J. Physiol. (1986), **372**, *pp.* 485–500
With 7 text-figures
Printed in Great Britain

REINNERVATION OF THE LATERAL GASTROCNEMIUS AND SOLEUS MUSCLES IN THE RAT BY THEIR COMMON NERVE

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(*Received 12 March 1985*)

SUMMARY

1. To determine whether there is any specificity of regenerating nerves for their original muscles, the common lateral gastrocnemius soleus nerve (l.g.s.) innervating the fast-twitch lateral gastrocnemius (l.g.) and slow-twitch soleus muscles was sectioned in the hind limb of twenty adult rats. The proximal nerve stump was sutured to the dorsal surface of the l.g. muscle and 4–14 months later, the contractile properties of the reinnervated l.g. and soleus muscles and their single motor units were studied by dissection and stimulation of the ventral root filaments. Contractile properties of normal contralateral muscles were examined for comparison and motor units were isolated in l.g. and soleus muscles for study in a group of untreated animals.

2. Measurement of time and rate parameters of maximal twitch and tetanic contractions showed that the rate of development of force increased significantly in reinnervated soleus muscles and approached the speed of l.g. muscles but rate of relaxation did not change appreciably. In reinnervated l.g. muscles, contraction speed was similar to normal l.g. muscles but relaxation rate declined toward the rates of relaxation in control soleus muscles.

3. After reinnervation by the common l.g.s. nerve, the proportion of slow motor units in l.g. increased from 10 to 31 % and decreased in soleus from 80 to 31 %. The relative proportions of fast and slow motor units in each muscle were the same as the proportions of fast and slow units in the normal l.g. and soleus muscles combined.

4. It was concluded that fast and slow muscles do not show any preference for their former nerves and that the change in the force profile of the reinnervated muscles is indicative of the relative proportions of fast and slow motor units: fast units dominate the contraction phase and slow units the relaxation phase of twitch and tetanic contractions of the muscle.

INTRODUCTION

During embryogenesis, motoneurones and muscles develop highly specific connexions based on the position of the neurone in the neuraxis, environmental cues

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and contact guidance of the developing axon, and possibly a biochemical identity for both cells (reviewed by Landmesser, 1980). Notwithstanding the considerable evidence for accurate guidance of developing nerve fibres toward their appropriate muscles, there is conflicting evidence that nerve fibres specifically seek out their original target end-organs in adult mammalian systems.

Early experiments attempted to determine whether there is nerve-muscle specificity in the adult by sectioning and resuturing a large nerve trunk in the lower limb and assessing target innervation (Sperry, 1941; Weiss & Hoag, 1946; Bernstein & Guth, 1961). Some investigators concluded that muscles showed no preference for their original nerve (Weiss & Hoag, 1946; Bernstein & Guth, 1961; Miledi & Stefani, 1969). In other experiments where a denervated fast muscle was presented with regenerating axons from both the original nerve and from the nerve to the slow soleus muscle, the fast muscle appeared to show a preference for its original nerve fibres (Hoh, 1975). Because many of these studies did not provide information on the characteristics of the muscles before and after reinnervation nor did they identify which axons innervated which muscles, it was not possible to clearly determine whether fast and slow muscle fibres were preferentially reinnervated by nerve fibres which belonged to fast and slow motor units, respectively.

Cross-innervation experiments demonstrated that adult mammalian muscle can readily be innervated by foreign nerves and some muscle properties may be altered under the influence of the nerve (Buller, Eccles & Eccles, 1960; Yellin, 1967). However, following cross-innervation, the muscle had only the choice between incorrect innervation or none at all. Self-reinnervation is a frequent experimental complication after cross-union of nerves to fast and slow muscles and this has been taken as evidence of preference by muscles for their former nerves (Close, 1969).

There is a simpler and more natural experimental situation in which muscles can choose self from foreign nerve reinnervation: the predominantly fast lateral gastrocnemius (l.g.) and predominantly slow soleus muscles of the triceps surae group of ankle extensor muscles are innervated in the hind limb by a common lateral gastrocnemius soleus branch of the tibial nerve (l.g.s.). A portion of the nerve innervates the l.g. muscle and the remaining nerve fibres pass through l.g. to innervate soleus. Thus, cutting the l.g.s. nerve and allowing it to reinnervate the denervated muscles presents the fast l.g. and slow soleus with opportunities to show selective or non-selective reinnervation for their original nerve fibres or foreign nerve fibres and the situation mimics that occuring during normal nerve repair after injury.

Specificity of reinnervation in whole muscles may better be characterized by description of their motor unit population (Burke, Levine, Tsairis & Zajac, 1973; Kugelberg, 1973; Gordon & Stein, 1982*a*; Gordon, Stein & Thomas, 1986). At the single motor unit level, proportions of fast and slow motor unit types in reinnervated cat muscles after resuture of a single muscle nerve were normal (Gordon & Stein, 1982*a*). This suggests that nerve fibres of slow and fast motor units were equally successful in regeneration and reinnervation of muscle. Nevertheless, nerve fibres did not make contact with their original muscle fibres but each motoneurone innervated muscle fibres that formerly belonged to several different motor units (Karpati & Engel, 1968; Kugelberg, Edstrom & Abbruzzese, 1970; Gordon & Stein, 1982*a*).

In the present study we have re-examined the question of selective reinnervation

by studying the properties of the synergistic l.g. and soleus muscles after suture of their common l.g.s. nerve. After section of the common l.g.s. nerve, the l.g. and soleus muscles may become reinnervated by regenerating axons of fast or slow motor units from either muscle or both. The muscles and their motor units were characterized by their contractile properties both before and after reinnervation. We found that 90 % of the l.g. muscle motor units are normally fast and 80 % of the soleus units are slow. After reinnervation, l.g. and soleus muscles contained equal proportions of fast and slow motor units. Nevertheless the two muscles did not become identical. The contractile properties of each muscle were not completely typical of either slow or fast muscle and changes in the contractile properties of muscle could be attributed to an altered proportion of motor units in reinnervated muscle. The results of the study therefore support the view that denervated muscles will accept motor innervation from any regenerating nerves and do not show preference for their original nerves. Preliminary accounts of the data have been published in abstract form (Gillespie, Gordon & Murphy, 1983*a*,*b*).

METHODS

Surgery and preparation

The l.g.s. nerve was cut before its entry into the l.g. muscle in twenty Sprague–Dawley rats of both sexes (175–200 g) under sodium pentobarbitone (Nembutal) anaesthesia (60 mg kg^{-1}) and aseptic conditions. The nerve was sewn to the dorsal surface of l.g. After 4–14 months the animals, now weighing 300–500 g, were again anaesthetized with an initial dose of Ketamine (100 mg kg^{-1}) followed by Nembutal (20 mg kg^{-1}) intraperitoneally. Anaesthesia was maintained with intravenous Nembutal (20 mg kg^{-1}) as necessary and the blood pressure was monitored through an arterial cannula inserted into the carotid artery. Both hind legs were prepared for observation by denervation of all muscles except the l.g. and soleus, and the tendons of these two muscles were tied separately with surgical Mersilene (gauge 0) for attachment to transducers (Grass FTO3C or FTO3B) for force recording. A laminectomy was performed and the ventral roots of L_4 and L_5 were separated from the spinal cord and prepared for stimulation of single motor units by division into fine filaments. In eight control animals of comparable age and weight one hind limb was similarly prepared for study of the motor unit population of their l.g. and soleus muscles.

The animal was placed in a prone position on a heating pad and the distal femur and calcaneus were secured with metal pins. The skin around the incisions of the spinal cord and the legs was drawn up and a paraffin pool kept around the spinal cord and the leg muscles. Spinal and rectal temperatures were maintained at 36–38 °C. The length of each muscle was adjusted to give maximum twitch force in response to a single square-wave pulse of 0·01 ms duration and amplitude 2 times threshold to the sciatic nerve.

Muscle and motor unit force

Twitch and tetanic contractions of control and reinnervated muscles were recorded in response to maximal stimulation of the sciatic nerve prior to division of the L4 and L5 ventral roots for study of reinnervated motor units in experimental animals and normal units in control animals. The electromyogram (e.m.g.) of the muscles was recorded by two fine (75 μm) silver wires inserted into the belly of the l.g. and soleus muscles. Force and e.m.g., were amplified, displayed on a Tektronix storage oscilloscope and digitized by an LSI-11 computer (Digital Equipment Corp., Marlboro, MA, U.S.A.). Up to thirty contractions were averaged on-line to improve the signal-to-noise ratio, and the data was stored for later analysis. The contractile characteristics were determined for the stored data as described in detail previously (Stein, Gordon & Shriver, 1982; Gordon & Stein, 1985) and illustrated in Fig. 1 for a normal l.g. muscle.

1. The contraction time is the time from the beginning of the rise of force to a peak force of a single isometric twitch at the optimum muscle length.

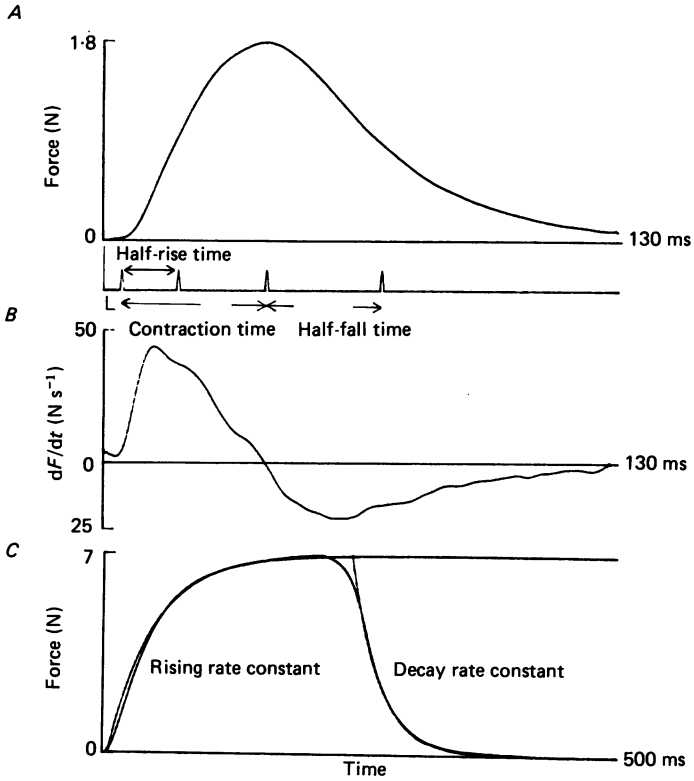

Fig. 1. Isometric force of lateral gastrocnemius muscle recorded in response to maximal stimulation of the motor nerves: *A*, twitch, *B*, the first differential of the twitch and *C*, tetanus in response to twenty pulses at 80 Hz. The following parameters were automatically computed from averaged computer records: *A*, twitch force (N), contraction time, half-rise time, half-fall time (ms) and decay rate constant (s^{-1}) of a single exponential fitted to the falling phase of the twitch (not shown) and to the tetanic contraction (*C*) (note that decay rate constant was similar for twitch and tetanic contractions), *B*, the first differential of the twitch force and *C*, the rising rate constant and decay rate constant obtained from single exponentials fitted to the rise and fall of tetanic force, respectively. (For further details see Methods.)

2. The maximum force is the peak amplitude of the force trace of a single isometric twitch at the optimum muscle length.

3. The half-rise time is the time from the onset of the rise of force to 50 % of the maximum force for the isometric twitch.

4. The half-fall time is the time required for the twitch force to decay from its peak to half its maximum value.

5. The maximum rate of rise of force during a twitch contraction is determined from the peak of the first differential of the twitch curve, divided by the twitch force to give a normalized rate of change of force with units of s^{-1}.

6. The rising rate constant is obtained by fitting an exponential to the rising phase of the tetanic contraction, as described in detail previously (Stein *et al.* 1982; Gordon & Stein, 1985).

7. The decay rate constant is obtained by fitting an exponential to a segment of the decay of the tetanus or the twitch. Rate of decay of force is the same for twitch and tetanic contractions and was obtained for both contractions (cf. Stein *et al.* 1982).

8. The maximum tetanic force was defined as the maximum force produced in response to a train of twenty pulses given at an interstimulus interval of one-third of the muscle contraction time.

Speed of contraction of soleus muscles were considerably slower than previously published results (Close, 1967). The temperature of the leg muscles in our muscles was 28–30 °C even though the spinal cord and rectal temperatures of the rat were maintained at 37 °C. Using a Q_{10} of 2·5 for contraction time of the twitch (Stein *et al.* 1982) to correct for temperature brings our values for soleus into agreement with those of Close's recorded at 37 °C.

The protocol used for recording of contractile properties of the muscles was described in detail for cat triceps surae muscles by Gordon & Stein (1982*a*). Briefly, force and e.m.g. of the whole muscle were recorded in response to one, five and twenty pulses applied at 2 × threshold voltage to the sciatic nerve or to the L4 and L5 nerve roots. Contractile properties of individual units were examined by division of ventral roots into filaments until stimulation of a filament produced a single all-or-none twitch response, which remained the same when the voltage was kept near threshold. We also checked that increasing the voltage to 4 or 5 × threshold did not recruit other motor units (no increases in the force or change in the shape of the e.m.g.). Motor unit force and e.m.g. were recorded for twitch and tetanic contractions in response to one pulse and five or twenty pulses at an interspike interval equal to one-third of the twitch contraction time. We often chose to record tetanic force in response to five rather than twenty pulses so as not to fatigue the motor unit during the three to thirty repetitions for computer averaging. In units in which tetanic force was recorded for both five and twenty pulses it was noted that in response to five stimuli, muscles developed 0·77 ± 0·11 % (mean ± S.D.) of the force developed in response to twenty stimuli. To determine the fatigability of the muscle fibres, single motor units were stimulated by thirteen pulses at an interstimulus interval of 25 ms (40 Hz) once per second for 2 min (Burke *et al.* 1973). The tetanic contraction was recorded at time 0, 1 and 2 min (see Fig. 6) and the fatigue index calculated as the ratio of the force developed at 2 and 0 min.

Statistical treatment

Significance was determined using analysis of variance. Significance of differences between individual means was determined by the technique of Gabriel as outlined by Sokal & Rohlf (1969). Where it was necessary to compare the proportion of motor unit types, the χ^2 test with Yate 's correction was employed. In all cases, $P < 0{\cdot}05$ was considered significant.

RESULTS

When twitch and tetanic contractions of reinnervated and contralateral control muscles are compared directly on the same axes in Fig. 2*A* and *B*, it is clear that reinnervated muscles exerted less force than the controls. Reinnervated l.g. developed an average of 2·7 ± 0·48 N (mean ± S.E. of mean) tetanic force and reinnervated soleus an average of 0·81 ± 0·19 N, which was 45 and 61 % of the normal contralateral muscles, respectively. This relatively low recovery of force is consistent with previous results in cat muscles after suture of the l.g.s. nerve to muscle (Gordon & Stein, 1982*b*). In order to make a direct comparison of the time course of the twitch and tetanic contractions, the forces were normalized and contractions of control and reinnervated muscles were plotted on the same axes in Fig. 2*C*. Examination of the twitch contractions on the left of Fig. 2*C* shows that the reinnervated l.g. develops force as rapidly as the control l.g. muscle but relaxes more slowly. The rising phase of the twitch contraction of the reinnervated soleus muscle resembles the rising phase of control and reinnervated l.g. muscles and is quite distinct from that of the control soleus muscle. The relaxation phase of the reinnervated soleus muscle also resembles the relaxation of reinnervated l.g. in that both are becoming intermediate with respect to control muscles.

The rapid development of twitch force of both reinnervated muscles and overlap of the rising phase of contraction with the control l.g. twitch suggests that both reinnervated l.g. and soleus muscles have become fast contracting, perhaps implying

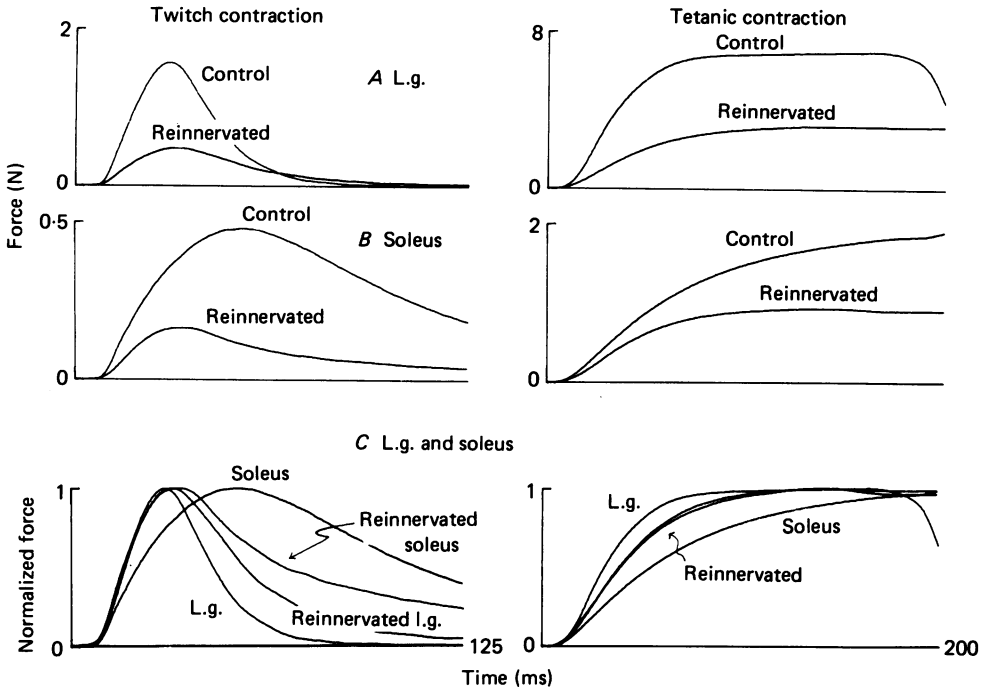

Fig. 2. Twitch (on the left) and tetanic contractions (on the right) of reinnervated and contralateral l.g. (*A*) and soleus (*B*) muscles are plotted on the same force and time axes (for each muscle), 11 months after unilateral l.g.s. nerve section and resuture. In *C*, all four muscles are plotted together to compare rise and fall of force in twitch contractions and the exponential rise of force in tetanic contractions. Because the fall of force follows a simple exponential which is identical for twitch and tetanic contractions (cf. Methods, Stein *et al.* 1982; Gordon & Stein, 1985) relaxation from tetanic contraction is not shown and the time scale for tetanic contractions was chosen to best compare the exponential rise of tetanic force (fitted rising exponentials are not shown but see Fig. 1). Rise of force in soleus is normally considerably slower than l.g. in twitch and tetanic contraction (compare rising rate constants of 16 s^{-1} and 50 s^{-1} for soleus and l.g. muscles shown here). Note that rise of force of reinnervated soleus and l.g. muscles are the same; for the twitch they are the same as the control l.g. and for the tetanus intermediate between the control soleus and l.g. Rate of relaxation of reinnervated muscles are intermediate between control soleus and l.g. muscles (compare decay rate constants of 17 s^{-1} and 86 s^{-1}).

that both are preferentially innervated by axons of fast motor units. However, the relaxation phase of the twitch as well as that for tetanic contractions (not shown on the time scale in Fig. 2*C*) was prolonged in the l.g. after reinnervation. Rate of relaxation slows in reinnervated l.g. and increases in reinnervated soleus. Therefore, it appears that, for this parameter of contractile speed, the reinnervated muscles are becoming intermediate between the normally fast l.g. and slow soleus. The rate of force development for the tetanic contractions, shown on the right of Fig. 2*C*, also suggests that the time course of the contractions of both muscles is becoming intermediate between that of either a predominantly fast or slow muscle.

The speeds of contraction and relaxation are compared for reinnervated and contralateral control muscles from twenty animals in Figs. 3 and 4. For all parameters used to measure rise of twitch force and tetanic force, reinnervated soleus muscles

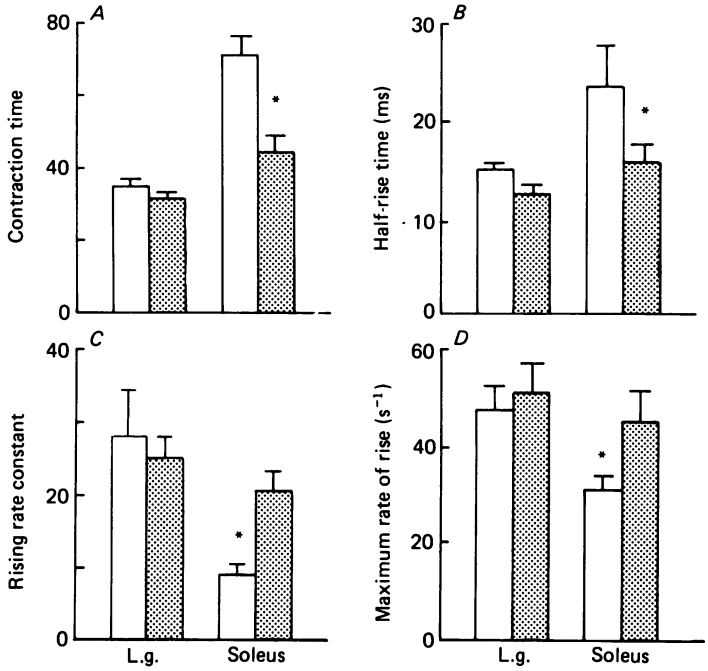

Fig. 3. The mean+S.E. of mean of *A*, the contraction time, *B*, half-rise time, *C*, the calculated maximum rate of rise of force and *D*, rising rate constant; parameters of the rising phase of twitch and tetanic contractions are compared for twenty control and reinnervated l.g. muscles and nineteen and fourteen control and reinnervated soleus muscles. The means of all parameters of contraction speed of reinnervated soleus muscles were significantly increased from control values (denoted by an asterisk) and became similar to mean values in control l.g. There is little change in the range of development of tension in the l.g. muscle after reinnervation. Open bars, control muscles; stippled bars, reinnervated muscles.

were significantly faster in developing force than contralateral soleus muscles. In contrast, rate of force development in reinnervated l.g. muscles was not significantly different from contralateral control muscles. Therefore, all reinnervated muscles became as fast contracting as contralateral l.g. muscles, as shown in the example of Fig. 2. The rate of relaxation of soleus and l.g. changed after reinnervation to values intermediate between control soleus and l.g. muscles (Fig. 4). Differences between means for reinnervated and contralateral control muscles were significant for l.g. but not soleus muscles (Fig. 4). Half-fall time of reinnervated l.g. increased and the decay rate constant was significantly reduced. Although reinnervated soleus appears to show a trend in the opposite direction the differences were not significant.

In summary, the contraction phase of the slow twitch soleus muscle was most affected by reinnervation by the cut and sutured common l.g.s. nerve while the relaxation phase was most affected in the reinnervated l.g. muscles. Only the rising phases of the contractions in reinnervated muscles suggest that axons of fast motor units may have preferentially reinnervated both muscles since both reinnervated muscles resembled the control fast l.g. muscle. It is clear that the relaxation rate of l.g. is slower than control which is consistent with some reinnervation by slow unit

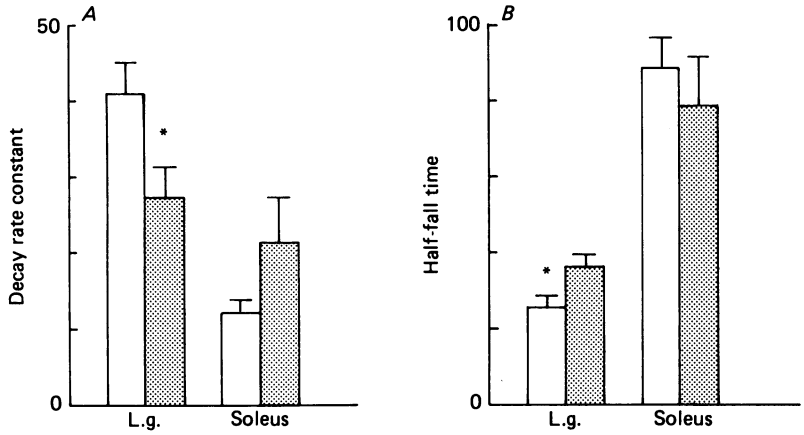

Fig. 4. The time course of the relaxation phase of contractions, described by mean + S.E. of mean of *A*, the decay rate constant (s^{-1}) for the fall in twitch and tetanic force and *B*, the half-fall time of the twitch. Control muscles are shown as open bars and reinnervated muscles by a stippled bar. (Asterisks denote significant differences between reinnervated and control muscles.)

axons. The intermediate relaxation rate of reinnervated soleus is also consistent with the presence of some slow motor units.

A reasonable explanation of the changes in the time course of the twitch and tetanic contractions is that fast muscle fibres tend to dominate the rise of force while the slow fibres in the muscle limit the rate of relaxation of the whole muscle. The prolonged relaxation time in reinnervated l.g. muscle implies more slow muscle fibres than normal and the increase in the rate of rise of force during contraction in reinnervated soleus muscle could result from an increased proportion of fast muscle fibres. To determine the reinnervation of the muscles more directly, single motor units were isolated and characterized.

Motor units in control and reinnervated muscles

The motor units sampled in normal l.g. and soleus muscles from untreated animals, and muscles which became reinnervated after l.g.s. nerve section and resuture, were classified into four types according to their contractile properties. The criteria used were fatigability measured by the fatigue index during a standard fatigue test, twitch contraction time and characteristic shape of tetanic contractions at 40 Hz stimulation. The types of motor units were fast fatigable (f.f.) (fatigue index < 0·25, contraction time < 30 ms), fast intermediate (f.i.) (0·25 < fatigue index < 0·75; contraction time < 30 ms), fast fatigue resistant (f.r.) (fatigue index > 0·75; contraction time < 30 ms), and slow (fatigue index > 0·75; contraction time > 0·30 ms).

Fatigue index. Motor units from normal and reinnervated muscles showed a similar range of fatigability and were separated by fatigue index as shown in Fig. 5 in which horizontal lines at fatigue index of 0·25 separate the fatigable f.f. units from the intermediate fatigable f.i. and at fatigue index of 0·75, separate f.i. units from the fatigue-resistant units.

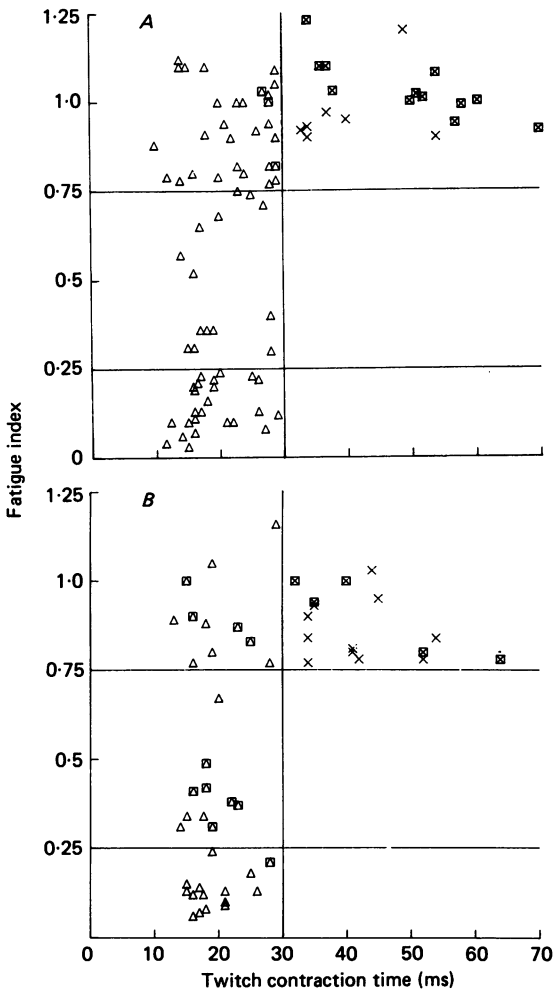

Fig. 5. Fatigue index of *A*, normally innervated and *B*, reinnervated motor units from l.g. and soleus muscles plotted as a function of their twitch contraction time. Those motor units with a fatigue index above the horizontal line drawn at 0·75 were considered to be fatigue resistant while those below the 0·25 fatigue index line were fatigable. Motor units between these two fatigue index limits showed intermediate fatigue characteristics. *A*, motor units with contraction times in the same range as fatigable units (fatigue index < 0·25) were considered fast and separated from units with contraction times outside of this range. Fast (△, l.g.; ⊠, soleus) and slow (×, l.g.; ⊠, soleus) units fell below 30 and above 30 ms, respectively. Units which showed the ' slow force profile ' shown in Fig. 6 as shown by × for l.g. units and ⊠ for soleus units, fell in the group of slow units with contraction times above 30 ms. The same criteria of separation of f.f., f.i., f.r. and slow units in normal muscles (*A*) were applied to reinnervated muscles (*B*).

Twitch contraction time. Fatigue-resistant units with contraction times in the same range as fatigable f.f. and intermediate f.i. units were separated on the x-axis in Fig. 5 from more slowly contracting units (cf. Fleshman, Munson, Sypert & Friedman, 1981; Kernell, Eerbeek & Verhey, 1983).

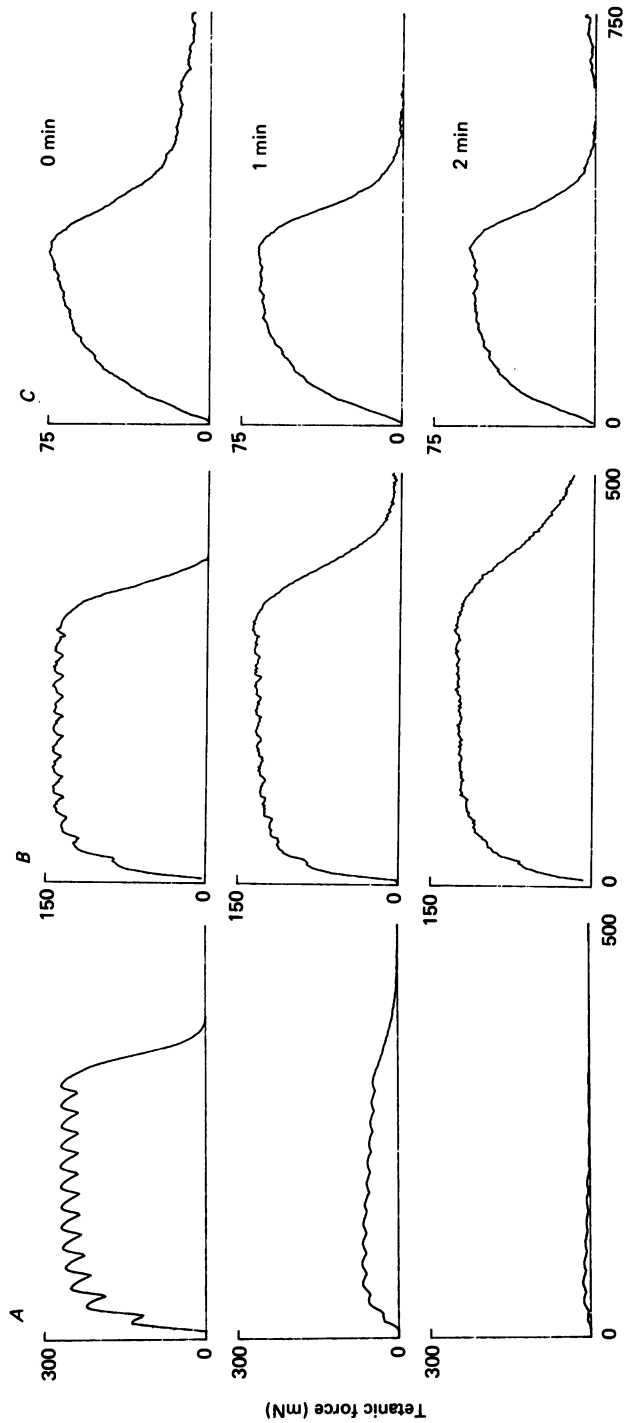

Fig. 6. Typical records of tetanic contractions of f.f., f.r. and slow motor units at 0, 1 and 2 min during repetitive stimulation with thirteen pulses at 40 Hz every second for 2 min.

Tetanic contractions. Tetanic contractions recorded from different units at the beginning and at 1 and 2 min during the repetitive stimulation at 40 Hz in the fatigue test, show characteristic differences (Fig. 6): f.r. and slow units in particular could be readily distinguished on the basis of reproducible differences in the rise of force during the 300 ms tetanus at 40 Hz. Fast units developed force rapidly in response to the first four to five stimulus pulses in a tetanic train to reach a plateau level of

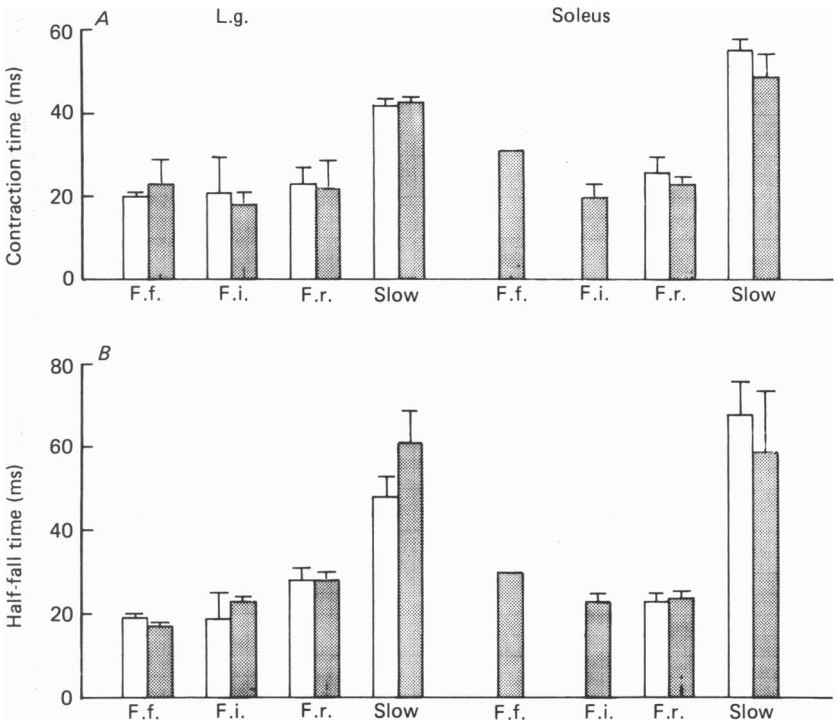

Fig. 7. Means (+S.E. of means) of *A*, the contraction time (ms) and *B*, the half-fall time (ms) of twitch contractions of motor units in control and reinnervated l.g. and soleus muscles. There are no significant differences between motor units from control and reinnervated muscles.

unfused contraction. In contrast, slow units always developed force gradually and force continued to rise without reaching a plateau during the 300 ms tetanus. The gradual rise in force in the slow units never showed the sudden rise of force in the tetani of fast units as they attained peak force. The units which showed the 'slow' force profile are shown by an × symbol in the graphs of Fig. 5 for l.g. units and ⊠ for soleus units. The contraction time of these units were consistently longer than that of the 'fast units' with no significant difference in twitch contraction time or half-fall time between reinnervated and control units, for each of the four unit types (Fig. 7). The point of division was clearly at a contraction time of 30 ms, with fast units below 30 ms and slow units above 30 ms.

Proportions. The distribution of motor units in the reinnervated l.g. and soleus are compared with the distribution in normal muscles in Table 1. Normally, l.g. muscles contain all four types of units with slow units representing the smallest proportion.

TABLE 1. Distribution of motor unit types in l.g. and soleus muscles after reinnervation by the sutured l.g.s. nerve compared with the normal distribution in untreated muscles.

	L.g. motor units		Soleus motor units	
	Number of units	Relative frequency (%)	Number of units	Relative frequency (%)
F.f.				
Control	25	34	0	—
Reinnervated	14	39	1	6
F.i.				
Control	12	16	0	—
Reinnervated	4	11	6	37
F.r.				
Control	30	41	3	20
Reinnervated	7	19	4	25
Slow				
Control	7	9	12	80
Reinnervated	11	31	5	31

Soleus contains only two motor unit types, f.r. and slow units, with the slow units comprising some 80% of the total. The distribution of units in the normally innervated l.g. muscles is significantly different from soleus muscles (χ^2 test with Yate 's correction $P<0{\cdot}01$). Following reinnervation by the common l.g.s. nerve, each of the l.g. and soleus muscles contained all motor unit types and both contained a similar proportion of slow motor units ($\approx 30\%$). The increase in relative proportion of slow units in l.g. and decrease in the soleus muscles were significant at the 5% level of confidence. Further, the over-all proportions of fast and slow units in the reinnervated l.g. and soleus muscles were no longer significantly different. It is apparent therefore that both muscles contained an equal number of fast and slow motor units after reinnervation.

DISCUSSION

A striking finding in this study is that the fast-twitch l.g. muscle and the slow-twitch soleus muscle each contain close to the same proportion ($\approx 30\%$) of slow motor units after reinnervation by regenerating axons from the cut and repaired l.g.s. nerve. In other words, reinnervated l.g. muscle contained a larger proportion of slow motor units and the reinnervated soleus contained a smaller proportion of slow motor units than normal controls. If the muscles showed no preference for their former nerve, the proportions of fast and slow motor units in each muscle should be the same as the proportions of each unit type in the two control muscles taken together. A simple calculation, shown in Table 2, uses the muscle twitch forces and the proportions of motor units contained in them to estimate the actual number of motor units in l.g. and soleus muscles. An estimate of twenty-three slow and forty-two fast fibres in the normal l.g.s. nerve predicts that, if reinnervated equally, both the l.g. and soleus muscles will contain 35% slow motor units. Our data shows that both reinnervated muscles contain $\approx 30\%$ slow motor units which is consistent with the conclusion that reinnervation was non-selective with respect to the proportion of successful nerve fibres in reinnervating each muscle.

TABLE 2. The number of fast and slow motor neurones in the common l.g.s. nerve can be estimated by determining the number of motor units in l.g. and soleus muscles indirectly by division of the total muscle force by the average motor unit force. The proportion of slow and fast motor units was obtained experimentally by classification of the units according to their contraction time and fatigue sensitivity. This calculation permits an estimate of the total number of fast and slow motor neurones in the l.g.s. nerve. Twenty-three out of sixty-five fibres innervate slow units in l.g. and soleus muscles taken together and constitute 35% of the nerve fibres in the l.g.s. nerve

	L.g.	Soleus	L.g.s. nerve
Muscle force (mN)	1650±173 (8)	291±30 (3)	—
Motor unit force (mN)	40±4·3 (74)	12±1·3 (15)	—
No. of motor units = muscle force/unit force	41	24	65
Proportion of slow units (%)	9	80	35
Total no. of slow units	4	19	23
Total no. of fast units	37	5	42

Reinnervation of soleus muscle fibres by original and foreign nerve fibres in a competitive situation confirms the experimental findings of Hoh (1975). But our results differ from his in that he reported that soleus nerve failed to reinnervate a fast-twitch muscle, the extensor digitorum longus (e.d.l.) when directed to it in competition with the original e.d.l. nerve. This apparent preference of a fast-twitch muscle for its original nerve fibres was not confirmed by Riley (1978) and was not observed in the fast l.g. muscle in the present study. The obvious difference in the two types of studies is in the method of directing regenerating axons from self and foreign nerves to denervated muscle. Hoh cut the soleus and e.d.l. nerves and sutured the proximal soleus and e.d.l. nerve stumps to the distal stump of e.d.l. nerve. The strategy used in the present study of using the proximal stump of the common nerve to l.g. and soleus muscles as the source of regenerating foreign and original nerve axons and applying them directly back to their muscles avoided the mismatching of the number of axons proximally and distally. We also avoided the problem of misdirection of regenerating fibres due to factors other than selectivity, by having the regenerating nerve fibres in their original anatomical position in the l.g.s. nerve trunk. Riley (1978) showed that cut nerves often failed to regenerate to the foreign muscle to which they were directed by suturing. In fact, nerve fibres frequently made no nerve–muscle contacts at all. End-to-end sutures of proximal and distal nerve stumps after transection are known to suffer mechanical stresses even when elaborate surgical precautions are taken (De Medinaceli & Freed, 1983; De Medinaceli, Freed & Wyatt 1983). These stresses are likely to be even greater in nerve sutures which purposely misdirect the proximal nerve stump away from its natural course to the distal nerve stump of another muscle as in competition experiments and cross-reinnervation studies. They may actually account, at least in part, for a frequent observation that nerve fibres reinnervate their original muscles despite cross-union of nerves (Dubowitz, 1967; Close 1969; Chan, Edgerton, Goslow, Kurata, Rasmussen & Spector, 1982).

Studies of competition of original foreign nerves for reinnervation of denervated muscles, in which the nerves are sutured directly to muscle fascia (Frank, Jansen, Lomo & Westgaard, 1975; Ip & Vrbova, 1983) are further complicated by variable distance of the two nerves from the former end-plates where regenerating axons have

a strong tendency to make synaptic connexions (Gutmann & Young, 1944; Gutmann & Hanzilikova, 1967; Sanes, Marshall & McMahan, 1978; Ip & Vrbova, 1983).

In conclusion, many extraneous factors influence regeneration and have complicated experiments designed to determine selectivity of reinnervation. By minimizing many of these factors, the present experimental paradigm provides strong support for the notion that specificity of nerve-muscle connexions during development is not retained in the adult.

The trophic influence of the nerve on muscle

Cross-innervation experiments have consistently led to the conclusion that the nerve can alter the contractile speed of the muscle (Buller *et al.* 1960; Close, 1967; Dubowitz, 1967; Dhoot, Perry & Vrbova, 1981) but the finding that the conversion of some muscle contractile properties towards those of the type of muscle formerly innervated by the nerve was incomplete, especially for slow muscle, suggested a limitation of the ability of the nerve to control muscle properties (Robbins, Karpati & Engel, 1969; Chan *et al.* 1982). Hypotheses presented to explain the intermediate contractile properties of whole muscle have been either that all fibres have undergone partial change to a similar degree and are all intermediate in type (Buller & Lewis, 1965; Close, 1969; Robbins *et al.* 1969; Sreter, Luff & Gergely, 1975; Hoh, Kwan, Dunlop & Kim, 1980), or that reinnervated muscle contained a mixture of fast and slow muscle units which are fully differentiated with respect to contraction time (Chan *et al.* 1982; Gordon & Stein, 1982*a*). Findings of this study provide clear evidence to support the latter suggestion and are consistent with recent studies showing complete conversion of slow to fast or fast to slow forms of the regulatory proteins of the troponin complex within single muscle fibres, after cross-innervation (Dhoot *et al.* 1981). In our study, the new nerve supply determined the contractile properties of individual muscle fibres so that the properties of each motor unit type were similar to control in reinnervated motor units as shown in Figs. 5–7. There was no evidence that reinnervated motor units had intermediate contractile speed characteristics at long times after reinnervation, but rather that the alterations in contractile properties of whole muscle are due to the altered proportions of each type of motor unit in the muscle. These proportions will dictate the over-all time characteristics of the muscle twitch as we have shown for the reinnervated l.g. and soleus muscles. The fast motor units in a muscle will cause a fast rate of twitch tension and the slow motor units will extend the duration of the twitch and control the rate of relaxation of the muscle.

Consequently, changes in whole muscle properties after reinnervation are inadequately described using the contraction time alone. Much more information can be provided by examination of the characteristics of the rising and falling phases of the twitch and tetanic contractions, and by examination of the motor unit population. Since the proportions of fast and slow motor units in a muscle are responsible for the contractile characteristics, the changes in whole muscle contraction and relaxation rates after reinnervation indicate changes in proportions of fast and slow units, respectively.

We would like to thank Dr R. B. Stein for his valuable comments on the manuscript and the Canadian Medical Research Council for support of this research. P. R. Murphy was a Post-doctoral Fellow of the Alberta Heritage Foundation for Medical Research and the work presented here forms part of the thesis accepted by the University of Alberta for the degree of Ph.D. to M. J. Gillespie.

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