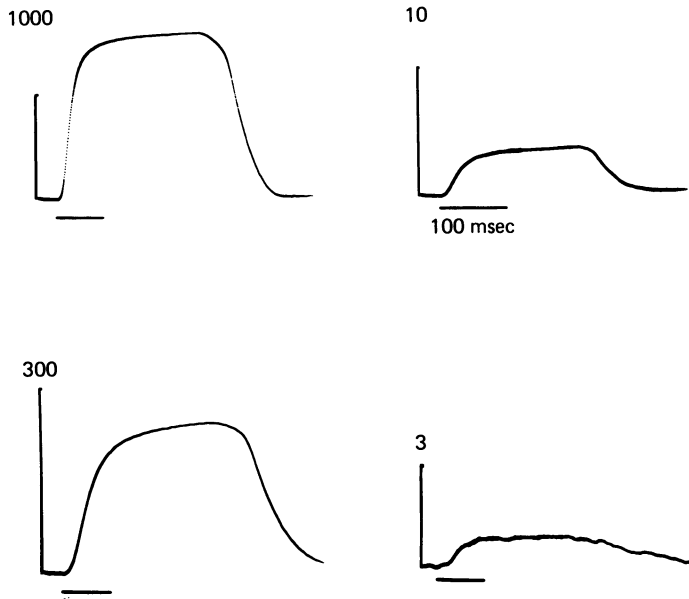


Fig. 1. Tetanic myograms of motor units from cross-reinnervated muscles: soleus (above) and flexor digitorum longus (below). The left and right columns illustrate motor units with large and small tensions. Horizontal bars all 100 msec, vertical calibration bars are labelled to indicate their tensions in mN.

which had a substantial amount of reinnervation from its own nerve: all other crosses were at least 95% pure. The soleus motor units consisted of 64 reinnervated by flexor digitorum longus axons and forty-four (in one muscle) reinnervated by flexor hallucis longus axons.

Confusion has been caused by incorrect naming of these two fast twitch muscles. Buller *et al.* (1960), Lewis & Buller (1965*a, b*) and Bagust *et al.* (1973) have reversed the names of flexor hallucis longus and flexor digitorum longus. The latter name is correctly applied (as in this paper) to the smaller, medial muscle of this pair.

In one experiment 109 α and γ cross-reinnervating soleus axons were isolated for measurement of conduction velocities. The distribution of conduction velocities has been presented in Fig. 1 of Bagust *et al.* (1974*a*), and showed a separation of α and γ axons as clear as is found in normal motor nerves. During other experiments some axons were isolated which later, at the time of analysis, were judged to be γ when compared with the total population; at most two of these presumed γ axons elicited detectable tension in the muscle when stimulated. It was concluded that after reinnervation γ axons rarely establish permanent functional connexions with extra-fusal muscle fibres under these conditions.

The distribution of α -axonal conduction velocities in flexor digitorum longus nerve was almost symmetrical: from a total of sixty-two axons, thirty-two had conduction velocities greater than the mean. In normal flexor digitorum longus nerve a slight asymmetry is seen, with sixty-nine axons having conduction velocities greater than the mean in a sample of 122. The small difference between the cross-reinnervating and normal axons was, however, not significant ($\chi^2 = 0.23$). Small changes in the

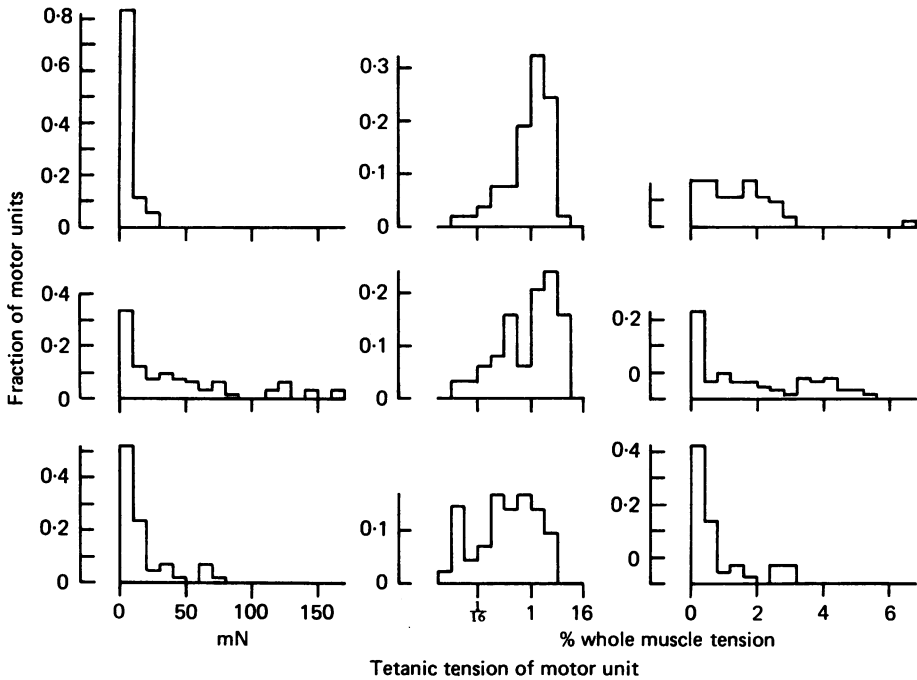


Fig. 2. Histograms showing frequency of occurrence of tetanic tensions in motor units of cross-reinnervated muscles. Top row from flexor digitorum longus muscle innervated by soleus nerve (fifty-three motor units, four muscles). Below are motor units from soleus muscles innervated by flexor digitorum longus nerve (middle row; sixty-three motor units, five muscles) or by flexor hallucis longus nerve (bottom row; forty-two motor units, one muscle). In the left-hand column the histograms are based on absolute tensions. In the right-hand and centre columns motor unit tension has been calculated as a percentage of the whole muscle tension, with linearly or logarithmically scaled bins.

distributions of soleus axonal conduction velocities were found but, again, were not significant ($\chi^2 = 0.9$).

Typical tetanic myograms illustrating the range of motor units are shown in Fig. 1, and some of the statistics of the cross-reinnervated motor unit populations are presented in Table 1.

Motor unit tensions

The distributions of the cross-reinnervated motor unit tetanic tension are shown in Fig. 2. The differences between the types of muscle are best illustrated by the absolute tensions (Fig. 2 left-hand column), whereas the range of tensions within muscles is more clearly shown when the motor unit tension is expressed as a percentage of the whole muscle tension (Fig. 2, right-hand column). The logarithmically scaled histograms (Fig. 2, centre column) have been added to display the distribution of small motor units.

The mean percentage tension of the motor units allows an estimate to be made of the average number of motor units per muscle. In each type of reinnervated muscle the number of motor units was reduced below the normal value. Thus the number

TABLE 1. Tetanic tensions and twitch times to peak of motor units and muscles. The figures in brackets are s.e. of means. Tension of motor units are given in absolute units and as a percentage of the tension of the muscle from which they were isolated functionally. Tension per unit area is estimated only approximately (the muscles were used for histology and were not available to allow dissection and direct measurement of fibre length). Fibre length was estimated from muscle length measured *in situ* multiplied by 0.44 (soleus) or 0.32 (flexor digitorum longus), these ratios of fibre length to muscle length were found in normal muscles by Edjtehadi & Lewis (1974). Whole muscle weight is expressed as a ratio of the weight of the contralateral unoperated muscle. The weighted mean motor unit twitch time to peak was calculated as the sum of the products of twitch time to peak and twitch tension divided by the sum of twitch tensions. Control muscles from Bagust *et al.* (1973) and Bagust (1974): flexor digitorum, soleus and flexor hallucis longus muscles respectively

Muscle . . .	Flexor digitorum longus	Soleus	Soleus
Nerve . . .	Soleus	Flexor digitorum longus	Flexor hallucis longus
Motor units			
Tension			
Number	53*	63	42
Mean			
(mN)	60.8 (0.73)	389 (51)	165 (33)
(%)	1.50 (0.17)	1.89 (0.21)	0.69 (0.13)
Largest			
(mN)	240	1660	750
(%)	6.7	5.3	3.1
Smallest			
(mM)	0.65	3.5	3.4
(%)	0.018	0.026	0.014
Time to peak (msec)			
Number	71	64	44
Mean	45.8 (0.95)	35.0 (1.41)	35.3 (1.7)
Weighted mean	46.5	37.1	31.4
Fastest	33	16.8	18.6
Slowest	75	80	58
Whole muscles (crossed)			
Number	4*	5	1
Tension			
(N)	4.33	21.9	24.3
(N/g)	7.1 (0.96)	6.0 (0.32)	9.4
(N/mm ²)	0.16	0.23	0.21
Weight ratio	0.40 (0.081)	1.41 (0.037)	1.2
Time to peak (msec)	54 (4.9)	39 (1.97)	36
Whole muscles (control)			
Tension (N/mm ²)	0.38	0.25	0.41
Time to peak (msec)	25.2 (0.76)	72 (2.5)	27.1

* Data excludes fifteen motor units from one muscle in which there was extensive self-reinnervation

of soleus α -motor axons innervating flexor digitorum longus muscle was about sixty-three, compared with an estimate of 145 from motor unit tensions in normal muscle (Bagust, 1974) and 131 from histological counts (Eccles & Sherrington, 1930; Boyd & Davey, 1966; Olson & Swett, 1966). A similar diminution was seen in both nerves crossed into soleus; flexor digitorum longus nerve contributing fifty-three α -motor axons, compared with estimates of 123 from normal flexor digitorum longus

motor units (Bagust *et al.* 1973) or 104 from axon counts (Olson & Swett, 1966; Boyd & Davey, 1966). The one flexor hallucis nerve innervating soleus had 145 α -motor axons, compared with normal histological counts of 214 (Olson & Swett, 1966; Boyd & Davey, 1966).

The mean absolute tensions also allow interesting comparisons. The mean value for cross-reinnervated flexor digitorum longus motor units of about 61 mN was considerably smaller than the value of about 390 mN for soleus motor units reinnervated by flexor digitorum longus nerve. This difference contrasts with the normal muscles in which mean motor unit tensions are approximately equal; 112 mN for soleus and 126 mN for flexor digitorum longus (Bagust, 1974; Bagust *et al.* 1973, respectively). The difference between the cross-reinnervated muscles can not be ascribed to differences in the numbers of axons reinnervating the two types of muscle which were very close (56 and 63) as they are in normal muscles (123 and 145). Furthermore, although there was no difference between the weights of the two sets of cross-reinnervated animals, muscle tension and therefore mean motor unit tension increase directly with body weight (Buller & Lewis, 1964*b*). The cross-reinnervated animals were larger than those of the normal series used for comparison (3.3 kg compared with 2.1 kg) but, despite being from larger cats, the cross-reinnervated flexor digitorum longus motor units produced, on average, only half the tension of normal flexor digitorum longus motor units. The combined effects of a reduced number of innervating axons and a smaller tension per motor unit result in muscles with very small tension, averaging 4.3 N in the tetanus compared with a value of 20–25 N expected for animals of this weight. In contrast, the tensions of cross-reinnervated soleus motor units (average 389 mN) were larger than would be predicted for normal solei; Bagust (1974) found 112 mN which value would be expected to increase to 190 mN as body weight increased from 2.1 kg (Bagust, 1974) to 3.5 kg. It is evident, therefore, that the absolute tension developed by a motor unit is, on average, dictated by the innervating nerve.

The changes in mean motor unit tension brought about by cross-reinnervation appeared to be due substantially to changes in muscle mass. The cross-reinnervated solei were consistently heavier and the flexors digitorum longi lighter than the contralateral controls, (Table 1: but note that the control muscles were unexposed and unstimulated during the period of experimental recording, and in normal animals recording from only one limb causes the stimulated muscles to increase in weight by some 20%, compared with controls in the contralateral leg: H. J. Finol, unpublished observations). The differences in mass were due, probably, to differences in fibre cross-sectional area, because no significant differences were seen between the lengths of the cross-reinnervated and control muscles. Tension per unit area was below normal value in both cross-reinnervated muscles (Table 1), but was higher in the soleus muscle indicating that nerve cross-union had reversed the normal difference between specific tensions of fast and slow muscle.

Fig. 2 illustrates the variation in motor unit tensions within muscles following cross-reinnervation. In both muscles the distributions were skewed to large values. Cross-reinnervated soleus had come to resemble normal flexor digitorum longus both in the range of motor unit tensions (compare Table 1 with the range of 0.024–3.8% for normal flexor digitorum longus described by Bagust *et al.* 1973) and in the general

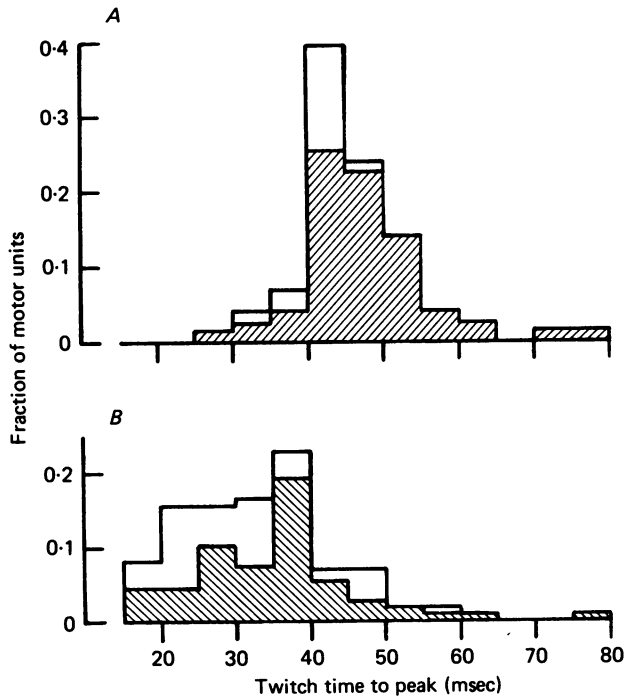


Fig. 3. Distribution of twitch times to peak in cross-reinnervated flexor digitorum longus (*A*) and soleus (*B*) muscles. In *A* cross-hatching indicates motor units from the muscles with only minor self-reinnervation. In *B*, cross-hatching indicates motor units from the muscle reinnervated by flexor digitorum longus nerve, and the plain areas those reinnervated by flexor hallucis longus nerve. The twitches were measured at the muscle length at which whole muscle tetani were maximal.

shape of the distribution histograms. This second resemblance is probably not significant since the flexor digitorum longus distribution was not modified by cross-reinnervation and the skewed pattern develops in soleus after self-reinnervation (Bagust & Lewis, 1974). The absolute tension of the largest cross-reinnervated motor unit was greater in soleus than flexor digitorum longus, in agreement with the mean values.

Contraction times

As is typical for cross-reinnervation in the cat, the conversion of the fast muscle was more complete than that of the slow muscle (Table 1). The distributions of motor unit twitch times to peak are illustrated in Fig. 3. Cross-reinnervation of flexor digitorum longus produced muscles which had slightly shorter twitch times to peak than normal soleus (cf. Buller & Lewis, 1965*a*). Correspondingly, although the motor unit twitch times to peak of flexor digitorum longus were prolonged by cross-reinnervation (Fig. 3*A*), they were shorter than the twitch times to peak of normal or self-reinnervated soleus; the shift was most marked at the upper end of the range. The symmetrical distribution about the mean which appeared in flexor digitorum longus after cross-reinnervation is characteristic of normal soleus muscle.

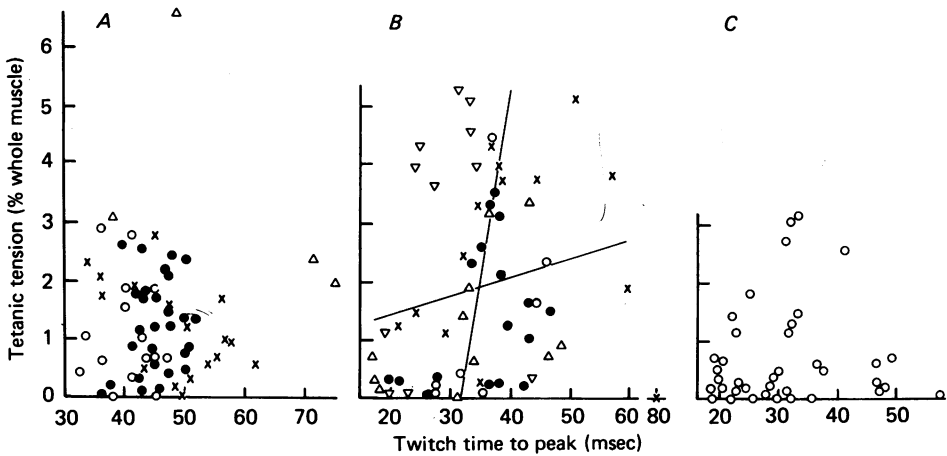


Fig. 4. Relationship between twitch time to peak and tetanic tension of motor units in cross-reinnervated flexor digitorum longus (*A*) and soleus (*B*, *C*) muscles. Reinnervating nerve was that to flexor digitorum longus in *B* and flexor hallucis longus in *C*. Symbols indicate individual animals, and the open circles in *B* are motor units from the muscle with much self-reinnervation. The lines indicate least-squares fitted regressions (time to peak on tension and vice versa) in the one case in which they might be significant ($r = -0.20$, $P < 0.1$) for the pooled data.

The twitch times of soleus motor units reinnervated by flexor hallucis longus nerve (plain columns in Fig. 3*B*) did not differ from those reinnervated by flexor digitorum longus nerve (cross-hatched columns in Fig. 3*B*). The shape of the distribution again has been converted to that characteristic of the reinnervating nerve; in this cross it is asymmetrical and skewed towards slow twitch times to peak reflecting the population of slower axons. The slowest motor units had longer times to peak than any seen in normal flexor digitorum longus (Bagust *et al.* 1973) and the fastest was slower than some 25% of motor units in the normal muscle. Thus the failure of complete conversion of cross-reinnervated soleus was seen at both ends of the range of component motor units.

Relationships between contraction time, tension and conduction velocity

A second factor was involved in the incomplete conversion of soleus, and this is illustrated by Fig. 4. In normal soleus muscle the fastest motor units develop more tension than the slowest; this is true of both slow and fast twitch muscle but is most marked in the latter, in which motor unit tensions increase logarithmically with decreasing time to peak (Bessou *et al.* 1963; Bagust *et al.* 1973). In cross-reinnervated soleus, by contrast, there was a trend in the opposite direction, such that the fastest motor units tended to have small tensions (Fig. 4*B*). Thus the fastest contracting motor units made a contribution to the total muscle response which was less than their number would suggest and much less than the contribution of the fast motor units in normal muscle. A further contrast with normal muscle was that in motor units of cross-reinnervated flexor digitorum longus no relationship was found between twitch time to peak and tetanic tension (Fig. 4*A*).

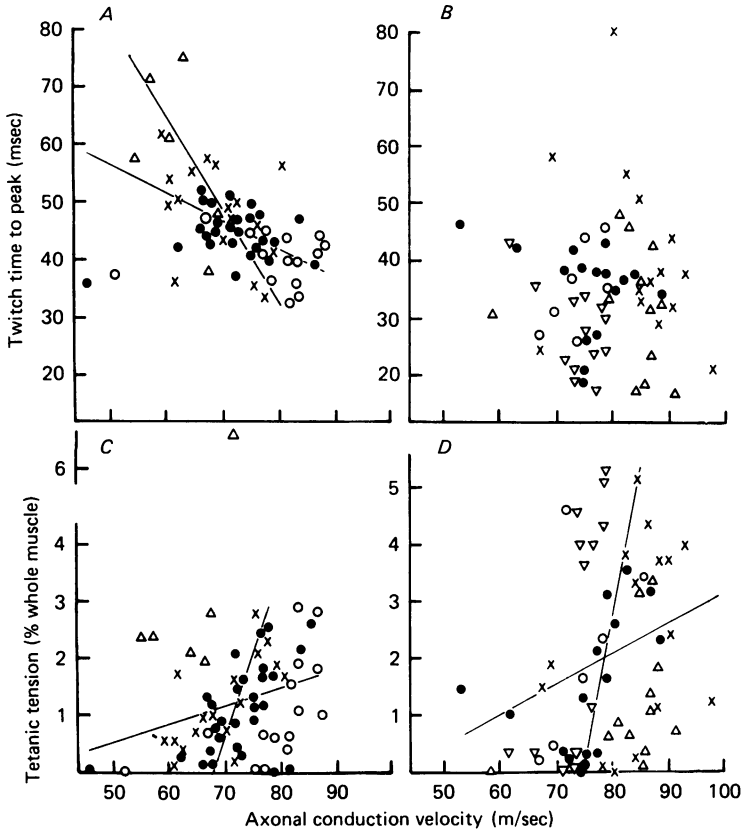


Fig. 5. Relationships of axonal conduction velocity to twitch time to peak (*A, B*) and to tetanic tension (*C, D*) of motor units of cross-reinnervated muscles. *A, C* are from flexor digitorum longus innervated by soleus nerve and *B, D* from soleus innervated by flexor digitorum longus nerve. Symbols and lines as in Fig. 4. The significance of the regressions were: *A*, $r = -0.56$, $P < 0.01$; *C*, $r = 0.33$, $P < 0.02$; *D*, $r = 0.30$, $P < 0.05$. Individual animals showed significant trends at various levels of significance: < 0.01 (\times , \bullet in *C*), < 0.02 (\bullet in *A*, ∇ in *D*), < 0.05 (\times in *A*, \circ in *B* and *C*).

The relatively small tensions of the fastest motor units was also reflected in the the failure to redevelop completely normal relations between axonal conduction velocity and contractile responses (Fig. 5). The greatest discrepancy was seen in soleus cross-reinnervated with flexor digitorum longus nerve (Fig. 5 *B*) or with flexor hallucis longus nerve (not illustrated). No relationship was found between motor unit twitch time to peak and axonal conduction velocity (a negative regression is seen in normal muscle). Tetanic tension, in contrast, increased with conduction velocity (Fig. 5 *D*) as normal. Both relationships in cross-reinnervated flexor digitorum longus (Fig. 5 *A, C*) were qualitatively similar to those in normal soleus, but quantitatively were less clear than in either normal muscle. Although the regressions of Fig. 5 *A, C, D*, are statistically significant, (see legend to Fig. 5), the relationships are not immediately apparent at first inspection of the scatter diagrams. A minority of the individual muscles showed a significant trend, and such correlation coefficients were

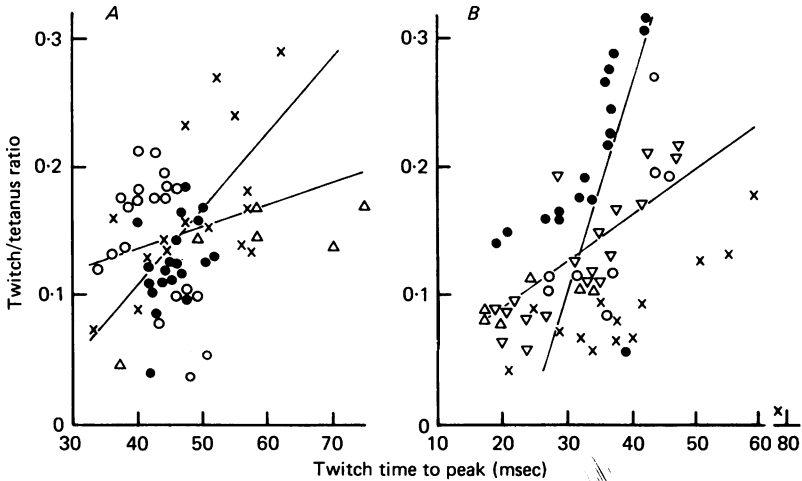


Fig. 6. Relationships between twitch time to peak (at motor unit tetanic optimum length) and the ratio of twitch tension to tetanic tension (maximum value) in motor units of cross-reinnervated muscles. *A*, flexor digitorum longus and *B*, soleus muscle. Symbols and lines as in Figs. 4 and 5. Significance of regressions: *A*, $r = 0.30$, $P < 0.05$; *B*, $r = 0.58$, $P < 0.01$. Most individual muscles gave regressions significant at the 0.01 level (\circ , \times in *A* and ∇ , Δ , \times in *B*), at the 0.02 level (\bullet in *B*) or the 0.05 level (Δ in *A* and \circ in *B*).

less significant than those derived after pooling all the data: the reverse situation is found in normal muscles (Bagust *et al* 1973; Bagust, 1974).

Twitch-tetanus ratio and optimal length

The ratio of twitch tension to tetanic tension, which is a measure of the degree of activation in the twitch, increased with twitch time to peak in both cross-reinnervated muscles (Fig. 6) as in normal muscles. If motor units with similar twitch times to peak from the two reinnervated muscles are compared, it is seen that those from soleus tended to have the higher twitch-tetanus ratio. The reverse is found in normal muscle so this is a further example of reversal of motor unit properties by cross-reinnervation.

In mammalian muscle the length at which twitch tension is maximal is longer than the length optimal for tetanic tension; for intact muscles the difference between twitch and tetanus optimal lengths is about 5–10% of fibre length. In normal soleus this whole muscle property is reflected in the motor units, but for flexor digitorum longus the difference between twitch and tetanus optima is much greater for the motor units than for the muscle (Lewis, Luck & Knott, 1972). These differences between the length-related properties of motor units of the two muscles were lost following cross-reinnervation. In cross-reinnervated flexor digitorum longus the motor unit twitch optimum length was on average 1.09 (s.d. = 1.12) mm longer than the tetanus optimum (4.5% of estimated fibre length). The corresponding difference for cross-reinnervated soleus was 4.40 (s.d. = 1.87) mm or 4.7% of fibre length.

DISCUSSION

In general the results of these experiments are clear cut: cross-reinnervation acts at the level of the individual motor unit. They add another argument against the idea that cross-reinnervation produces changes by differential atrophy of, say, the slow muscle fibres of cross-reinnervated soleus: the cross-reinnervated soleus contains many motor units with shorter contraction times than are found in normal soleus,

just as cross-reinnervated fast muscle has motor units slower than any in the control muscle. Such differences must be due to transformation of the properties of individual muscle fibres.

Some aspects of normal motor unit organization are not seen in the cross-reinnervated muscles. Most of these discrepancies are a consequence of reinnervation rather than of nerve crossing in that they are seen in self-reinnervated muscle. The most obvious example of this is the distribution of motor unit tensions: reinnervated muscles consist of a few abnormally large motor units and an abnormally large number of small ones.

The shape of the tension histograms of the reinnervated muscles might be postulated to be the result of a random distribution of muscle fibres between the reinnervated axons. A quantitative prediction of a random model is difficult because no record was kept of the number of axons which ran in a muscle nerve but which did not elicit tension in the muscle. A guess of 5% would not be unreasonable, and values between 0 and 20% were assumed to predict Poisson distributions of motor unit tensions from the mean values. In all cases the experimental distributions differed significantly from any of the predictions of the simple random reinnervation model.

The changes in the whole muscle tensions per unit area indicate that the packing density of myofibrils in fibres is influenced by innervation in the cat just as it is in the rat (Hoh, 1974).

The most remarkable discrepancy was the relation between twitch time to peak and tetanic tension. In normal fast and slow muscle, the fastest motor units have the highest tension. This correlation was lost after cross-reinnervation and may even be reversed in the cross-reinnervated soleus. A similar, but less clear, reversal was observed in self-reinnervated soleus, and two hypotheses were offered (Bagust & Lewis, 1974). One was that there was selective reinnervation (large axons being less able to reform connexions with muscle fibres). The other was that the fastest contracting muscle fibres can only be induced in the reinnervated muscle by appropriate connexions (between the fastest axons and the fastest muscle fibres) and that the probability of such a match was low. The present results allowed a quantitative test of the first hypothesis. No significant change in the distribution about the mean of axonal conduction velocities was found in either cross-reinnervated muscle, which argues against selective reinnervation and loss of fastest axons.

A third hypothesis should be added: that there is a change in motoneurons after reinnervation of soleus such that those with the fastest conducting axons are no longer capable of branching extensively and maintaining large motor units. The consequence of the inverted relationship is clear, the twitch of cross-reinnervated soleus is incompletely converted, in part, because the fast contracting motor units make a small contribution to the whole muscle response, in part because the conversion is incomplete at the motor unit or muscle fibre level.

In cross-reinnervated flexor digitorum longus motor units the most important discrepancy was in the motor axons which had originally innervated soleus muscle. The conduction velocities of cross-reinnervating soleus axons were higher than those of soleus axons self-reinnervating soleus muscle (Lewis *et al.* 1978). This change would

seem to be due to an influence of the muscle on the axons reinnervating it. Despite this modification of axon properties, the fast muscle shows the more complete conversion by cross-reinnervation.

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