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HOW FLEXIBLE IS THE NEURAL CONTROL OF MUSCLE PROPERTIES?

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SUMMARY

The issue addressed in this paper is to what extent are selected physiological properties and associated protein systems of muscle fibres controlled or regulated by neuronal systems. One extreme position would be that all muscle proteins are controlled completely by the neural system that innervates the muscle. The opposite position would be that none of the muscle proteins are under neural influence. Although the concept that there is complete neural control of all proteins has generally received more support, it is more likely that there is only partial neural control of some proteins. Identical physiological, morphological and metabolic properties of all muscle fibres within a motor unit would suggest a complete neural control of all protein systems in muscle fibres. However, evidence against this idea is provided by the marked heterogeneity in the activities of two enzymes, alpha glycerophosphate dehydrogenase and succinic dehydrogenase (SDH), and in the wide variations in muscle fibre cross-sectional areas among fibres of the same motor unit in the cat soleus and tibialis anterior.

Buller, Eccles & Eccles (1960) convincingly showed that certain properties of a muscle were dependent, at least in part, on the source of its innervation. This interrelationship was demonstrated by denervating a fast and a slow muscle and then surgically reuniting the proximal nerve segments to the foreign distal nerve stump. After approximately 6 months, the effects of this cross-reinnervation procedure on the contractile responses of the muscles were studied. The finding of primary importance to the topic of this paper is that after cross-reinnervation, the isometric contraction time of the slow soleus muscle had become more like that of the fast muscle and *vice versa*.

Since this classic report, there have been numerous studies which have essentially verified these initial findings. Many of the subsequent papers have addressed two major questions regarding the phenomenon of neural control of muscle properties. First, how does the nervous system exert its control over certain physiological and biochemical properties? Three principal means of neuronal control have been proposed: (1) the pattern (quality) of impulses; (2) the total number (quantity) of

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impulses; and, (3) a neurotrophic (nonactivity-related) mechanism (Buller *et al.* 1960). All three possibilities remain viable today. However, the most promising evidence suggests that impulse frequency (Salmons & Sreter, 1976) and quantity of impulses (Sreter, Pinter, Jolesz & Mabuchi, 1982; Jolesz & Sreter, 1981) could be responsible, at least in part, for differences in the speed-related properties of slow and fast muscles.

A second issue that has been addressed repeatedly since the initial cross-reinnervation studies is the extent to which the neural innervation influences muscle properties. Are all physiological, metabolic and morphological properties of muscle fibres under complete control of the nerve? Alternatives to unilateral control by the nerve would be that some properties are controlled completely or in part by one or more non-neural system(s) (Fig. 1). In our opinion, the evidence suggests that the neural systems share the control of muscle properties with other control systems such as the muscle's own intrinsically-based genetic system.

The fact that all physiological properties of a cell are largely manifestations of the kind, number and location of proteins within the cell emphasizes the importance of understanding the factors that control protein synthesis and degradation. A direct

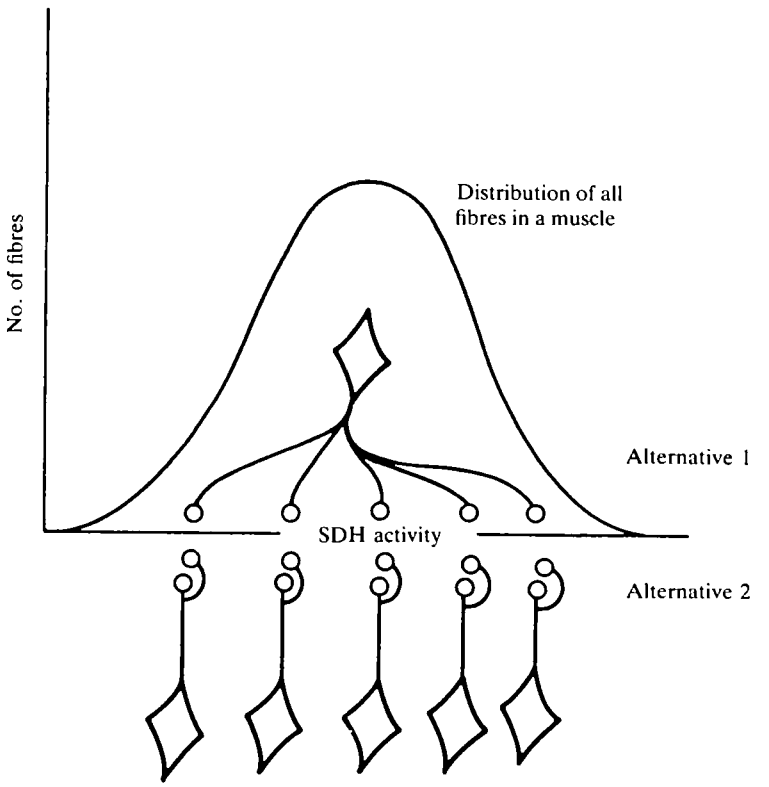

Fig. 1. Theoretically, any given property [e.g. succinate dehydrogenase (SDH) activity of muscle fibres within a muscle] could vary as much within a motor unit as across motor units. In contrast, all fibres within a motor unit could have identical properties and the variability within a muscle could be due to differences between units.

assessment of these proteins is represented in those studies that have dealt with measurements of muscular enzymic activities (e.g. the determination of the specific activities of myosin adenosine triphosphatase, ATPase, and succinic dehydrogenase, SDH). Further understanding is also gained by determining the kinds of proteins found in a muscle (e.g. the proportion of slow and fast myosin light chains, Salmons & Sreter, 1976).

Potential sources of protein regulation within a muscle fibre are illustrated in Fig. 2. In addition to neural input, an obvious and potentially independent source of control intrinsic to the muscle fibre is *via* its nuclei. Given that muscle fibres contact other cells types, extrinsic control systems other than neural also may be present. Thus, indirect factors (e.g. hormonal, as well as more local factors such as the milieu of a fibre's nuclei) may exert a control that is independent of neural influence. For a multinucleated cell, another possible source of interaction is the coordination of protein regulation among the nuclei within a fibre. If a muscle fibre has similar properties throughout its length, it would seem that all nuclei within that fibre must be synchronized in their control of protein metabolism. The relative importance of these control or regulatory mechanisms, individually or in combination, remains to be determined. It is our opinion that each source has a certain potential for altering protein metabolism at several levels within the muscle fibre. Therefore, attempts to assign importance, or to identify a single controlling mechanism may be inappropriate.

To address the central theme of how comprehensive the neural influence over muscle fibre protein metabolism is, control at three levels will be considered: (1) whole muscle, (2) inter-motor unit and (3) intra-motor unit. In addition, perturbations of the neural input to skeletal muscle have been achieved through different experimental models to determine the stability of the protein-coordinating systems. Specifically, self-reinnervation, cross-reinnervation and spinal transection

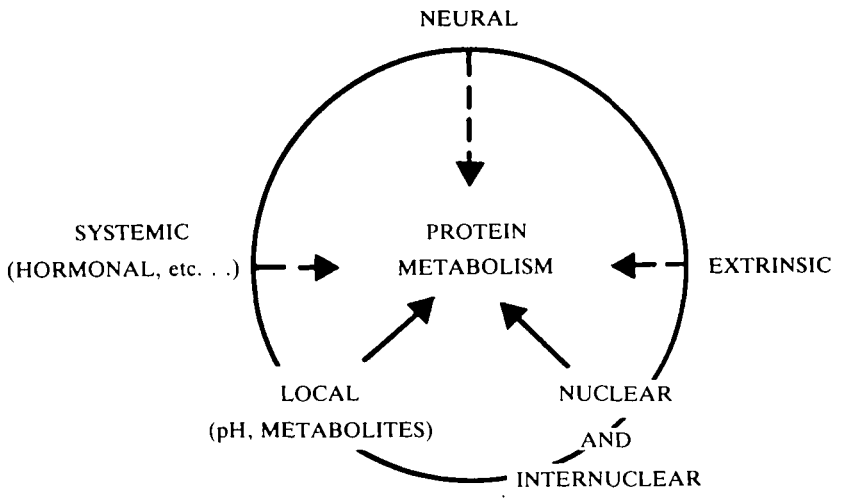

Fig. 2. A scheme illustrating potential sources of influence on muscle fibre proteins.

have been studied in sufficient detail to be discussed at one or more of the levels outlined.

LEVEL 1: CONTROL OF WHOLE MUSCLE PROPERTIES

Several months after cross-reuniting their nerves, a slow muscle can be converted toward a fast muscle and *vice versa* as shown by contraction time, half-relaxation time, and maximum velocity of shortening (Buller *et al.* 1960; Close, 1969). Similar trends in conversion are evident for some closely associated biochemical properties, e.g. myosin ATPase, myofibrillar ATPase (Buller, Mommaerts & Seraydarian, 1969), myosin light chain patterns (Salmons & Sreter, 1976) and metabolic properties, e.g. glycolytic enzymes (Prewitt & Salafsky, 1967). In contrast, the mitochondrial enzyme, malate dehydrogenase, is not reduced in a muscle after it is cross-reinnervated with a nerve that normally innervates a low oxidative fast muscle (Prewitt & Salafsky, 1967). This observation suggests that the oxidative capacity of a muscle is somewhat independent of the quantity and/or quality of neural input.

Similarly, complete spinal cord transection at a low thoracic level results in a conversion of the mechanical and biochemical properties of both slow and fast ankle extensors toward a 'faster' muscle (Roy *et al.* 1984). Consistent with the concept of independence of the oxidative activities of muscle fibres from neural input seen in the cross-innervation model is the observation that no changes in the citrate synthetase activity or the fatigue index in the cat soleus or medial gastrocnemius muscles were observed 6–12 months after cordotomy (Baldwin *et al.* 1984). Thus, the completeness or degree of the conversion process produced by the altered neural input resulting from these models remains unclear.

One limitation of whole muscle analyses is that the response to altered neural input is not uniform in all muscle fibres. The histochemical analysis of muscle fibres following cross-reinnervation clearly illustrates this point. Although one can find generalized statements implying that the expected complete histochemical conversions were observed (Romanul & Van Der Meulen, 1967), more recent evidence suggests that only a small proportion of soleus muscle fibres develop the characteristics of a typical fast muscle fibre following as long as 1 year of cross-reinnervation (Chan *et al.* 1982). A final point is that data derived from cross-reinnervated whole muscle analyses should be interpreted with some reservation. Although it can be determined with reasonable certainty that the reinnervation is only from the foreign nerve, a greater amount of uncertainty exists as to whether the foreign reinnervation is complete, that is, whether all muscle fibres received some innervation by the foreign nerve.

LEVEL 2: CONTROL BETWEEN DIFFERENT MOTOR UNITS WITHIN A MUSCLE

Analysis of the physiological properties of motor units avoids some of the limitations inherent in the determination of whole muscle properties. For example, we know that a relatively normal range in several motor unit properties can be re-

established after self-reinnervation and cross-reinnervation, e.g. in tetanic tension, contraction time and conduction velocity (Bagust & Lewis, 1974; Bagust, Lewis & Westerman, 1981; Chan *et al.* 1982). It can also be established with reasonable certainty whether a motor unit's innervation source is from the original (self-reinnervated) or from a foreign (cross-reinnervated) nerve trunk.

Up to a year after cross-reinnervation of the slow, fatigue-resistant soleus with a nerve that originally innervated a fast, fatigable muscle, those cross-reinnervated motor units which developed 'fast' properties remained fatigue-resistant. In concurrence with no increase in fatigability, the oxidative potential of the soleus muscle fibres as shown histochemically by the SDH reaction, was maintained (Edgerton, Goslow, Rasmussen & Spector, 1980*a*). Similar findings have been observed in soleus motor units from spinal transected cats (Edgerton *et al.* 1980*b*). These results suggest that the source of control of this oxidative enzyme and those systems responsible for the fatigue response of a motor unit may be intrinsic to the muscle. An alternative explanation is that the normal neural pattern of the motoneurones is modified by the cross-reinnervation procedure. If, indeed, this is the case, then cross-reinnervation is not a useful experimental model to study the neural influence on muscle.

It is generally agreed that the speed-related contractile properties reflect protein systems not closely aligned with the mitochondrial systems discussed above. Changes in contraction time of cross-reinnervated motor units are in agreement with the interpretation that there could be complete neural control of muscle fibre properties. However, a rather consistent indicator of 'speed' of contraction, the sag property, has been reported not to change after cross-reinnervation (Gauthier, Burke, Lowey & Hobbs, 1983), although Chan *et al.* (1982) reported that about 40 % of the cross-reinnervated fibres of the soleus muscle showed some 'sag'. In normal muscles, Burke and colleagues (Burke, Levine, Tsairis & Zajac, 1973) have used the presence or absence of sag in subtetanic contractions as a criterion for the classification of a motor unit as fast or slow, respectively. The occasionally complete conversion of motor unit contraction time and relaxation time and whole muscle maximum shortening velocity (Buller *et al.* 1969; Close, 1969) after cross-reinnervation and the variable response of a sag-like property suggest that some of these speed-related systems are under partial neural control. Furthermore, the fact that contraction time and sag are tightly coupled normally but are not necessarily coupled after cross-reinnervation, suggests that the underlying protein systems responsible for these two properties are merely coincidentally related in normal muscles.

LEVEL 3 : CONTROL AMONG FIBRES WITHIN A MOTOR UNIT

The analysis of single muscle fibres belonging to the same motor unit permits the use of an additional and more stringent criterion to determine the extent of the neural control. The concept of complete neural control of muscle would be consistent with the observation that all the fibres of a muscle unit are essentially invariant in their physiological, morphological and metabolic characteristics.

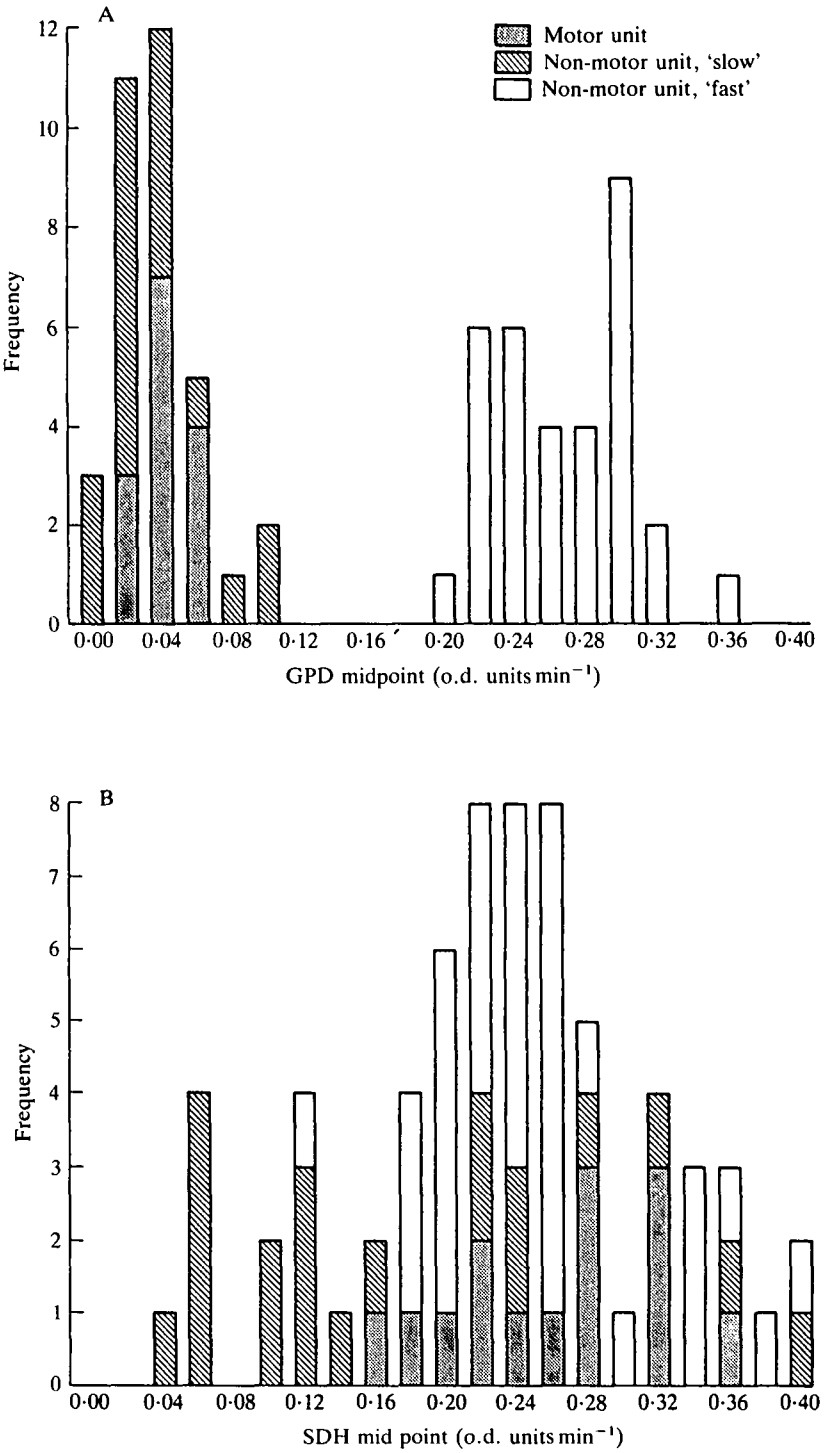
A
Motor unit
Non-motor unit, 'slow'
Non-motor unit, 'fast'
Frequency
0 2 4 6 8 10 12
0·00 0·04 0·08 0·12 0·16 0·20 0·24 0·28 0·32 0·36 0·40
GPD midpoint (o.d. units min⁻¹)
B
Frequency
0 1 2 3 4 5 6 7 8
0·00 0·04 0·08 0·12 0·16 0·20 0·24 0·28 0·32 0·36 0·40
SDH mid point (o.d. units min⁻¹)

Fig. 3

The initial studies of Kugelberg & Edstrom (1968) and Edstrom & Kugelberg (1968) in the rat tibialis anterior, of Mayer & Doyle (1970) in the cat tibialis anterior and extensor digitorum longus, and of Burke *et al.* (1973) in the cat medial gastrocnemius and soleus (Burke, Levine, Saloman & Tsairis, 1974) suggest a homogeneity for ATPase staining and for a variety of oxidative histochemcial reactions for fibres within a motor unit. However, the general conclusion of homogeneity should be tempered by the qualitative method used in determining a muscle fibre's metabolic profile. Although the homogeneity of the ATPase stain at selected pH values can be used in the assignment of individual fibres to a specific type, visual inspection of mitochondrial and/or glycolytic enzyme reactions cannot be used with the same certainty when categorizing fibres into types.

The development of the concept of motor unit homogeneity has a curious history. Interestingly, a study often quoted to support the concept of homogeneity reported a three- to five-fold range in fibre area within a motor unit (Kugelberg & Edstrom, 1968). The idea of metabolic homogeneity also may be questioned. For example, we have used data from Fig. 7 of Kugelberg & Lindegren (1979) to estimate the variability in SDH staining density in fibres within a given motor unit. The coefficient of variation (CV) of individual fibre SDH activity from nine motor units ranged between 3 and 17 % with a mean variation of approximately 10 %. These data strongly suggest that some metabolic heterogeneity exists between the fibres of a motor unit. Although the technique (microphotometry) utilized to generate these data is described as being quantitative, no verification was presented, leaving this as a cause for a conservative interpretation.

Nemeth, Pette & Vrbová (1981) addressed the question of metabolic homogeneity of a motor unit by performing biochemical assays on single fibres of a motor unit identified by glycogen depletion. Fibres were dissected from the muscle, identified as having a critical level of glycogen, and assayed for malate dehydrogenase activity. Contrary to our interpretation of the previously discussed data of Kugelberg & Lindegren (1979), Nemeth *et al.* (1981) reported that the variability among fibres of the same unit was actually less than within a muscle fibre along its length and less than the measurement error. The data of Nemeth *et al.* (1981) suggest that all fibres within a unit have identical malate dehydrogenase activities and would be consistent with the concept that there is complete neural control of this protein in muscle.

Using a different quantitative procedure (Castleman, Chui, Martin & Edgerton, 1984) however, we have found considerably more variation in another oxidative enzyme (i.e. SDH, within a motor unit). In addition, we have found similar degrees of variability for a glycolytic enzyme [i.e. alpha-glycerophosphate dehydrogenase (GPD)] and for fibre cross-sectional area (CSA).

Fig. 3. Distribution of (A) alpha-glycerophosphate dehydrogenase (GPD) and (B) succinate dehydrogenase (SDH) activity in a normal soleus muscle in which a motor unit had been repetitively stimulated. Enzyme activities were determined using an image processing system and reported as optical density units per minute (o.d. units min^{-1}). Comparisons were made between (1) depleted fibres (motor unit), (2) surrounding non-depleted fibres which stained lightly for alkaline myosin ATPase, pH = 8·8 (non-motor unit, 'slow'), and (3) surrounding non-depleted fibres which stained heavily for alkaline myosin ATPase (non-motor unit, 'fast').

In five normal adult cats, a muscle unit from the normally homogeneous cat soleus was depleted of its glycogen through repetitive stimulation of its motoneurone.

Table 1. *Summary of means and variations of cross-sectional area, GPD and SDH activities of muscle fibres within and across motor units*

		N	$\bar{X}$	S.D.	CV
Area (μm^2)					
	MU	92	2148	456	21
	NMU	278	2681	585	22
GPD (o.d. units min^{-1})					
	MU	14	0·045	0·016	34
	NMU-'S'	20	0·040	0·024	62
	NMU-'F'	20	0·281	0·034	12
SDH (o.d. units min^{-1})					
	MU	14	0·256	0·058	22
	NMU-'S'	20	0·174	0·115	66
	NMU-'F'	20	0·259	0·076	29

GPD, alpha glycerophosphate dehydrogenase; SDH, succinate dehydrogenase; MU, muscle fibres of a motor unit identified by glycogen depletion; NMU, muscle fibres within the region of the identified motor unit fibres, but not part of the motor unit; NMU-'S' those NMU fibres that stained lightly for myofibrillar ATPase, after alkaline preincubation; NMU-'F', those NMU fibres that stained darkly for myofibrillar ATPase, after alkaline preincubation; coefficient of variance, CV; mean, $\bar{X}$; number of fibres, *N*; standard deviation, S.D.

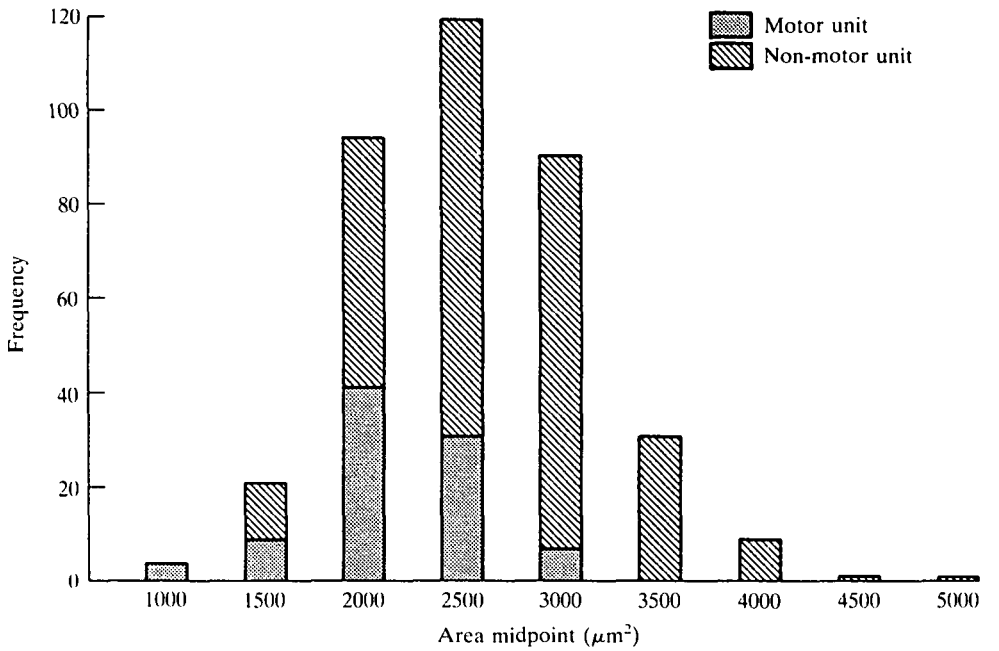

Fig. 4. Distribution of fibre areas (μm^2) in a depleted soleus muscle unit (motor unit) and in surrounding non-depleted fibres (non-motor unit). Fibre areas were determined from a periodic acid stained cross-section using an image processing computer.

Additional soleus units ($N = 3$) were depleted in adult cats that had undergone complete spinal cord transection (T12–T13) 4 months earlier. The units from the spinal transected cats were of particular interest because the resultant muscular changes were consistent with a coordinated conversion of motoneuronal and muscle fibre physiological and biochemical properties from that of slow to fast motor units (T. P. Martin, T. Cope, S. C. Bodine & V. R. Edgerton, unpublished observations). In all units studied, the range in SDH and GPD reactions and in fibre CSA within a unit was only slightly less than the variation among fibres of the same type but belonging to different units (Figs 3, 4). This variability was reflected in the coefficients of variation (Table 1). Thus, in contrast to the results reported by Nemeth *et al.* (1981) for the rat extensor digitorum longus, our results for the cat soleus showed marked variabilities among fibres of the same motor unit.

To further examine the homogeneity of muscle fibres within a unit, we have studied the cat tibialis anterior muscle because it contains a greater range of motor unit types. Relative to the data in Table 1, our conclusion is that although the variability in fibre GPD and SDH (Fig. 3) within a unit may be slightly less than the variability across units, it, at least, spans the range that generally would be expected of muscle fibres of the same 'type'. A similar conclusion can be drawn for fibre CSA (Fig. 4) except that the range within a unit was larger than the range seen for GPD or SDH.

The implications of these results are that there is some, but not complete, neural control of muscle fibre CSA, GPD activity and SDH activity within a motor unit. Given these results, a modification of the concept of the completeness of the neural control of muscle fibre properties is appropriate. Additionally, these results suggest some alternatives to the concept of the interaction of neural activation and muscular responses. If CSA and SDH activity are determined by the neuromuscular activity patterns or by the amount of muscular activity, then all fibres within a unit should have similar properties. Alternatively, all muscle fibres may not be activated identically in response to each action potential generated by the motoneurone. This, however, is contrary to current views. In summary, it appears that the neural influence on muscle properties is shared with other control systems, and that these systems are not dependent completely on activation features.

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