

ISOMETRIC CONTRACTIONS OF MOTOR UNITS
IN SELF-REINNERVATED FAST AND SLOW TWITCH
MUSCLES OF THE CAT

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SUMMARY

1. Isometric contractions of motor units have been studied in self-reinnervated soleus and flexor digitorum longus muscles, the motor nerves of which had been transected 6 months earlier.

2. The distributions of motor unit tetanic tensions in both muscles were skewed towards large values and showed greater variance than normal: there were motor units which produced more tension and others which developed less tension than in control muscles. Mean tensions of motor units are compared with those of the controls.

3. The variance of times to peak of motor units in both muscles was significantly smaller than normal.

4. Conduction velocities of axons were slower than normal above the point of nerve section. A number of axons did not functionally cross this point and those that did conducted slowly below the neuroma.

5. Many of the correlations seen in normal muscle between axon conduction velocity, motor unit tetanic tension, twitch time to peak and ratio of twitch to tetanic tension were demonstrated in reinnervated muscle.

6. We suggest that the pattern of motor unit organization in self-reinnervated muscle can in part be due to random regrowth of axons but is largely produced by specific processes related to axon size which are not identical in fast and slow muscle.

INTRODUCTION

Cat limb muscles have a complex pattern of motor units in which axonal conduction velocity is related to the isometric tetanic tension and twitch speed of the muscle fibres (Bessou, Emonet-Dénand & Laporte,

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1963). When motor units have been compared in detail some differences have been shown between the patterns in a fast twitch and slow twitch muscle (Bagust, Knott, Lewis, Luck & Westerman, 1973; Bagust, 1974). The motor unit patterns are not fully developed until some time after birth (Bagust, Lewis & Westerman, 1974). We wished to know whether it is possible, in the adult, for reinnervation to restore the normal pattern of motor unit organization and in particular the characteristic differences between fast and slow muscle. It is known that there are no major changes in the properties of whole muscle contractions following self-reinnervation (e.g. Buller & Lewis, 1965*b*). But before reinnervation can occur there is considerable disruption of the normal anatomy and physiology of the muscle and nerve fibres. After nerve section peripheral nerve branches degenerate and regrowing axons establish new end-plate sites on muscle fibres (Gwyn & Aitken, 1966). An axon does not necessarily re-establish connexions with the muscle fibres which it had originally innervated (Kugelberg, Edström & Abbruzzese, 1970). The denervated muscle fibres do not degenerate but atrophy (Tower, 1935) and undergo a change in twitch speed in the third week (Lewis, 1972). The first axons re-establish functional connexions only after the fourth week (Eccles, Eccles & Kozak, 1962). An examination of motor unit patterns might therefore reveal more subtle differences between normal and reinnervated muscle than the study of whole muscle contractions had done. The investigation might also give information about the mechanisms by which motor nerves determine the contractile properties of muscle fibres and organize the muscle into motor units.

We have therefore examined flexor digitorum longus (FDL) and soleus muscles of cats 6 months after transection of their motor nerves. The characteristics of their motor units are reported in this paper. Throughout the description comparisons are made with control groups of motor units from normal muscles. The normal data used is that presented for FDL by Bagust, Knott, Lewis, Luck & Westerman (1973) and for soleus by Bagust (1974).

METHODS

Young cats weighing more than 1 kg were anaesthetized with an i.p. injection of sodium pentobarbitone (35 mg/kg body wt.). Using aseptic surgery a lateral incision was made in the skin of one lower leg through which the nerve to either soleus or FDL was transected. The cut ends of soleus nerves were tied together with fine thread to prevent separation. The wound was closed in layers, sodium penicillin powder was spread subcutaneously and procaine penicillin was injected systemically. The animals were housed in a pen (floor 2 m by 3 m, walls 2 m high and made of 1 in wire mesh) which allowed reasonable activity. Within a week of the operation the cats were able to run and climb as well as normal animals and no abnormality of

ankle or toe movement was detected. Six to seven months later the mean animal weight was 1.8 kg (s.d. = 0.3 kg). They were anaesthetized with sodium pentobarbitone, 40 mg/kg body wt. of injected i.p., smaller doses were administered i.v. to maintain anaesthesia.

Methods of preparation were similar to those described earlier by Buller & Lewis (1965*b*) and Bagust, Knott, Lewis, Luck & Westerman (1973). We took care to free the muscle nerve for stimulation only above the neuroma at the site of transection. This reduced the possibility of damage to axons which may have reached the muscle by paths outside the main nerve trunk. In some experiments fine stimulating electrodes were brought down on to the nerve trunk below the neuroma. At the beginning of each experiment, twitches of the whole muscle were measured at a number of lengths with an unbonded wire strain gauge (Ether UF 1-16). During the course of the experiment muscle twitches at optimum length were measured and experiments terminated when tension fell to less than 80% of the initial value. Motor units were functionally isolated at random by splitting ventral roots and isometric twitch and tetanus tension-length curves were measured on the same dynamometer used for whole muscle contractions. Terminally a less sensitive tension recorder (Statham, G 1-80) was substituted to measure whole muscle tetanus tension-length curves. Details of the methods were similar to those described by Bagust, Knott, Lewis, Luck & Westerman (1973), Bagust, Lewis & Westerman (1974) and Bagust (1974) for motor units of normal unoperated FDL and soleus muscles. In the results, the normal muscle motor units described in these two papers have been used as controls for comparison with the motor units of the self-reinnervated muscles. The wet weights of reinnervated and contralateral muscles were measured for comparison.

RESULTS

Thirty-three motor units were functionally isolated from five soleus muscles and twenty-six motor units from five FDL muscles, only one muscle being examined in any one animal. The periods of reinnervation were not significantly different in the two sets and the over-all mean was 207 days (s.d. = 19 days). The mean maximum tetanic tension of FDL (12.6 N; s.d. = 4.7 N) was also close to that of soleus (13.1 N; s.d. = 4.9 N). These tensions were less than would be expected in normal animals of the same weight (Buller & Lewis, 1965*a*). The deficit was largely due to a reduction in muscle weight since the tensions per unit mass were normal (20 N/g in FDL and 7.6 N/g in soleus). The mean wet weight of the self-reinnervated muscles was 92% of that of the contralateral muscles.

The distributions of motor unit tetanic tensions are shown in Fig. 1 (left) and several differences from those in normal muscles are seen. Most obvious is the fact that in both reinnervated muscles the distribution of motor unit tensions was significantly skewed towards large values ($P < 0.1\%$). This contrasts with control soleus muscles in which motor units are normally distributed. Control FDL motor units do have an asymmetrical distribution but the variance in the reinnervated muscles was found to be significantly greater than that of the controls (variance ratio = 2.4; degrees of freedom or d.f. = 25 and 60; $P < 1\%$). Logarithm-

mic transforms allowed a similar comparison to be made in soleus. Again the variance in the reinnervated muscles was greater (ratio = 13.9; d.f. = 29 and 65; $P < 1\%$). These variances are examined in greater detail in Table 1.

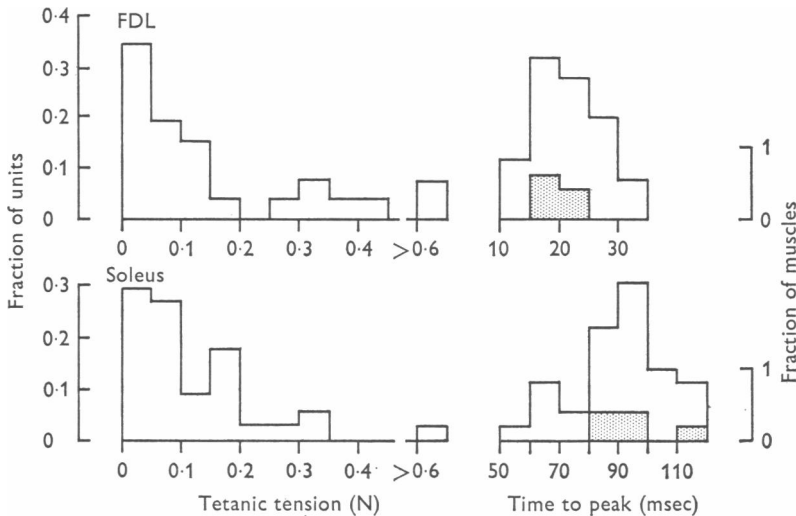


Fig. 1. Distributions of thirty-three motor units from reinnervated soleus (below) and twenty-six from reinnervated FDL muscles. On the left are the motor unit tetanic tensions and on the right twitch times to peak, each motor unit was measured at its own tetanic optimum length. Superimposed on the right are the distributions of whole muscle twitch times (cross-hatched) with a separate scale shown on the far right.

The greater variance of motor unit tensions in reinnervated muscles compared with normals was due to differences at both ends of the range. At the lower end there were some motor units which developed less tension than those seen in normal muscles. This was most clear in soleus, the smallest motor unit of which developed 1.2 mN tetanic tension (0.0093% of the parent muscle tetanic tension) but was also seen in FDL in which the lowest motor unit tension was 2.2 mN (0.013%). These were smaller than the corresponding values in normal muscle. At the upper end of the range the greatest tension developed by a motor unit in the reinnervated muscles exceeded the largest tension of the motor units in normal muscle. This was more marked if the tension of the motor units was expressed as a percentage of that of the parent muscle. Then the largest motor unit tension seen in FDL (14.1%) and in soleus (4.3%) were three or four times larger than those found in the control muscles. However the excess was less when tensions were expressed in absolute units: in reinnervated soleus the largest

motor unit tension (0.53 N) was about twice that in normal muscle and in FDL there was only a small discrepancy (the largest motor unit developed 0.88 N tetanic tension).

Mean motor unit tension gives an estimate of the number of motor axons which innervate a muscle. The mean tetanic tension of the reinnervated soleus motor units was 1.00% of the whole muscle tension (s.e. of mean = 0.17%, $n = 33$) and for FDL was 1.66% (s.e. of mean = 0.57%, $n = 26$). These figures had large standard errors which were largely a result of the skewed distributions with a small number of very large motor units. They lead to an estimate that on average 100 axons reinnervated the soleus and sixty the FDL muscles which are less than estimates in normal muscles (150 and 124 respectively). The mean motor unit tensions were larger in reinnervated muscles than in the controls and the differences were significant by a t test both in soleus ($t = 2.6$; d.f. = 42.3, allowing for unequal variances; $P = 1\%$) and in FDL ($t = 2.4$; d.f. = 33.0; $P < 5\%$). These differences could be due to differences between animals rather than a consequence of reinnervation (Bagust, Knott, Lewis, Luck & Westerman, 1973). The analysis of variance in both FDL and soleus confirmed that this was possible (Table 1).

During the search for motor units, a number of axons were isolated which were single as judged by an all-or-none antidromic action potential, but which gave no muscle tension or electromyogram when stimulated in the ventral root filament. The conduction times of these axons were within the range found for those axons which excited the muscle. In experiments in which we attempted to stimulate below the neuroma no antidromic action potential could be recorded from the ventral root. Moreover, stimulation of the nerve above the neuroma in these animals elicited the same tension as stimulation below, indicating that there were few axons crossing the neuroma which did not have functional connexions within the muscle. We have made no systematic measurements but we found axons which did not conduct below the neuroma in three of the six experiments in which we stimulated distally. It would be of interest to know if these axons might have re-established connexions with the muscle fibres later. If taken into account they would reduce the estimates of mean motor unit tension to 1.56% in FDL and 0.88% in soleus.

The distributions of times to peak of twitches of motor units and the whole muscles are shown in Fig. 1 (right). The mean value for FDL muscles (18.8 msec) was similar to that of normal muscles; the mean for reinnervated soleus muscles (94 msec) was greater than that for the control muscles but the difference was not significant ($t = 0.95$).

At first sight the distributions of the motor unit twitch times to peak appeared to be similar to those of the normal controls but there was signifi-

cantly less scatter in the reinnervated muscles. This can be seen from the range of motor unit twitch times to peak which was 14–32 msec in reinnervated FDL and 54–119 msec in soleus. The variances of motor unit twitch times were shown (Table 1) to be significantly less in reinnervated muscles than in the control series ($P < 5\%$ in FDL, $P < 2\%$ in soleus). In FDL this reduction in scatter following reinnervation is remarkable because the whole muscle twitches showed more variability in times to peak than in the control group. The variance ratio (control to reinnervated) of whole muscle twitch times to peak was 3.1 (d.f. = 4 and 18) which was significant at the 5% level.

TABLE 1. Analyses of variance of motor unit responses. Tetanic tensions were expressed as a percentage of the tension of the parent muscle; twitch times to peak were measured in msec. Logarithmic transforms have been used for three sets in order to normalize the distributions. It is assumed that the variances may be partitioned into three groups: that between motor units within a muscle, that between muscles within a set (control or reinnervated) and that between the two sets. The figures below are variances (with degrees of freedom in brackets) or, in every third row, the variance ratio (with its significance in brackets, n.s. indicates that the results were not significant at the 5% level)

	Within muscles	Within sets	Between sets
FDL log tension (%)			
Reinnervated	0.432 (21)	0.609 (4)	
Control	0.166 (55)	0.536 (5)	0.055 (1)
Ratio	2.6 (< 1%)	1.13 (n.s.)	
Soleus log tension (%)			
Reinnervated	0.393 (26)	1.43 (3)	
Control	0.036 (62)	0.051 (3)	0.411 (1)
Ratio	11.1 (\leq 1%)	1.43 (n.s.)	
FDL log time to peak (msec)			
Reinnervated	0.0109 (21)	0.0254 (4)	
Control	0.0255 (65)	0.0792 (7)	0.0016 (1)
Ratio	2.1 (< 5%)	3.1 (n.s.)	
Soleus time to peak (msec)			
Reinnervated	148 (30)	938 (4)	
Control	297 (92)	1347 (7)	1262 (1)
Ratio	2.0 (< 2%)	1.4 (n.s.)	

The mean conduction velocity of motor unit axons was 78 m/sec (s.d. = 10 m/sec; $n = 23$) for FDL and in soleus was 59 m/sec (s.d. = 14 m/sec; $n = 33$). These values were lower than those obtained from normal animals; the differences were significant for FDL ($t = 4.0$; $P < 0.1\%$) and for soleus ($t = 3.1$; $P < 1\%$). In some experiments action potentials were also initiated below the neuroma and these had conduction times greater by 0.2 to 4.5 msec (mean 0.9 msec). The additional conduction distance

was not more than 20 mm, indicating that many of the regrowing segments of the nerve axons conducted very slowly.

The relationships found in normal muscles between motor unit axonal conduction velocity, twitch time to peak, tetanic tension and ratio of twitch to tetanic tension were seen, in part, in reinnervated muscles. First, motor unit tetanic tension increased with axonal conduction velocity in both types of reinnervated muscle ($P < 1\%$, Table 2) as in normal

TABLE 2. Correlations between various characteristics of motor units of reinnervated muscles, lines fitted by the least squares method. n.s. indicates results not significant at the 5% level

	FDL			Soleus		
	Slope	<i>n</i>	<i>P</i>	Slope	<i>n</i>	<i>P</i>
Regressions on conduction velocity (m/sec)						
Of twitch time to peak (msec)	-0.020	23	n.s.	-0.040	33	n.s.
Of tetanic tension (log %)	0.034	26	< 1%	0.024	31	< 1%
Regressions on twitch time to peak (msec)						
Of tetanic tension (log %)	-0.066	26	< 1%	0.024	33	< 1%
Of twitch-tetanus ratio	0.0087	21	< 1%	0.0012	33	n.s.

muscle. Secondly, no relationship was found between twitch time to peak and conduction velocity in the pooled data from reinnervated muscles, although in two of the ten muscles a correlation significant at the 5% level was present. In these two, time to peak decreased with increasing conduction velocity of the axon as in normal muscles. Thirdly, in reinnervated FDL (Fig. 2*B*), motor unit tetanic tension decreased with increasing twitch time to peak ($P < 1\%$, Table 2), this is similar to the relationship in normal muscle. However, in soleus the opposite relation was found with the same degree of significance, thus soleus motor units with fast twitches developed less tension than average in reinnervated muscles. Examination of Fig. 2*D* indicates that this correlation depends upon the fact that the four motor units with the shortest time to peak all developed little tension.

Fig. 2 also presents the data as cumulative tension curves. The latter were obtained by summing tetanic tensions of motor units ranked in order of their twitch times to peak, starting with the slowest. It can be seen that the shapes of the curves for FDL and soleus were dissimilar. These differences resembled those seen between comparable curves derived from normal FDL and soleus motor units.

In motor units of normal adult muscles the ratio of peak twitch tension to maximum tetanic tension is positively correlated with twitch time to peak. This relationship was also seen in reinnervated FDL muscles ($P < 1\%$, Table 2) but not in soleus. Fig. 3 illustrates these points and also

indicates the distribution of twitch-tetanus ratios. The ranges of twitch-tetanus ratios were 0.19–0.41 in reinnervated FDL and 0.17–0.37 in soleus; these were 2.5 and 1.5 times smaller than those in the corresponding control muscles. The variance in the reinnervated FDL motor unit twitch-tetanus ratios was significantly lower than that of normal motor units

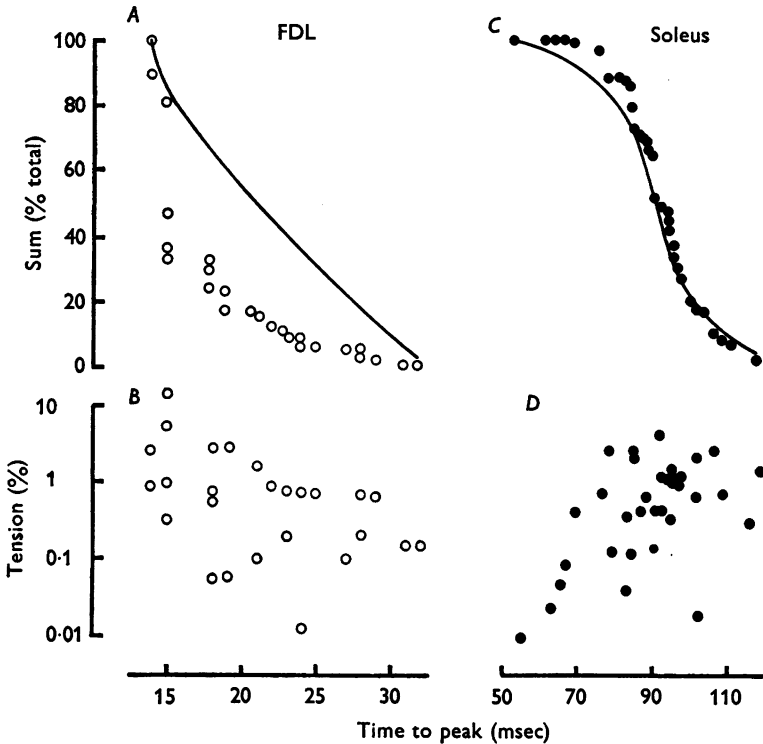


Fig. 2. *B* and *D* are plots of motor unit tetanic tension against twitch times to peak for reinnervated soleus (*D*) and FDL. *A* and *C* are cumulative curves derived from these. The motor units have been ranked from slowest to fastest and their tensions summed. The intermediate sums of tension have been plotted against twitch time as circles. A model has also been made in which it was assumed that each motor unit had the same tension. The continuous lines have been fitted to the cumulative sums derived from this model. Tensions are expressed as a percentage of the parent muscle tetanic tension.

(variance ratio = 3.8; d.f. = 20 and 68; $P < 1\%$). The corresponding variance ratio in soleus was not significant. The mean motor unit twitch-tetanus ratios of 0.30 in FDL and 0.25 in soleus were similar to those in the control muscles.

In many motor units of FDL twitch tension and therefore twitch-tetanus ratio increased with muscle length up to maximum *in situ* length (Bagust, Knott, Lewis, Luck & Westerman, 1973). Soleus motor units typically had a maximum twitch-tetanus ratio at a length close to the muscle optimum length and the ratio was smaller at both longer and shorter lengths. This point of difference between fast and slow has been found in control muscles.

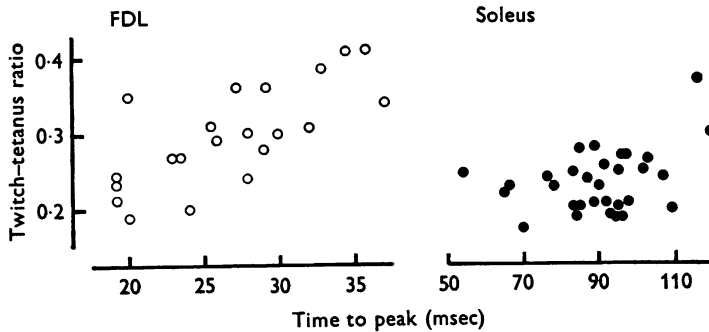


Fig. 3. Relationship between twitch time to peak and the ratio of twitch to tetanic tensions for FDL (left) and soleus motor units in reinnervated muscles.

DISCUSSION

Motor units in reinnervated muscles have been identified histochemically in rats (Kugelberg *et al.* 1970) and recorded electromyographically in man (Yahr, Herz, Moldaver & Grundfest, 1950). These studies show abnormal arrangements in that a motor unit may contain large or small groups of muscle fibres, which suggest that regrowing motor axons branch within a small region of the muscle and capture groups of adjacent muscle fibres. The distributions of motor unit tensions described earlier are certainly compatible with a reinnervation process in which nerve axons arrive at the muscle at random times and then branch and form end-plates. In this model, those which arrive first would branch most extensively and form the largest motor units; those arriving later would find fewer muscle fibres without innervation. The similarity between the distributions of motor unit tensions in FDL and soleus, which contrasts with the picture in normal development of motor unit patterns (Bagust, Lewis & Westerman, 1974), would be a strong argument for random reinnervation. But there are several difficulties in adopting such a simple model. Although the range of motor unit tensions was greater than normal, there were no motor units which developed very much larger absolute tensions than those in normal muscles. Perhaps there is a maximum innervation ratio (the number of

fibres which can be innervated by one axon). Some of the observations of Eccles & Sherrington (1930) may support this, since they found that in semitendinosus (and less obviously in other muscles) mean motor unit tension was almost constant, independent of the number of axons innervating a muscle, although the number varied fivefold in semitendinosus nerves. This hypothesis might be extended to suggest that the maximum innervation ratio is related to axonal diameter. The relationship between axonal conduction velocity and motor unit tension, which reappears in reinnervated muscle, would be explained by this.

In other ways the differences between normal fast and slow muscle have been restored. Clearly this is true of mean motor unit twitch time to peak, and also is seen in the responses of motor unit contractions to change of muscle length. Most interesting is the restoration of some of the relationships between the characteristics of the motor units, one of which has already been discussed. The exceptions to this were the lack of any clear-cut association of motor unit twitch time to peak with axonal conduction velocity in either muscle or with twitch-tetanus ratio in soleus. These anomalies may be due to the difficulties of proving the correlations in pooled data or in small samples from one animal (Bagust, 1974) and the negative finding may not be important. More difficult to explain is the abnormal relationship between motor unit twitch speed and tetanic tension in reinnervated soleus. The fact that the fastest motor units were smaller than average could be explained by selective reinnervation so that the large axons (normally associated with fast muscle fibres) had a lower than average chance of forming synapses on slow muscle fibres. Selective regeneration need not be invoked if the results of cross-reinnervation in the cat are taken into account (Buller & Lewis, 1965*b*). These showed that the nerve to FDL produced a change in the twitch time to peak of soleus but only to a value intermediate between that of the fast and of the slow muscle. If this applied to a fast conducting axon reinnervating slow twitch muscle fibres, the resulting motor unit would have an intermediate twitch time. The fastest motor units could then only result from fast conducting axons establishing connexions with a high proportion of muscle fibres which were originally fast. Since both fast axons and fast fibres are uncommon in slow muscle the chance of this happening would be low, therefore these motor units would be small. This argument depends on the assumption that characteristics of the twitch speed of a muscle fibre is retained during denervation, but this has been shown to be true at the whole muscle level (Lewis, 1972).

Bagust, Knott, Lewis, Luck & Westerman (1973) have suggested that the correlations between the variables of motor unit contraction can be explained if it is assumed that there is independent control of two aspects

of the muscle fibre response (the force-velocity relationship and the duration of active state following a single stimulus). One of a set of possible models would be one in which all the muscle fibres within a muscle contain the same myosin (or one of a limited range: Weeds & Pope, 1971) whilst the duration of active state varies from the fibres of one motor unit to those of another. This would result in the correlation between motor unit twitch time to peak and twitch-tetanus ratio. It would be predicted from this hypothesis that any change in the neural influence which altered only the duration of active state in the motor units would produce parallel changes in the twitch time and the twitch-tetanus ratio. This is compatible with the changes in the self-reinnervated muscles in which there is a smaller than normal range for both these variables. It would be interesting to know if there were complete restoration after a longer period of reinnervation.

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